Microbiology (1998), **144**, 2377–2406 Printed in Great Britain

SGM SPECIAL LECTURE

Biochemistry of methanogenesis: a tribute to Marjory Stephenson

Rudolf K. Thauer

Tel: +49 6421 178200. Fax: +49 6421 179209. e-mail: thauer@mailer.uni-marburg.de

Max-Planck-Institut für terrestrische Mikrobiologie, Karl-von-Frisch-Straße, D-35043 Marburg, and Laboratorium für Mikrobiologie, Fachbereich Biologie, Philipps-Universität, Karl-von-Frisch-Straße, D-35032 Marburg, Germany

1998 Marjory Stephenson Prize Lecture

(Delivered at the 140th Ordinary Meeting of the Society for General Microbiology, 31 March 1998)

Keywords: methanogenesis and methanogenic archaea, methyl-coenzyme M reductase and its crystal structure, coenzyme F_{430}, coenzyme M, coenzyme B

Historical overview

In 1933, Stephenson & Stickland (1933a) published that they had isolated from river mud, by the single cell technique, a methanogenic organism capable of growth in an inorganic medium with formate as the sole carbon source.

$$4HCOO^- + 4H^+ \rightarrow CH_4 + 3CO_2 + 2H_2O \qquad \Delta G^{0\prime} = -144{\cdot}5 \text{ kJ mol}^{-1}$$

Methane formation from formate was shown to occur in a stepwise manner, by the preliminary decomposition of formic acid into CO_2 and H_2 followed by a reduction of CO_2 by H_2, suggesting that formate was not an intermediate in the reduction of CO_2 to methane.

$$HCOO^- + H^+ \rightarrow H_2 + CO_2 \qquad \Delta G^{0\prime} = -3{\cdot}5 \text{ kJ mol}^{-1}$$
$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad \Delta G^{0\prime} = -131 \text{ kJ mol}^{-1}$$

Cell suspensions of the micro-organism catalysed the reduction of methylene blue with H_2, indicating that the methanogen contained an enzyme which activates molecular hydrogen.

$$H_2 \rightarrow 2e^- + 2H^+ \qquad E_0' = -414 \text{ mV}$$

This enzyme had been discovered by Stephenson & Stickland (1931a) 2 years before in a number of bacterial species and was named by them 'hydrogenase'.

The paper by Stephenson & Stickland (1933a) is considered to mark the beginning of the modern era for study of methanogenesis (Wolfe, 1993). It is the first report on the isolation in pure culture of a methanogen and the first study of an enzyme and of reactions involved in methanogenesis.

Besides CO_2, the cell suspensions of the methanogen isolated by Stephenson & Stickland (1933a) catalysed the reduction of carbon monoxide, formaldehyde (added as hexamethylenetetramine) and methanol to methane with H_2, albeit at much lower rates than the reduction of CO_2. It is significant that the cell suspensions did not yield methane from compounds of two or more carbon atoms. The cell suspensions did, however, slowly reduce sulphate with H_2 to H_2S, a property not encountered by any other methanogen isolated since then. It therefore seems possible that, in spite of all efforts to purify it, the culture obtained by Stephenson & Stickland (1933a) was contaminated with one of the sulphate reducers abundantly present in the river mud (Stephenson & Stickland, 1931b) from which the methanogen was isolated (Elsden & Pirie, 1949).

The following review summarizes what we presently know about the biochemistry of methanogenesis, emphasis being put on the enzyme methyl-coenzyme M reductase catalysing the methane-forming reaction proper. It is a tribute to Marjory Stephenson, who discovered hydrogenase in these micro-organisms. Marjory Stephenson died 50 years ago on 12 December 1948 at the age of only 63 (Elsden & Pirie, 1949; Robertson, 1949; Woods, 1950; Elsden, 1981). She had worked for 30 years in the Cambridge Biochemical Laboratory headed by Frederick Gowland Hopkins (Stephenson, 1948) on various aspects of the chemistry of bacteria (Stephenson, 1932, 1933, 1934, 1935). The 3rd edition of her book on *Bacterial Metabolism* (Stephenson, 1949) is a treasury of knowledge very worth reading even today, 50 years later. Marjory Stephenson was the second woman to be elected to the Royal Society of London (Mason, 1992, 1996) and a cofounder of the Society for General Microbiology, of which she was the second president (Postgate, 1995).

Except when otherwise noted, the free energy changes given for methanogenic reactions were calculated from the free energies of formation from the elements of the substrates and products with non-gaseous compounds at 1 M aqueous solution and gaseous compounds in the gaseous state at 1 atmosphere pressure (101 kPa). The free energy changes of formation were taken from Thauer *et al.* (1977).

In this review, with the exception of a few papers describing discoveries, only publications on the biochemistry of methanogenesis are cited that have appeared since 1993: these contain reference to earlier and often more important papers, many of which have come from the laboratories of A. J. Kluyver (Kamp *et al.*, 1959), H. A. Barker (Barker, 1956), T. Stadtman (Stadtman, 1967; Grahame & Stadtman, 1993), R. Wolfe (Wolfe, 1991, 1996), J. D. Ferry (Ferry, 1993b, 1995, 1997a), G. Gottschalk (Deppenmeier *et al.*, 1996), A. Klein (Sorgenfrei *et al.*, 1997a), J. Reeve (Reeve *et al.*, 1997) and G. Vogels (Keltjens & Vogels, 1993). A historical overview of methanogenesis has recently been published by Wolfe (1993). The reader is also referred to the book on *Methanogenesis: Ecology, Physiology, Biochemistry & Genetics* edited by Ferry (1993a), to the review by Daniels (1993) and by Blaut (1994) and three more recent reviews by the author (Weiss & Thauer, 1993; Thauer *et al.*, 1993; Thauer, 1997).

Methanogens: where they live, what they do and who they are

Methane is an end product of the microbial decomposition of organic matter in anaerobic freshwater environments such as lake sediments and the intestinal tract of animals. Estimates are that presently about 1 % of the plant material formed per year by photosynthesis from CO_2 is remineralized via methane, more than 10^9 tons of the combustible gas being intermediarily generated. Roughly two-thirds of the methane diffuses into aerobic zones, where it is oxidized by methanotrophic bacteria. A few per cent is buried, leading to the formation of methane deposits, but most of the rest escapes to the atmosphere where it is photochemically converted to CO_2. The concentration of atmospheric methane has increased steadily during the last 300 years from 0·7 p.p.m. to 1·7 p.p.m., owing probably in large part to the expanded cultivation of rice and ruminant life stock (Conrad, 1996). This is of concern since methane is a potent greenhouse gas.

In anaerobic freshwater sediments, plant material such as glucose from cellulose is completely decomposed to CO_2 and CH_4.

$$\text{Glucose} \rightarrow 3CO_2 + 3CH_4 \qquad \Delta G^{0\prime} = -418{\cdot}1 \text{ kJ mol}^{-1}$$

This reaction is not catalysed by single micro-organisms but by syntrophic associations of micro-organisms. First the glucose is fermented to acetate, CO_2 and H_2 or to acetate, formate and H_2:

$$\text{Glucose} + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + 2CO_2 + 4H_2 \qquad \Delta G^{0\prime} = -215{\cdot}7 \text{ kJ mol}^{-1}$$

$$\text{Glucose} + 2H_2O \rightarrow 2CH_3COO^- + 2HCOO^- + 4H^+ + 2H_2 \qquad \Delta G^{0\prime} = -208{\cdot}7 \text{ kJ mol}^{-1}$$

These fermentations are brought about by strictly anaerobic bacteria and/or protozoa. In a second step, the products of glucose fermentation are then converted to methane, the rate of conversion being such that the concentrations of acetate (<1 mM), formate (<0·1 mM) and H_2 (<1 μM) in the anaerobic sediments remain very low (Zinder, 1993).

$$CH_3COO^- + H^+ \rightarrow CO_2 + CH_4 \quad \Delta G^{0\prime} = -36 \text{ kJ mol}^{-1}$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad \Delta G^{0\prime} = -131 \text{ kJ mol}^{-1}$$

$$4HCOO^- + 4H^+ \rightarrow CH_4 + 3CO_2 + 2H_2O \qquad \Delta G^{0\prime} = -144{\cdot}5 \text{ kJ mol}^{-1}$$

The organisms mediating these methane-forming reactions were originally thought to be bacteria, but are now recognized as belonging to a separate phylogenetic domain, the *Archaea*. Methanoarchaea are highly specialized. They can only use acetate, H_2 and CO_2, formate and/or other C_1 compounds such as methanol, methylthiols and methylamines as energy substrates, the C_1 compounds being either disproportionated to CO_2 and CH_4 or reduced to methane with H_2 as exemplified for methanol:

$$4CH_3OH + 2H_2O \rightarrow 3CH_4 + 1CO_2 + 4H_2O \qquad \Delta G^{0\prime} = -106{\cdot}5 \text{ kJ (mol } CH_4)^{-1}$$

$$CH_3OH + H_2 \rightarrow CH_4 + H_2O \quad \Delta G^{0\prime} = -112{\cdot}5 \text{ kJ mol}^{-1}$$

Methanogenesis is the only way that methanogenic archaea can obtain energy for growth and these are the only organisms known to produce methane as a catabolic end product.

Despite the high specialization, not all methanogens are phylogenetically closely related. This is reflected, for example, in differences in cell wall composition (Sprott & Beveridge, 1993; Kandler & König, 1998). Methanogens can also have very different optimal growth conditions; thus some thrive optimally at temperatures below 20 °C and others at or near the temperature of boiling water (Boone *et al.*, 1993).

Methanogens taxonomically all belong to the archaeal kingdom of *Euryarchaeota*. They are classified in five orders each phylogenetically related to another as distantly as the *Cyanobacteriales* to the *Proteobacteriales*. The five orders are: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanopyrales* and *Methanosarcinales*. Of these, only the *Methanosarcinales* can ferment acetate to CO_2 and CH_4 and grow on methanol, methylthiols or methylamines as sole energy source. In turn, hyperthermophilic species are only found among the *Methanobacteriales*, *Methanococcales* and *Methanopyrales* (Boone *et al.*, 1993).

The DNA sequence of the total genome has, until now, been determined for two methanoarchaea, for *Methanococcus jannaschii* (Bult *et al.*, 1996) and for *Methanobacterium thermoautotrophicum* (strain ΔH) (Smith *et al.*, 1997). Recently, the sequence for *Archaeoglobus fulgidus* has also been published (Klenk *et al.*, 1997). This sulphate-reducing archaeon is phylogenetically most closely related to the *Methanosarcinales* with which it has many biochemical features in common

Table 1. Reactions and enzymes known to be involved in methane formation from acetate in the *Methanosarcinales* (Ferry, 1993b, 1995, 1997a)

H-S-CoM, coenzyme M; H-S-CoB, coenzyme B; H_4SPT, tetrahydrosarcinapterin, which is the modified tetrahydromethanopterin (for structures see Fig. 3) present in the *Methanosarcinales* (Gorris & van der Drift, 1994).

Reaction*	Enzyme (gene)	Most recent literature
Acetate + CoA → acetyl-CoA + H_2O $\Delta G^{0\prime} = +35{\cdot}7$ kJ mol^{-1}†	Acetate kinase (*ack*) and phosphotransacetylase (*pta*) in *Methanosarcina* spp. or acetate thiokinase (*acs*) in *Methanosaeta* spp.	Latimer & Ferry (1993); Singh-Wissmann & Ferry (1995); Eggen *et al.* (1991a)
Acetyl-CoA + H_4SPT → CH_3-H_4SPT + CO_2 + CoA + 2[H] $E_0' = -200$ mV‡ $\Delta G^{0\prime} = +41{\cdot}3$ kJ mol^{-1}‡§	Carbon-monoxide dehydrogenase/acetyl-CoA synthase‖ (*cdhABCXDE*)	Grahame (1993); Sowers *et al.* (1993); Lu *et al.* (1994); Grahame & DeMoll (1995, 1996); Eggen *et al.* (1991b, 1996); Grahame *et al.* (1996); Maupin-Furlow & Ferry (1996a, b)
CH_3-H_4SPT + H-S-CoM → CH_3-S-CoM + H_4SPT $\Delta G^{0\prime} = -30$ kJ mol^{-1}¶	Methyl-H_4SPT:coenzyme M methyltransferase# (energy conserving) (*mtrEDCBAFGH*)	Fischer *et al.* (1992); Lu *et al.* (1995); Lienard *et al.* (1996); Lienard & Gottschalk (1998)
CH_3-S-CoM + H-S-CoB → CoM-S-S-CoB + **CH_4** $\Delta G^{0\prime} = -45$ kJ mol^{-1}**	Methyl-coenzyme M reductase†† (*mcrBDCGA*)	Thauer *et al.* (1993); Springer *et al.* (1995)
CoM-S-S-CoB + 2[H] → H-S-CoM + H-S-CoB $E_0' = -200$ mV** $\Delta G^{0\prime} = -40$ kJ mol^{-1}§	Heterodisulphide reductase‡‡ (*hdrDE*)	Heiden *et al.* (1993); Peer *et al.* (1994); Künkel *et al.* (1998)

* $\Delta G^{0\prime}$ values add up to -38 kJ mol^{-1}, which is very similar to the $\Delta G^{0\prime} = -36$ kJ mol^{-1} calculated for $CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$ from free energy of formation data (Thauer *et al.*, 1977). See also the first footnotes to Tables 2 and 3.

† From Thauer *et al.* (1977).

‡ From Thauer (1990); CO_2 in the gaseous state at 10^5 Pa. E_0' was determined by Grahame & DeMoll (1995) to be -270 mV and $\Delta G^{0\prime}$ to be $+28$ kJ mol^{-1}, values not consistent with the overall thermodynamics of methanogenesis from acetate (see first footnote), from CO_2 (Table 2) and from methanol (Table 3). At first sight, a $\Delta G^{0\prime} = +41{\cdot}3$ kJ mol^{-1} appears unrealistically endergonic. Note, however, that in the reaction four products are formed from two substrates. $\Delta G'$ is thus highly concentration dependent. At 0·1 mM concentrations of all substrates and products ($pH_2 = 10$ Pa), $\Delta G' = -2{\cdot}6$ kJ mol^{-1} and $E' = -430$ mV (Thauer, 1990).

§ Calculated for 2[H] = $1H_2$ at 10^5 Pa.

‖ The nickel iron–sulphur corrinoid enzyme is isolated from *Methanosarcina* spp. as a multienzyme complex composed of five different subunits (Grahame & DeMoll, 1996; Maupin-Furlow & Ferry, 1996a, b); ferredoxin appears to be the physiological electron acceptor (Fischer & Thauer, 1990; Peer *et al.*, 1994; Ferry, 1995, 1997a). The encoding genes in *Methanosarcina thermophila* are organized in a transcription unit, *cdhABCXDE*, X being an open reading frame of unknown function (Maupin-Furlow & Ferry, 1996b). CdhA and CdhC are predicted to be nickel iron–sulphur proteins and CdhD and CdhE to harbour the corrinoid prosthetic group.

¶ From Weiss *et al.* (1994).

Membrane-associated multienzyme complex composed of eight different subunits MtrA–H, MtrA harbouring a corrinoid prosthetic group (Lienard & Gottschalk, 1998). Energy conservation proceeds via an electrochemical Na^+ potential (Lienard *et al.*, 1996).

** From Thauer *et al.* (1993).

†† The enzyme is isolated as a multienzyme complex of three different subunits, McrABG, in an $\alpha_2\beta_2\gamma_2$ configuration.

‡‡ Membrane-associated enzyme purified from methanol-grown *Methanosarcina barkeri* is a complex of two different subunits, HdrDE (Künkel *et al.*, 1997), and from acetate-grown cells is a complex of eight different subunits, HdrDE and EchABCDEF, Ech being an *E. coli* hydrogenase-3-type hydrogenase (Künkel *et al.*, 1998). HdrE is a *b*-type cytochrome (Künkel *et al.*, 1997, 1998).

(Schwörer *et al.*, 1993a; Kunow *et al.*, 1993, 1994, 1995; Thauer & Kunow, 1995; Vorholt *et al.*, 1995, 1997a).

Noteworthy is a recent hypothesis for the origin of eukaryotic cells from strictly hydrogen-dependent autotrophic methanoarchaea (Martin & Müller, 1998; see also Vogel, 1998).

Energy metabolism of methanoarchaea

The reactions and enzymes known to be involved in methane formation from acetate, from CO_2 and from methanol are summarized in Tables 1–3.

The energy metabolism of methanogens can be viewed

Table 2. Reactions and enzymes known to be involved in methane formation from CO_2 (Thauer *et al.*, 1993)

MFR, methanofuran; H-S-CoM, coenzyme M; H-S-CoB, coenzyme B; H_4MPT, tetrahydromethanopterin, which is a tetrahydrofolate (H_4F) analogue found instead of H_4F in the *Methanobacteriales* (for structures see Fig. 3). In the other orders, modified forms of H_4MPT are present: tetrahydrosarcinapterin in the *Methanosarcinales* and *Methanococcales* and tatiopterin and/or thermopterin in the *Methanomicrobiales* (Gorris & van der Drift, 1994). F_{420} is a 5′ deazaflavin derivative found in relatively high concentrations in methanogenic archaea (Gorris & van der Drift, 1994). See also the legend to Table 4.

Reaction*	Enzyme (gene)	Most recent literature
CO_2 + MFR + 2[H] → formyl-MFR $E_0' = -530$ mV† $\Delta G^{0'} = +16$ kJ mol^{-1}‡	Formylmethanofuran dehydrogenase (*fwdHFGDACB* and *fmdECB*)§	Schmitz *et al.* (1994); Bertram & Thauer (1994); Bertram *et al.* (1994a, b); Wasserfallen (1994); Hochheimer *et al.* (1995, 1996); Vorholt *et al.* (1996, 1997b); Vorholt & Thauer (1997); Holm & Sander (1997)
Formyl-MFR + H_4MPT → formyl-H_4MPT + MFR $\Delta G^{0'} = -4{\cdot}4$ kJ mol^{-1}	Formylmethanofuran:H_4MPT formyltransferase (*ftr*)	Shima *et al.* (1995, 1996); Lehmacher (1994); Ermler *et al.* (1997a); Kunow *et al.* (1996)
Formyl-H_4MPT + H^+ → methenyl-H_4MPT$^+$ + H_2O $\Delta G^{0'} = -4{\cdot}6$ kJ mol^{-1}	Methenyl-H_4MPT cyclohydrolase (*mch*)	Klein *et al.* (1993a); Vaupel *et al.* (1996, 1998)
Methenyl-H_4MPT$^+$ + $F_{420}H_2$ → methylene-H_4MPT + F_{420} + H^+ $\Delta G^{0'} = +5{\cdot}5$ kJ mol^{-1}‖	F_{420}-dependent methylene-H_4MPT dehydrogenase (*mtd*)	Klein *et al.* (1993b); Kunow *et al.* (1993); Klein & Thauer (1995, 1997); Mukhopadhyay *et al.* (1995)
Methenyl-H_4MPT$^+$ + H_2 → methylene-H_4MPT + H^+ $\Delta G^{0'} = -5{\cdot}5$ kJ mol^{-1}	H_2-forming methylene-H_4MPT dehydrogenase (*hmd*)	Schwörer *et al.* (1993b); Schleucher *et al.* (1994, 1995); Klein *et al.* (1995a, b); Berkessel & Thauer (1995); Hartmann *et al.* (1996a, b); Thauer *et al.* (1996)
Methylene-H_4MPT + $F_{420}H_2$ → CH_3-H_4MPT + F_{420} $\Delta G^{0'} = -6{\cdot}2$ kJ mol^{-1}‖	F_{420}-dependent methylene-H_4MPT reductase (*mer*)	Vaupel & Thauer (1995); Nölling *et al.* (1995a); Kunow *et al.* (1993)
CH_3-H_4MPT + H-S-CoM → CH_3-S-CoM + H_4MPT $\Delta G^{0'} = -30$ kJ mol^{-1}¶	Methyl-H_4MPT:coenzyme M methyl-transferase (energy conserving)# (*mtrEDCBAFGH*)	Stupperich *et al.* (1993); Gärtner *et al.* (1993, 1994); Weiss *et al.* (1994); Harms *et al.* (1995); Harms & Thauer (1996a, 1997, 1998)
CH_3-S-CoM + H-S-CoB → CoM-S-S-CoB + CH_4 $\Delta G^{0'} = -45$ kJ mol^{-1}	Methyl-coenzyme M reductase (*mcrBDCGA* and *mrtBDGA*)**	Bonacker *et al.* (1993); Goubeaud *et al.* (1997); Shima *et al.* (1997); Ermler *et al.* (1997b); Becker & Ragsdale (1998)
CoM-S-S-CoB + 2[H] → H-S-CoM + H-S-CoB $E_0' = -200$ mV $\Delta G^{0'} = -40$ kJ mol^{-1}††	Heterodisulphide reductase (*hdrA*, *hdrBC*)‡‡	Hedderich *et al.* (1994); Setzke *et al.* (1994)

* $\Delta G^{0'}$ values from Thauer *et al.* (1993). They add up to $-130{\cdot}7$ kJ mol^{-1}, which is almost identical to the $\Delta G^{0'} = -131$ kJ mol^{-1} calculated for $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ from free energy of formation data (Thauer *et al.*, 1977).

† From Bertram & Thauer (1994); determined at 60 °C; CO_2 in the gaseous state at 10^5 Pa.

‡ Calculated for 2[H] = $1H_2$ at 10^5 Pa. The direct electron donor is probably a polyferredoxin (Vorholt *et al.*, 1996). The endergonic reduction of CO_2 with H_2 to formylmethanofuran is driven by the electrochemical Na^+ potential involving reversed electron transport (Kaesler & Schönheit, 1989a, b).

§ *fwd* denotes the genes encoding the tungsten enzyme and *fmd* the molybdenum enzyme in *Methanobacterium thermoautotrophicum* (Hochheimer *et al.*, 1996). The latter enzymes are isolated from *Methanobacterium thermoautotrophicum* as multienzyme complexes composed of the subunits FwdABC and FwdA + FmdBC, respectively. FwdB and FmdB contain bound molybdopterin-guanine dinucleotide and tungsten and molybdenum, respectively (Hochheimer *et al.*, 1996).

‖ $H_2 + F_{420} \rightarrow F_{420}H_2$; $\Delta G^{0'} = -11$ kJ mol^{-1}; the E_0' of the $F_{420}/F_{420}H_2$ couple is -360 mV (Thauer *et al.*, 1993) and that of the H^+/H_2 couple is -414 mV (Thauer *et al.*, 1977).

to consist of two parts (Fig. 1): an oxidative part in which coenzyme M (H-S-CoM, 2-thioethanesulfonate) and coenzyme B (H-S-CoB, 7-thioheptanoylthreonine-phosphate) (for structures see Fig. 4) are oxidized to the heterodisulphide CoM-S-S-CoB; and a reductive part in which the heterodisulphide of coenzyme M and coenzyme B is re-reduced.

Oxidative part

In this part, methyl-coenzyme M is a central intermediate (Taylor & Wolfe, 1974a, b; Shapiro & Wolfe, 1980; Lovley *et al.*, 1984). It is formed from coenzyme M and acetate, CO_2 or reduced C_1 compounds such as methanol, methylthiols and methylamines via the pathways shown in Fig. 2. Methyl-coenzyme M is subsequently reduced with coenzyme B to methane with the concomitant formation of the heterodisulphide of coenzyme M and coenzyme B (Bobik *et al.*, 1987; Ellermann *et al.*, 1988).

$$CH_3\text{-S-CoM} + \text{H-S-CoB} \rightarrow CH_4 + \text{CoM-S-S-CoB} \qquad \Delta G^{0\prime} = -45\ \text{kJ mol}^{-1}$$

As far as known the exergonic reaction, which is catalysed by methyl-coenzyme M reductase and which proceeds essentially irreversibly, is not coupled with energy conservation (Thauer *et al.*, 1993).

In methanogenesis from CO_2 (Escalante-Semerena *et al.*, 1984) and from acetate (Fischer & Thauer, 1989), N^5-methyltetrahydromethanopterin (CH_3-H_4MPT) or N^5-methyltetrahydrosarcinapterin (CH_3-H_4SPT) is an intermediate in methyl-coenzyme M formation (Fig. 2). [Tetrahydromethanopterin (H_4MPT) is the tetrahydrofolate (H_4F) analogue found instead of H_4F in the *Methanobacteriales* (for structures see Fig. 3). In the other orders, modified forms of H_4MPT are present: tetrahydrosarcinapterin in the *Methanosarcinales* and *Methanococcales* and tatiopterin and/or thermopterin in the *Methanomicrobiales* (Gorris & van der Drift, 1994; see also White, 1998).] The methyl group of CH_3-H_4MPT is transferred to coenzyme M in an exergonic reaction ($\Delta G^{0\prime} = -30$ kJ mol^{-1}) coupled with energy conservation via an electrochemical sodium potential across the cytoplasmic membrane (Becher *et al.*, 1992a, b; Becher & Müller, 1994; Müller & Gottschalk, 1994; Lienard *et al.*, 1996; Lienard & Gottschalk, 1998). Despite this fact, methyl-coenzyme M formation from acetate or CO_2 is most probably not associated with a net phosphorylation of ADP since the first step in methanogenesis from acetate (Table 1) and that from CO_2 (Table 2) are endergonic reactions which in order to proceed are predicted to consume more energy than is conserved in the methyl transfer reaction.

Reductive part

Since the generation of the heterodisulphide in the oxidative part of the energy metabolism apparently is not coupled with **net** ATP formation, the energy required for growth of methanogens must be generated in the reductive part, the exergonic reduction of the heterodisulphide (Fig. 1), and indeed it has been shown that the reduction of the heterodisulphide is coupled with phosphorylation of ADP via the chemiosmotic mechanism involving an electrochemical H^+ potential as intermediate (Peinemann *et al.*, 1990; Deppenmeier *et al.*, 1990a, b, 1991; Sparling *et al.*, 1993). Dependent on the methanogenic growth substrate, the electrons for the reduction derive from the oxidation of either H_2, the carbonyl group of acetyl-CoA, formate, ethanol or 2-propanol or of one of the reduced C_1 compounds methanol, methylthiols or methylamines. Under standard conditions, the free energy change associated with heterodisulphide reduction with H_2 is -40 kJ mol^{-1}, with formate is $-43{\cdot}5$ kJ mol^{-1}, with ethanol (acetate as product) is -35 kJ mol^{-1} and with methanol (CO_2 as product) is -34 kJ mol^{-1}, which is sufficient to drive the phosphorylation of 1 mol ADP ($\Delta G^{0\prime} = +31{\cdot}8$ kJ mol^{-1}) (Thauer *et al.*, 1977). The reduction of the heterodisulphide with acetyl-CoA ($\Delta G^{0\prime} = \pm 0$ kJ mol^{-1}) (calculated from E_0' values given in Table 1) and with 2-propanol ($\Delta G^{0\prime} = -15$ kJ mol^{-1}) is much less exergonic. Under the conditions in the cell, the free energy change ΔG is, however, probably considerably more negative since the reduction is 'pushed' by irreversible formation of the heterodisulphide from methyl-coenzyme M and coenzyme B. Such a coupling is not unusual: e.g. in the arginine dihydrolase pathway operative in many bacteria and archaea, the energy-conserving formation of ornithine, CO_2 and NH_4^+ from citrulline ($\Delta G^{0\prime} = -19$ kJ mol^{-1}) is also 'pushed' by the preceding reaction, the irreversible hydrolysis of arginine to citrulline and NH_4^+ ($\Delta G^{0\prime} = -37{\cdot}7$ kJ mol^{-1}) (Thauer *et al.*, 1977). There is evidence that heterodisulphide reduction with H_2 is coupled with the synthesis of more than 1 mol ATP (Deppenmeier *et al.*, 1996).

¶ From Weiss *et al.* (1994).

Membrane-associated multienzyme complex composed of eight different subunits, MtrA–H, MtrA harbouring a corrinoid prosthetic group (Harms *et al.*, 1995). Energy conservation proceeds via an electrochemical Na^+ potential (Gärtner *et al.*, 1994). For literature on the enzyme complex from *Methanosarcina* spp., see Table 1.

** *mcr* denotes the genes encoding the isoenzyme I and *mrt* the isoenzyme II. Both isoenzymes are isolated as multienzyme complexes composed of three different subunits, McrABG or MrtABG, in an $\alpha_2\beta_2\gamma_2$ configuration. For literature on the enzyme complex from *Methanosarcina* spp., see Table 1.

†† Calculated for 2[H] = H_2 at 10^5 Pa.

‡‡ The enzyme is purified from *Methanobacterium thermoautotrophicum* in a complex with the F_{420}-non-reducing hydrogenase (Setzke *et al.*, 1994).

Table 3. Reactions and enzymes known to be involved in methane and CO_2 formation from methanol in *Methanosarcina* spp. (Keltjens & Vogels, 1993)

Only methanogens of the family *Methanosarcinaceae* are capable of methanol disproportionation to CH_4 and CO_2. For abbreviations, see Tables 1 and 2.

Reaction*	Enzyme (gene)	Most recent literature
1. Methane formation		
$CH_3OH + H\text{-}S\text{-}CoM \rightarrow CH_3\text{-}S\text{-}CoM + H_2O$ $\Delta G^{0\prime} = -27{\cdot}5$ kJ mol^{-1}	Methanol:coenzyme M methyltransferase (*mtaA* + *mtaBC*)	Sauer *et al.* (1997); Sauer & Thauer (1997, 1998)
$CH_3\text{-}S\text{-}CoM + H\text{-}S\text{-}CoB \rightarrow CoM\text{-}S\text{-}S\text{-}CoB + CH_4$ $\Delta G^{0\prime} = -45$ kJ mol^{-1}	Methyl-coenzyme M reductase (*mcrBDCGA*)	See Table 1
$CoM\text{-}S\text{-}S\text{-}CoB + 2[H] \rightarrow H\text{-}S\text{-}CoM + H\text{-}S\text{-}CoB$ $E_0' = -200$ mV $\Delta G^{0\prime} = -40$ kJ mol^{-1}†	Heterodisulphide reductase (*hdrDE*)	Heiden *et al.* (1994); Künkel *et al.* (1997)
2. CO_2 formation		
$CH_3\text{-}OH + H\text{-}S\text{-}CoM \rightarrow CH_3\text{-}S\text{-}CoM + H_2O$ $\Delta G^{0\prime} = -27{\cdot}5$ kJ mol^{-1}	Methanol:coenzyme M methyltransferase (*mtaA* + *mtaBC*)	Sauer *et al.* (1997); Sauer & Thauer (1997, 1998)
$CH_3\text{-}S\text{-}CoM + H_4SPT \rightarrow H\text{-}S\text{-}CoM + CH_3\text{-}H_4SPT$ $\Delta G^{0\prime} = +30$ kJ mol^{-1}	Methyl-H_4SPT:coenzyme M methyltransferase (energy conserving) (*mtrEDCBAFGH*)	See Table 1
$CH_3OH + H_4SPT \rightarrow CH_3\text{-}H_4SPT + H_2O$ $\Delta G^{0\prime} = +2{\cdot}5$ kJ mol^{-1}	Enzyme has not yet been identified unambiguously	Keltjens & Vogels (1993)
$CH_3\text{-}H_4SPT + F_{420} \rightarrow$ methylene-H_4SPT + $F_{420}H_2$ $\Delta G^{0\prime} = +6{\cdot}2$ kJ mol^{-1}‡	F_{420}-dependent methylene-H_4SPT reductase (*mer*)	Ma & Thauer (1990); Te Brömmelstroet *et al.* (1991)
Methylene-H_4SPT + F_{420} + $H^+ \rightarrow$ methenyl-H_4SPT^+ + $F_{420}H_2$ $\Delta G^{0\prime} = -5{\cdot}5$ kJ mol^{-1}	F_{420}-dependent methylene-H_4SPT dehydrogenase (*mtd*)	Enßle *et al.* (1991); Te Brömmelstroet *et al.* (1991)
Methenyl-$H_4SPT^+ + H_2O \rightarrow$ formyl-H_4SPT + H^+ $\Delta G^{0\prime} = +4{\cdot}6$ kJ mol^{-1}	Methenyl-H_4SPT cyclohydrolase (*mch*)	Te Brömmelstroet *et al.* (1990); Vaupel *et al.* (1998)
Formyl-H_4SPT + MFR $\rightarrow$ formyl-MFR + H_4SPT $\Delta G^{0\prime} = +4{\cdot}4$ kJ mol^{-1}	Formylmethanofuran:H_4SPT formyltransferase (*ftr*)	Kunow *et al.* (1996)
Formyl-MFR $\rightarrow CO_2$ + MFR + 2[H] $E_0' = -530$ mV $\Delta G^{0\prime} = -16$ kJ mol^{-1}†§	Formylmethanofuran dehydrogenase (*fmdEFACDB*)	Vorholt *et al.* (1996)

* $\Delta G^{0\prime}$ values from Thauer *et al.* (1993) and Keltjens & Vogels (1993). They add up to $\Delta G^{0\prime} = -319$ kJ mol^{-1} for $4CH_3OH + 2H_2O \rightarrow 3CH_4 + 1CO_2 + 4H_2O$, which is very close to $\Delta G^{0\prime} = -319{\cdot}5$ kJ mol^{-1} calculated from free energy of formation data (Thauer *et al.*, 1977).

† Calculated from $2[H] = 1H_2$ at 10^5 Pa.

‡ $F_{420}H_2 \rightarrow F_{420} + H_2$; $\Delta G^{0\prime} = +11$ kJ mol^{-1}; the E_0' of the $F_{420}/F_{420}H_2$ couple is -360 mV (Thauer *et al.*, 1993) and that of the H^+/H_2 couple is -414 mV (Thauer *et al.*, 1977).

§ The direct electron acceptor is probably a polyferredoxin (Vorholt *et al.*, 1996). The exergonic dehydrogenation of formylmethanofuran is coupled with energy conservation via an electrochemical Na^+ gradient (Kaesler & Schönheit, 1989a, b). See also Table 2.

Heterodisulphide reduction with H_2

The reduction of the heterodisulphide with the primary electron donors involves at least two enzymes, a dehydrogenase and a heterodisulphide reductase, and an electron transport chain connecting the two enzymes as schematically exemplified for heterodisulphide reduction with H_2.

$H_2 \rightarrow 2e^- + 2H^+$ $\qquad E_0' = -414$ mV

$2e^- + 2H^+ + CoM\text{-}S\text{-}S\text{-}CoB \rightarrow H\text{-}S\text{-}CoM + H\text{-}S\text{-}CoB$ $\qquad E_0' = -200$ mV

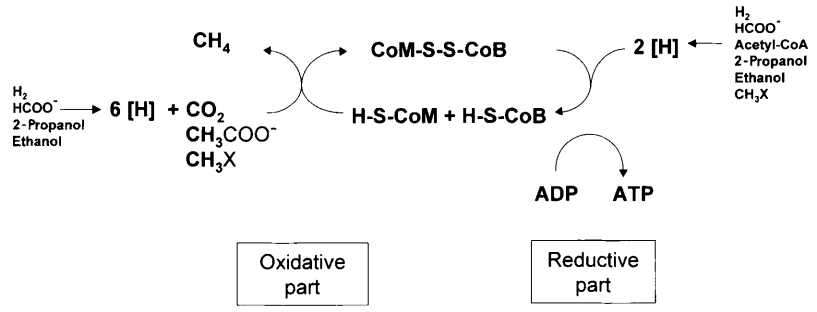

Fig. 1. Energy metabolism of methanogenic archaea. In the oxidative part, coenzyme M (H-S-CoM) and coenzyme B (H-S-CoB) are oxidized to the heterodisulphide CoM-S-S-CoB by CO_2, acetate or reduced C_1 compounds (CH_3-X) such as methanol, methylthiols and methylamines, which in turn are reduced to CH_4; in the reductive part, the heterodisulphide is reduced to coenzyme M and coenzyme B, the electron transport from the electron donors being coupled with phosphorylation.

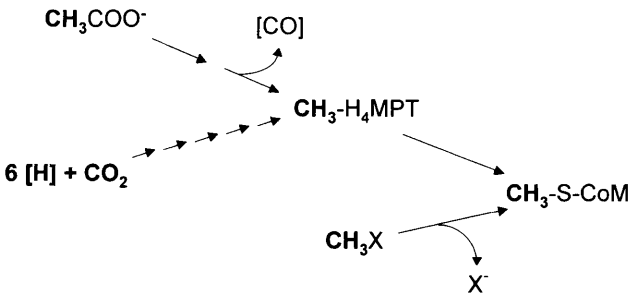

Fig. 2. Pathways of methyl-coenzyme M (CH_3-S-CoM) formation from acetate, CO_2 and reduced C_1 compounds (CH_3-X) such as methanol, methylthiols and methylamines. H-S-CoM, coenzyme M; [CO], enzyme-bound CO; CH_3-H_4MPT, N^5-methyltetrahydromethanopterin. Tetrahydromethanopterin (H_4MPT) is a tetrahydrofolate (H_4F) analogue found instead of H_4F in the *Methanobacteriales* (for structures see Fig. 3). In the other orders, modified forms of H_4MPT are present: tetrahydrosarcinapterin in the *Methanosarcinales* and *Methanococcales* and tatiopterin or thermopterin in the *Methanomicrobiales* (Gorris & van der Drift, 1994).

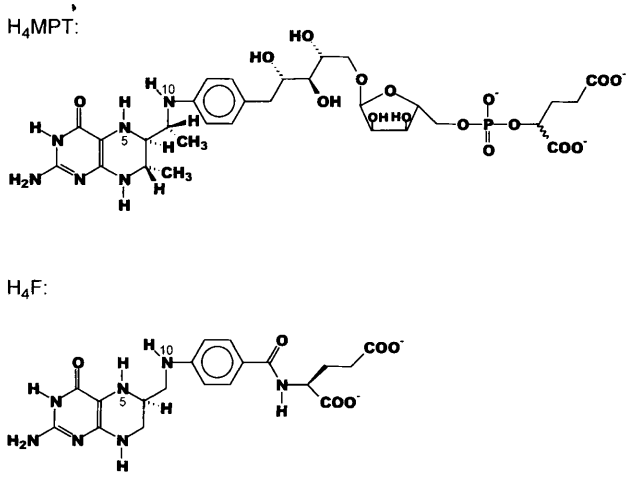

Fig. 3. Structures of tetrahydromethanopterin (H_4MPT) (Gorris & van der Drift, 1994; Schleucher *et al.*, 1994) and of tetrahydrofolate (H_4F). The functionally most important difference between H_4MPT and H_4F is that H_4MPT has an electron-donating methylene group conjugated to N^{10} via the aromatic ring whereas H_4F has an electron-withdrawing carbonyl group in this position. As a consequence, the redox potential $E'_0 = -390$ mV of the N^5,N^{10}-methenyl-H_4MPT/N^5,N^{10}-methylene-H_4MPT couple is by 90 mV more negative than the $E'_0 = -300$ mV of the N^5,N^{10}-methenyl-H_4F/N^5,N^{10}-methylene-H_4F couple and the redox potential $E'_0 = -320$ mV of the N^5,N^{10}-methenyl-H_4MPT/N^5,N^{10}-methylene-H_4MPT couple is by 120 mV more negative than the $E'_0 = -200$ mV of the N^5,N^{10}-methenyl-H_4F/N^5,N^{10}-methylene-H_4F couple (Thauer *et al.*, 1996). Tetrahydrosarcinapterin (H_4SPT) is a H_4MPT derivative with a glutamyl group attached to the hydroxyglutaryl group in the side chain.

The heterodisulphide reductase (Hdr), first discovered by Hedderich & Thauer (1988), is a novel type of disulphide reductase catalysing the reduction of the heterodisulphide in two successive one-electron steps and probably involving thiyl radical intermediates (Künkel *et al.*, 1997; Heim *et al.*, 1998). The enzyme complex, composed of at least two different subunits, has been characterized from *Methanobacterium thermoautotrophicum* (Hedderich *et al.*, 1990, 1994; Setzke *et al.*, 1994), from *Methanosarcina barkeri* (Heiden *et al.*, 1993, 1994; Künkel *et al.*, 1997, 1998) and from *Methanosarcina thermophila* (Peer *et al.*, 1994). Heterodisulphide reductase HdrDE from *Methanosarcina* spp. is a cytochrome *b* (HdrE)-containing enzyme (Heiden *et al.*, 1993, 1994; Künkel *et al.*, 1997); methanophenazine is most probably the direct electron donor, the oxidation of reduced methanophenazine with heterodisulphide being coupled with energy conservation (Abken *et al.*, 1998a, b; Bäumer *et al.*, 1998). The enzyme HdrABC from *Methanobacterium* spp. is an iron–sulphur protein (Hedderich *et al.*, 1994; Setzke *et al.*, 1994). The direct electron donor is not yet known (see Nölling *et al.*, 1995c).

Hydrogenotrophic methanogens can contain up to four different types of hydrogenases and of each type, several isoenzymes (Thauer *et al.*, 1993; Sorgenfrei *et al.*, 1997a, b; Künkel *et al.*, 1998; for topology see Braks *et al.*, 1994): (i) F_{420}-reducing Ni/Fe-hydrogenase (Vaupel & Thauer, 1998); (ii) F_{420}-non-reducing hydrogenase of unknown physiological electron acceptor – in *Methanosarcina* spp., one of the subunits from the enzyme is a cytochrome *b* (Deppenmeier, 1995; Deppenmeier *et al.*, 1995; Kumazawa *et al.*, 1994; Kemner & Zeikus, 1994a, b) and in *Methanobacterium* spp., which are devoid of cytochromes, the electron accceptor is possibly either a 44 kDa polyferredoxin (Reeve *et al.*, 1989;

Table 4. Dehydrogenases catalysing the reduction of F_{420} and the oxidation of $F_{420}H_2$ in the energy metabolism of methanogenic archaea

F_{420} is a 5′ deazaflavin derivative found in relatively high concentration in methanogenic archaea (Gorris & van der Drift, 1994). F_{420} in methanogens is converted to inactive F_{390} by adenylation and F_{390} to F_{420} by deadenylation in response to different growth conditions (Vermeij *et al.*, 1994, 1995, 1996)

Enzyme	Organism	Most recent literature
1. Reduction of F_{420}		
F_{420}-reducing hydrogenase	All hydrogenotrophic methanogens	Sorgenfrei *et al.* (1997a, b); Vaupel & Thauer (1998)
F_{420}-dependent formate dehydrogenase	Methanogens growing on formate	Nölling & Reeve (1997); Grahame & Stadtman (1993)
F_{420}-dependent methylene-H_4MPT dehydrogenase	Methanogens growing on methanol, methylthiols or methylamines	Keltjens & Vogels (1993)
F_{420}-dependent methylene-H_4MPT reductase	Methanogens growing on methanol, methylthiols or methylamines	Keltjens & Vogels (1993)
H_2-forming methylene-H_4MPT dehydrogenase plus F_{420}-dependent methylene-H_4MPT dehydrogenase	Most hydrogenotrophic methanogens of the orders *Methanobacteriales*, *Methanococcales* and *Methanopyrales*	Thauer *et al.* (1996); Afting *et al.* (1998)
F_{420}-dependent alcohol dehydrogenase	*Methanogenium liminatans*, *Methanoculleus thermophilicus*	Klein *et al.* (1996); Widdel & Frimmer (1995); Berk *et al.* (1996)
NADP-dependent alcohol dehydrogenase plus F_{420}-dependent NADP reductase	*Methanogenium organophilum*, *Methanobacterium palustre*, *Methanocorpusculum* spp.	Berk *et al.* (1996); Berk & Thauer (1997); Widdel & Frimmer (1995)
2. Oxidation of $F_{420}H_2$		
F_{420}-dependent methylene-H_4MPT dehydrogenase	Methanogens reducing CO_2 to methane	See Table 2
F_{420}-dependent methylene-H_4MPT reductase	Methanogens reducing CO_2 to methane	See Table 2
$F_{420}H_2$ dehydrogenase complex	*Methanosarcina mazei*, *Methanolobus tindarium*	Haase *et al.* (1992); Abken & Deppenmeier (1997); Abken *et al.* (1998a, b)
F_{420}-dependent hydrogenase	Methanogens of the orders *Methanococcales* and *Methanobacteriales* growing on formate and lacking $F_{420}H_2$ dehydrogenase complex	Vaupel (1993)

Hedderich *et al.*, 1992; Nölling *et al.*, 1995c) or a 45 kDa flavoprotein (Wasserfallen *et al.*, 1995; Nölling *et al.*, 1995c); (iii) *Escherichia coli* hydrogenase-3-type Ni/Fe-hydrogenase of unknown physiological electron acceptor (Künkel *et al.*, 1998); and (iv) a metal-free hydrogenase, the H_2-forming methylenetetrahydromethanopterin dehydrogenase, which together with the F_{420}-dependent methylenetetrahydromethanopterin dehydrogenase catalyses the reduction of F_{420} with H_2 (Thauer *et al.*, 1996; Reeve *et al.*, 1997). Both enzymes are induced during growth under conditions of nickel deprivation (Afting *et al.*, 1998).

There are indications that at least two of the hydrogenases can be involved in heterodisulphide reduction with H_2: with inside-out vesicles from *Methanosarcina mazei* it has been shown that both F_{420}-dependent (Deppenmeier *et al.*, 1990a, b) and F_{420}-independent (Deppenmeier *et al.*, 1991) reduction of the heterodisulphide with H_2 are coupled with the phosphorylation of ADP (Deppenmeier *et al.*, 1996; Abken *et al.*, 1998a, b).

Heterodisulphide reduction with $F_{420}H_2$

Cell extracts of *Methanosarcina mazei* and of *Methanolobus tindarius* catalyse the reduction of heterodisulphide with reduced F_{420}, the coenzyme of many dehydrogenases in methanogenic archaea (Table 4). (F_{420} is a 5′ deazaflavin derivative with an $E_0' = -360$ mV present in relatively high concentrations in these organisms; Gorris & van der Drift, 1994.) For the reduction of the heterodisulphide with $F_{420}H_2$, besides heterodisulphide reductase an additional enzyme is required: *Methanosarcina* spp. (Deppenmeier *et al.*, 1990a, b; Abken & Deppenmeier, 1997) and *Methanolobus tindarius* (Deppenmeier *et al.*, 1990b; Haase *et al.*, 1992) have been shown to contain a membrane-associated $F_{420}H_2$ dehydrogenase complex catalysing

the reduction of methanophenazine with $F_{420}H_2$, the reduction being coupled with phosphorylation of ADP (Abken *et al.*, 1998a, b; Bäumer *et al.*, 1998). Such an $F_{420}H_2$ dehydrogenase complex is also found in *Archaeoglobus* sp. (Kunow *et al.*, 1993, 1994; Klenk *et al.*, 1997), but is apparently lacking in *Methanococcus* spp. (Bult *et al.*, 1996) and *Methanobacterium* sp. (Smith *et al.*, 1997) as deduced from the genome sequences. In methanogens lacking the $F_{420}H_2$ dehydrogenase complex and not growing on H_2 and CO_2, heterodisulphide reduction with $F_{420}H_2$ probably proceeds via H_2 as intermediate (Vaupel, 1993).

Coupling with ATP synthesis

As indicated, methyl transfer from N^5-methyltetrahydromethanopterin to coenzyme M in the oxidative part of the energy metabolism is coupled with the build-up of an electrochemical Na^+ gradient (Becher *et al.*, 1992a, b; Lienard *et al.*, 1996). In turn, the reduction of the heterodisulphide in the reductive part is coupled with electrogenic H^+ translocation (Peinemann *et al.*, 1990; Deppenmeier *et al.*, 1990a, b, 1991; Sparling *et al.*, 1993). *Methanosarcina* spp. have been shown to contain an H^+-translocating A_1A_0 ATPase (Wilms *et al.*, 1996; Inatomi, 1996; Ruppert *et al.*, 1998) and there is indirect evidence also for the presence of an Na^+-translocating F_1F_0 ATPase (Becher & Müller, 1994). *Methanobacterium* spp. and *Methanococcus* spp. harbour only one A_1A_0 ATPase as deduced from the genome sequences (Bult *et al.*, 1996; Smith *et al.*, 1997). It is not known whether this ATPase is Na^+- or H^+-translocating. There is evidence for both (Chen & Konisky, 1993; Sparling *et al.*, 1993; Smigan *et al.*, 1994). Methanogens contain an Na^+/H^+ antiporter capable of interconversion of the electrochemical H^+ and Na^+ potentials (Schönheit & Beimborn, 1985; Deppenmeier *et al.*, 1996).

The stoichiometry of coupling of methanogenesis with ADP phosphorylation appears not to be constant. Evidence is available that during growth of methanogens on H_2 and CO_2 the growth yield [g (mol CH_4)$^{-1}$] increases with decreasing H_2 concentrations (Schönheit *et al.*, 1980; Morgan *et al.*, 1997; for regulation see Pihl *et al.*, 1994; Nölling *et al.*, 1995a, b; Nölling & Reeve, 1997), indicating that at low H_2 concentrations coupling of heterodisulphide reduction with ADP phosphorylation is tighter than at high H_2 concentrations. With decreasing H_2 concentrations the free energy change associated with CO_2 reduction to methane decreases from -131 kJ mol^{-1} at 10^5 Pa H_2 to only approximately -30 kJ mol^{-1} at the H_2 concentrations prevailing in methanogenic ecosystems where the H_2 partial pressure is only between 1 and 10 Pa.

Other heterodisulphide-generating reactions

Methyl-coenzyme M reduction with coenzyme B is not the only reaction in which the heterodisulphide is regenerated. Most methanogens contain a fumarate reductase which catalyses the reduction of fumarate with H-S-CoM and H-S-CoB to succinate and CoM-S-S-CoB (Bobik & Wolfe, 1989; Heim *et al.*, 1998). This reaction is involved in the biosynthesis of 2-oxoglutarate, which, in the methanogens containing the fumarate reductase, is synthesized via reductive carboxylation of succinyl-CoA (Tersteegen *et al.*, 1997; Thompson *et al.*, 1998).

Analogy between heterodisulphide reduction and sulphur reduction

Many anaerobic bacteria and archaea can grow with elemental sulphur as terminal electron acceptor in the energy metabolism (Schauder & Kröger, 1993). Sulphur reduction to H_2S is coupled with energy conservation as evidenced by growth on H_2 and S^0. In elemental sulphur, which at room temperature is S_8, the sulphur atoms are connected via S-S bonds just as the two sulphurs in heterodisulphide. The redox potential of the S^0/H_2S couple and that of the heterodisulphide/H-S-CoM + H-S-CoB couple are both in the order of -200 mV (Thauer *et al.*, 1977). Methanogens and sulphur-reducing micro-organisms thus have in common that they both live at the expense of S-S bond reduction. Methanogens differ, however, from sulphur reducers in that they are independent of an external sulphur source as electron acceptor since they can reoxidize the 'reduced sulphur' with CO_2 or one of the other methanogenic carbon substrates, which themselves are concomitantly reduced to methane.

Methyl-coenzyme M, an intermediate unique to methanogenesis

Coenzyme M and methyl-coenzyme M have, until now, only been found in methanogenic archaea (Balch & Wolfe, 1979). The same holds true for the enzymes catalysing the formation of methyl-coenzyme M (Table 5) and for methyl-coenzyme M reductase. All the other enzymes and coenzymes involved in methanogenesis, maybe with exception of coenzyme B, are also present in sulphate-reducing archaea (Vorholt *et al.*, 1995, 1997a; Klenk *et al.*, 1997). Recently, tetrahydromethanopterin and tetrahydromethanopterin-dependent enzymes were even found in a methylotrophic α-proteobacterium (Chistoserdova *et al.*, 1998), indicating that some of the methanogenic enzymes and coenzymes are not as unique to the archaeal kingdom as previously thought. Also of interest is the recent finding that several non-methanogens, including *Bacillus subtilis*, contain genes encoding a putative heterodisulphide reductase, the function of which remains to be elucidated (Heim *et al.*, 1998).

Coenzyme M is the smallest coenzyme known to date. The enzymes catalysing its methylation and reductive demethylation are highly specific for its structure. 2-Selenoethanesulphonate and 3-thiopropionate can in part substitute for H-S-CoM in its function, 3-thiopropanesulphonate cannot (Gunsalus *et al.*, 1978; Wackett *et al.*, 1987; Tallant & Krzycki, 1996, 1997).

Table 5. Reactions and enzymes known to be involved in the formation of methyl-coenzyme M (Keltjens & Vogels, 1993)

For abbreviations, see Table 2.

Reaction	Enzyme system	Most recent literature
CH_3-H_4MPT + H-S-CoM → CH_3-S-CoM + H_4MPT $\Delta G^{0'} = -30$ kJ mol^{-1}*	N^5-Methyl-H_4MPT:coenzyme M methyltransferase (energy conserving) (MtrA–H)	Stupperich *et al.* (1993); Gärtner *et al.* (1993, 1994); Weiss *et al.* (1994); Harms *et al.* (1995); Harms & Thauer (1996a, 1997, 1998); Lu *et al.* (1995); Lienard *et al.* (1996); Lienard & Gottschalk (1998)
CH_3-OH + H-S-CoM → CH_3-S-CoM + H_2O $\Delta G^{0'} = -27{\cdot}5$ kJ mol^{-1}	Methanol:coenzyme M methyltransferase (MtaA + MtaBC)†	Sauer *et al.* (1997); Sauer & Thauer (1997, 1998)
CH_3-NH_3^+ + H-S-CoM → CH_3-S-CoM + NH_4^+ $\Delta G^{0'} = -5{\cdot}1$ kJ mol^{-1}‡	Monomethylamine:coenzyme M methyltransferase (MtbA + MtmBC)†	Burke & Krzycki (1995, 1997); Burke *et al.* (1998)
$(CH_3)_2NH_2^+$ + H-S-CoM → CH_3-S-CoM + $CH_3NH_3^+$ $\Delta G^{0'} = -2{\cdot}5$ kJ mol^{-1}‡	Dimethylamine:coenzyme M methyltransferase (MtbA + MtbB + ?)	Wassenaar *et al.* (1996, 1998)
$(CH_3)_3NH^+$ + H-S-CoM → CH_3-S-CoM + $(CH_3)_2NH_2^+$ $\Delta G^{0'} = -6{\cdot}3$ kJ mol^{-1}‡	Trimethylamine:coenzyme M methyltransferase (MtbA/MtaA + MttBC)†	Ferguson & Krzycki (1997); Wassenaar *et al.* (1996)
$(CH_3)_4N^+$ + H-S-CoM → CH_3-S-CoM + $(CH_3)_3NH^+$ $\Delta G^{0'} = ?$	Tetramethylamine:coenzyme M methyltransferase (MtqA + MtqB + MtqC)†	Asakawa *et al.* (1998); Tanaka (1994)
CH_3-S-CH_3 + H-S-CoM → CH_3-S-CoM + CH_3-S-H $\Delta G^{0'} \approx 0$ kJ mol^{-1}	Methylthiol:coenzyme M methyltransferase§ (MtsAB)†	Paul & Krzycki (1996); Tallant & Krzycki (1996, 1997)

* From Weiss *et al.* (1994).

† For activity in cell extracts the presence of a methyltransferase-activating protein (MAP), reduced ferredoxin and ATP are additionally required (Daas *et al.*, 1993, 1994, 1996a, b). MtaA (= MT2-M), MtbA (= MT2-A), MtqA and MtsA exhibit methylcobalamin:coenzyme M methyltransferase (MT2) activity (Grahame, 1989; Yeliseev *et al.*, 1993; Ferguson *et al.*, 1996; Wassenaar *et al.*, 1996) and show sequence similarity (LeClerc & Grahame, 1996; Harms & Thauer, 1996b; Paul & Krzycki, 1996; Asakawa *et al.*, 1998). MtaA and MtaB are zinc enzymes (Sauer & Thauer, 1997); MtaC, MtmC, MttC, MtqC and MtsB have been shown to be corrinoid proteins harbouring 6-hydroxybenzimidazolyl cobamide as prosthetic group.

‡ Obtained from $\Delta G^{0'}$ for methylamine reduction to methane with H_2 calculated from the free energies of formation from the elements tabulated in Thauer *et al.* (1977) and from $\Delta G^{0'}$ of -85 kJ mol^{-1} for methyl-coenzyme M reduction with H_2 to CH_4 and coenzyme M (Thauer *et al.*, 1993).

§ Also catalyses the methylation of coenzyme M with methylmercaptopropanol (95%), methylmercaptopropionate (80%), mercaptomethanol (10%), methylmercaptoethanol (8%) and methyliodide (170%) (Tallant & Krzycki, 1997) and the methylation of mercaptoethanol (27%) and 2-mercaptopropanol (66%) (Tallant & Krzycki, 1996).

Methyl-coenzyme M reductase, its substrate and inhibitors

Methyl-coenzyme M and coenzyme B are the substrates of methyl-coenzyme M reductase, methane and heterodisulphide its products (Bobik *et al.*, 1987; Ellermann *et al.*, 1988) (Fig. 4). From the methyl-coenzyme M analogues tested in cell extracts of *Methanobacterium thermoautotrophicum*, only 2-(methylthio)propionate ($K_m = 1{\cdot}3$ mM; $V_{max} = 1{\cdot}3$ mU mg^{-1}), ethyl-coenzyme M ($K_m = 1{\cdot}3$ mM; $V_{max} = 7{\cdot}4$ mU mg^{-1}), methyl-seleno-coenzyme M ($K_m = 0{\cdot}3$ mM; $V_{max} = 35$ mU mg^{-1}) and difluoromethyl-coenzyme M ($K_m = 2{\cdot}5$ mM; $V_{max} = 20$ mU mg^{-1}) were reduced (methyl-coenzyme M: $K_m = 0{\cdot}1$ mM; $V_{max} = 11$ mU mg^{-1}) (Wackett *et al.*, 1987). Interestingly, trifluoromethyl-coenzyme M and trifluoromethyl-seleno-coenzyme M (Wackett *et al.*, 1987) and 2-(dimethylsulphonium)ethanesulphonate (Gunsalus *et al.*, 1978) were not reduced, neither were 2-(methoxy)ethanesulphonate, propyl-coenzyme M and 3-(methylthio)propanesulphonate; these compounds were even inhibitors (Gunsalus *et al.*, 1978; Wackett *et al.*, 1987). Other inhibitors based on the methyl-coenzyme M structure are 2-bromoethanesulphonate ($K_i = 8$ μM) and 2-chloroethanesulphonate ($K_i = 70$ μM) (Gunsalus *et al.*, 1978), 3-bromopropanesulphonate ($K_i = 50$ nM), 4-bromobutanesulphonate ($K_i = 6$ μM) and 2-azidoethanesulphonate ($K_i = 1$ μM)

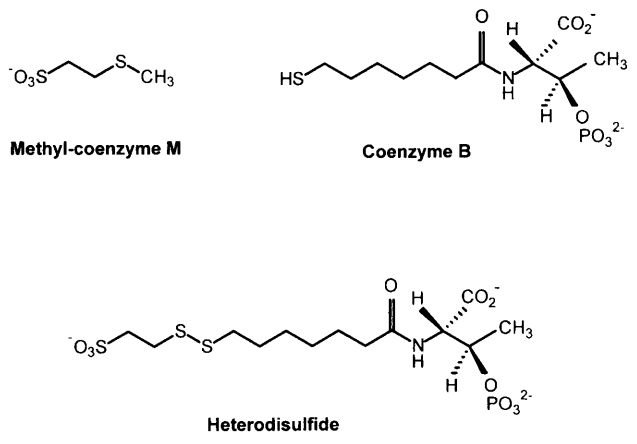

Fig. 4. Structures of methyl-coenzyme M (Taylor & Wolfe, 1974a, b), coenzyme B (Noll *et al.*, 1986) and of the heterodisulphide CoM-S-S-CoB (Ellermann *et al.*, 1988). For biosynthesis of coenzyme M and coenzyme B, see White & Zhou (1993), White (1994) and Howell *et al.* (1998).

(Ellermann *et al.*, 1989), allyl-S-coenzyme M (K_i = 20 μM) and cyano-S-coenzyme M (K_i = 30 μM) (Wackett *et al.*, 1987) and bromomethanesulphonate (K_i = 1·5 μM) (Olson *et al.*, 1992). Note that the apparent K_m and the K_i values have been determined with enzyme preparations with very low specific activity (apparent V_{max} values). There is evidence, however, that they should pertain also for the fully active enzyme (Brenner *et al.*, 1993).

Methyl-coenzyme M reductase is also highly specific for coenzyme B, the aliphatic armlength of the coenzyme apparently being crucial to its function. *N*-6-Thiohexanoyl-O-phospho-L-threonine (1%) can only to a minor extent and *N*-8-thiooctanoyl-O-phospho-L-threonine (0%) not at all substitute for the *N*-7-thioheptanoyl-O-phospho-L-threonine (100%) as electron donor; rather both compounds are inhibitors (K_i = 0·1 μM and 15 μM, respectively). This is also true for 7-bromoheptanoyl-O-phospho-L-threonine (K_i = 5 μM) and 7-(methylthio)heptanoyl-O-phospho-L-threonine (K_i = 7 μM) (Ellermann *et al.*, 1988) and 6-(methylthio)hexanoyl-O-phospho-L-threonine (K_i = 9 μM) (Olson *et al.*, 1992). Dephospho-coenzyme B and the D-epimer are neither active nor inhibitors (Ellermann *et al.*, 1988).

It has been proposed and evidence has been reported that the physiological electron donor for methyl-coenzyme M reduction could possibly be a larger molecule which contains coenzyme B covalently bound through a mixed anhydride linkage to a sugar moiety (Sauer *et al.*, 1990; Sauer, 1991). This is presently no longer considered to be very likely. Recently, methyl-coenzyme M reductase was isolated from *Methanobacterium thermoautotrophicum* under very mild conditions and then crystallized. The purified enzyme contained coenzyme B bound in its active site. In the crystal structure, the threonine phosphate moiety of coenzyme B could clearly be resolved. The phosphate group of the threonine phosphate moiety was not covalently bound to any other molecule (Ermler *et al.*, 1997b).

Molecular properties of methyl-coenzyme M reductase and of its isoenzyme

Methyl-coenzyme M reductase (MCR) has an apparent molecular mass of approximately 300 kDa. It is composed of three different subunits, α (McrA), β (McrB) and γ (McrG), in an $\alpha_2\beta_2\gamma_2$ arrangement (Gunsalus & Wolfe, 1980). The enzyme contains 2 mol of tightly but not covalently bound coenzyme F_{430} mol^{-1} (Ellefson *et al.*, 1982), which is a nickel porphinoid, the structure of which is shown in Fig. 5. The enzyme as purified generally also contains 2 mol H-S-CoM (Hartzell *et al.*, 1987) and 2 mol H-S-CoB (Noll & Wolfe, 1986). The enzyme can be reconstituted from its subunits in the presence of coenzyme F_{430}, albeit with very low specific activity (Hartzell & Wolfe, 1986).

Methanobacterium thermoautotrophicum, *Methanothermus fervidus* and *Methanococcus jannaschii* (probably all members of the *Methanobacteriales* and *Methanococcales*) have been shown to contain besides MCR a genetically distinct isoenzyme of methyl-coenzyme M reductase designated MRT (Rospert *et al.*, 1990; Brenner *et al.*, 1993; Lehmacher & Klenk, 1994; Bult *et al.*, 1996; Nölling *et al.*, 1996). The expression of the two isoenzymes is differentially regulated by the growth conditions (Bonacker *et al.*, 1992; Pihl *et al.*, 1994; Reeve *et al.*, 1997; Pennings *et al.*, 1997).

The genes encoding the three subunits of the isoenzyme I (MCR) form a transcription unit (*mcrBDCGA*) which additionally contains two open reading frames encoding two polypeptides, McrC and McrD, of molecular masses below 20 kDa (Cram *et al.*, 1987; Bokranz & Klein,

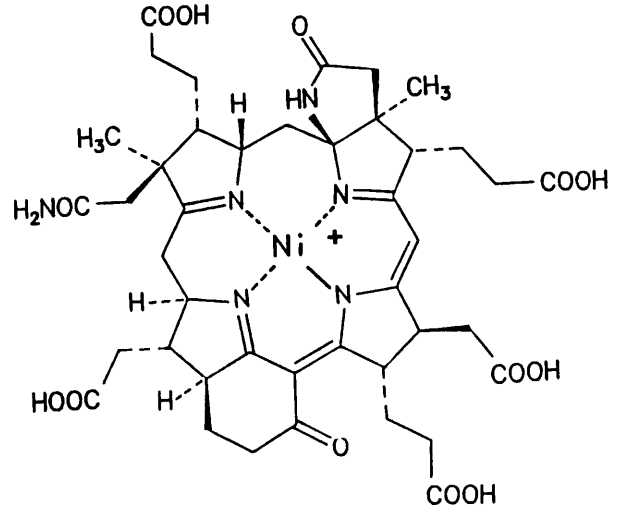

Fig. 5. Structure of coenzyme F_{430} in the Ni(II) oxidation state (Pfaltz *et al.*, 1982; Färber *et al.*, 1991). For the biosynthesis of coenzyme F_{430}, see Thauer & Bonacker (1994).

1987; Bokranz *et al.*, 1988). McrD, which can be detected in cell extracts (Sherf & Reeve, 1990) and which is associated with MCR from *Methanococcus vannielii* (Stroup & Reeve, 1993a, b), appears not to be required for MCR activity (Ellermann *et al.*, 1989; Rospert *et al.*, 1991; Goubeaud *et al.*, 1997). In members of the *Methanobacteriales*, the genes encoding the three subunits of the isoenzyme II (MRT) also form a transcription unit (*mrtBDGA*) which, however, contains additionally only one open reading frame encoding a protein with sequence similarity to McrD (Nölling *et al.*, 1996). In *Methanococcus jannaschii*, *mrtD* is separated from the *mrtBGA* operon by approximately 37 kbp. The function of the *mcrC*, *mcrD* and *mrtD* gene products is not known (Reeve *et al.*, 1997). A possibility could be that they are involved in post-translational modification of the α subunit, which, in the native enzyme, is extensively modified as revealed by crystal structure analysis: an arginine (4-methyl-Arg$^{\alpha 271}$), a histidine (1-*N*-methyl-His$^{\alpha 257}$), a cysteine (S-methyl-Cys$^{\alpha 452}$) and a glutamine (2-methyl-Gln$^{\alpha 400}$) are methylated and instead of a glycine there appears to be a thioglycine (Gly$^{\alpha 445}$) (Ermler *et al.*, 1997b).

Coenzyme F_{430}, the prosthetic group of methyl-coenzyme M reductase

The first report on coenzyme F_{430} (Fig. 5) is by Gunsalus & Wolfe (1978), who observed in cell extracts of *Methanobacterium thermoautotrophicum* a low-molecular-mass non-fluorescent compound with an absorption maximum at 430 nm. In 1979, Schönheit and others discovered that growth of methanogens is dependent on nickel. Diekert *et al.* (1980a) and Whitman & Wolfe (1980) then showed that the nickel was in part required for the synthesis of F_{430}, in which it was incorporated, and that F_{430} contained mol^{-1} 1 mol nickel. In the same year, biosynthetic evidence was provided by Diekert *et al.* (1980b) that F_{430} is a nickel tetrapyrrole, the structure of which was subsequently elucidated in collaboration with the group of A. Eschenmoser at the ETH Zürich (Pfaltz *et al.*, 1982; Färber *et al.*, 1991). Finally, in 1982 it was found that F_{430} is the prosthetic group of methyl-coenzyme M reductase (Ellefson *et al.*, 1982). Until now, coenzyme F_{430} has been found only in methanogenic archaea in which it is always present (Diekert *et al.*, 1981) and in which its only function appears to be the prosthetic group of methyl-coenzyme M reductase.

The presence of a ligand nickel atom is the striking feature of coenzyme F_{430}. Nickel brings up to five the number of different metals found in the centre of biological cyclic tetrapyrroles. Up to the discovery of F_{430}, the different metals known to occur were iron in haems and in sirohaem, magnesium in chlorophylls, cobalt in corrinoids and copper in turacin, the pigment of turaco bird feathers. Another striking feature is the state of reduction of the pyrrole ring system. One encounters the interesting fact, possibly related to its anaerobic evolution and function, that coenzyme F_{430} is by far the most highly reduced or saturated amongst all known tetrapyrroles (Fig. 6). Coenzyme F_{430} has only five double bonds, of which two pairs are conjugated, but they are separated by two single bonds and so do not form a larger conjugated bond system. The yellow colour of coenzyme F_{430}, in contrast to the red colour of porphyrins and corrinoids, is no doubt related to this low degree of conjugation (Friedmann *et al.*, 1991).

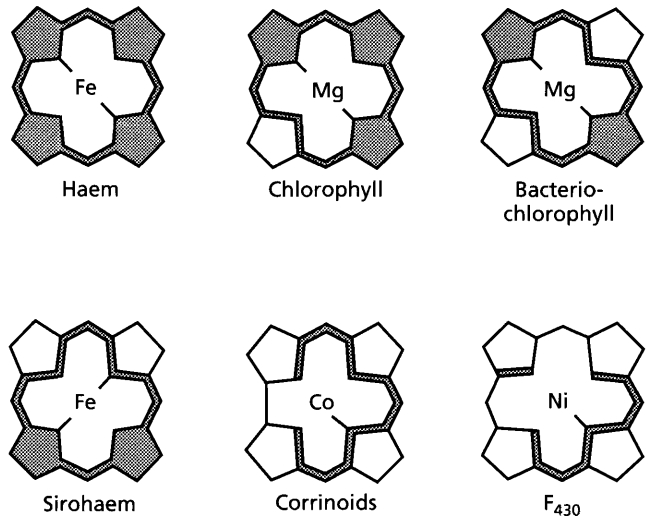

Fig. 6. Degree of unsaturation and conjugation in various macrocyclic tetrapyrroles. Shaded areas show double bonds or resonating conjugated bond systems. Upper line (from left to right): haems, plant-type chlorophylls, bacteriochlorophylls *a* and *b*; lower line (from left to right): sirohaem, corrinoids, coenzyme F_{430} (Eschenmoser, 1988).

Biosynthetically, coenzyme F_{430} is derived from dihydrosirohydrochlorin, which is also the biosynthetic precursor of sirohaem and B_{12} (Thauer & Bonacker, 1994; Hungerer *et al.*, 1996).

Coenzyme F_{430}, properties relevant to the catalytic mechanism of methyl-coenzyme M reductase

Coenzyme F_{430} is released from methyl-coenzyme M reductase upon denaturation with perchloric acid or trichloroacetic acid. In the released coenzyme, nickel is in the Ni(II) oxidation state. For the understanding of the function of coenzyme F_{430} in methyl-coenzyme M reductase, the following properties of the non-protein-bound cofactor are considered to be of importance (Jaun, 1993; Won *et al.*, 1993; Telser, 1998; see also Stolzenberg & Zhang, 1997; Pandiyan *et al.*, 1997; Schmid *et al.*, 1996, 1997; Jäger & Rudolph, 1997). They are discussed in comparison to those of cobalamin (B_{12}) (Kräutler *et al.*, 1998) (Fig. 7).

F_{430} as isolated in the Ni(II) oxidation state can be reduced to the Ni(I) oxidation state. In aqueous solution, the standard redox potential E_0' of the Ni(II)/Ni(I) couple is between −600 mV and −700 mV (Jaun & Pfaltz, 1986; Holliger *et al.*, 1993) and is thus in the same

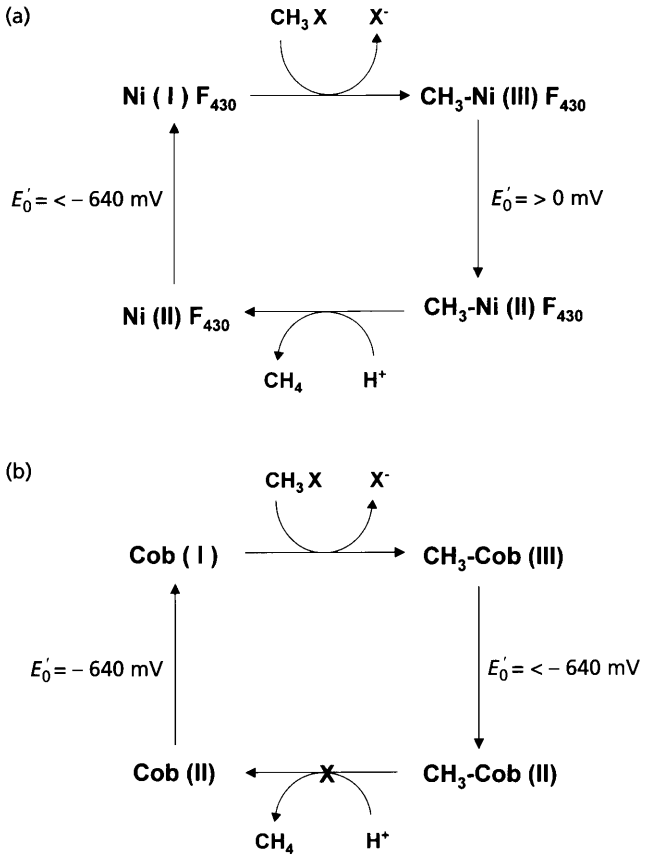

Fig. 7. Properties of protein-free coenzyme F_{430} (a) and of cobalamin (b) explaining why the former is a good methyl group reduction catalyst and cobalamin a good methyl group transfer catalyst. CH_3X, methyltosylate, methylhalides or methylsulphonium ions (Jaun & Pfaltz, 1988; Lin & Jaun, 1992).

order as that of the cob(II)alamin/cob(I)alamin couple, which is −640 mV (Lexa & Saveant, 1983; Daas *et al.*, 1995).

Ni(I) in F_{430} can be methylated with methyl iodide, methyltosylate or methylsulphonium ions (but not with methylthioethers) (Jaun & Pfaltz, 1988; Lin & Jaun, 1992), yielding a methyl-Ni(II) metalloorganic compound (Lin & Jaun, 1991). With methyltosylate, primarily methyl-Ni(III) F_{430} must be formed (Jaun, 1993), just as methylcob(III)alamin is formed upon methylation of cob(I)alamin. Methyl-Ni(III) F_{430} is, however, a very labile compound which is predicted to be almost instantaneously reduced to methyl-Ni(II) F_{430} by any electron donor available (Jaun, 1993). Upon methylation of Ni(I) F_{430}, only methyl-Ni(II) F_{430} rather than methyl-Ni(III) F_{430} can be detected (Lin & Jaun, 1991). Based on the E_0' of the Ni(III) F_{430}/Ni(II) F_{430} redox couple (Jaun, 1990), the E_0' of the methyl-Ni(III)/methyl-Ni(II) couple is estimated to be much more positive than 0 V. In this property, F_{430} differs significantly from cobalamin, the methylated form of which [methylcob(III)alamin] is stable and resistant towards reduction to methylcob(II)alamin with electron donors more positive than −650 mV. Methylation of Ni(I) F_{430} to methyl-Ni(III) F_{430} is a nucleophilic substitution which is predicted to proceed with inversion of stereoconfiguration (Lin & Jaun, 1992).

Methyl-Ni(II) F_{430} spontaneously protonolyses to yield CH_4 and Ni(II) F_{430} (Lin & Jaun, 1991). It is an electrophilic substitution which is predicted to proceed with retention of stereoconfiguration. To the contrary, methylcob(II)alamin dissociates to a methyl radical and cob(I)alamin rather than to protonolyse to methane and cob(II)alamin.

These properties of F_{430} indicate that the nickel porphinoid is a good methyl group reduction catalyst and differs in this property from cobalamin, which is a good methyl transfer catalyst. They are probably relevant to the catalytic mechanism of methyl-coenzyme M reductase: there is evidence that the enzyme only becomes active upon reduction of its prosthetic group from the Ni(II) to the Ni(I) oxidation state (Goubeaud *et al.*, 1997). Furthermore, there is evidence that methyl-coenzyme M reductase catalyses the reduction of the methyl group of methyl-coenzyme M to methane with inversion of stereoconfiguration, consistent with Ni(I) F_{430} methylation and methyl-Ni(II) F_{430} protonolysis as steps in the catalytic cycle (Ahn *et al.*, 1991).

Activation of methyl-coenzyme M reductase by reduction to the Ni(I) oxidation state

Methyl-coenzyme M reductase isolated from cells of methanogenic archaea is more or less inactive (Gunsalus & Wolfe, 1980; Brenner *et al.*, 1993). The enzyme purified from *Methanobacterium thermoautotrophicum* ΔH requires the addition of at least two proteins, designated A2 and A3a, of ATP and of Ti(III) citrate for activation *in vitro*. Even in their presence, however, only less than 0·1% of the specific activity *in vivo* is reached.

$$\text{MCR (inactive)} \xrightarrow[\text{Ti(III); pH 7}\ \text{ATP}]{A_2, A_{3a}} \text{MCR (0·1\% active)}$$

The sequence of *atw*, the A2-encoding gene, predicts that A2 is a 59 kDa ATP-binding protein related to the ABC family of proteins that participates in energy-dependent transport processes (Kuhner *et al.*, 1993).

It was mentioned above that MCR as isolated in its inactive form contains tightly bound 2 mol coenzyme M (Hartzell *et al.*, 1987). When in the presence of the activating system (A proteins, ATP and an electron donor), the inactive enzyme was incubated with [*methyl*-^{3}H, *thio*-^{35}S]methyl-coenzyme M and component B, the coenzyme M moiety of methyl-coenzyme M was found to slowly exchange into the enzyme-bound coenzyme M during turnover, 150–160 mol CH_4 (mol enzyme)$^{-1}$ having to be formed before the exchange into the 2 mol bound coenzyme M was complete (Hartzell *et al.*, 1987). In the absence of the activating system and of component

B or in the presence of 2-bromoethanesulphonate, neither an exchange nor methane formation was observed. Apparently exchange was dependent on enzyme turnover. The finding of complete exchange indicates that all the MCR molecules present must have been activated under the assay conditions. The finding that only 0·1 % of the maximal possible specific activity was reached (see above) and that it took more than 100 turnovers for complete exchange indicates that the MCR molecules present were not all activated at the same time and that they lost activity again after several turnovers.

The enzyme purified from the Marburg strain of *Methanobacterium thermoautotrophicum* shows residual activity (0·1 U mg^{-1}) in the absence of A2, A3a and ATP (Ankel-Fuchs & Thauer, 1986). In the presence of an appropriate reductant, the specific activity even increases to 1 U mg^{-1}, which is 2 % of that expected from *in vivo* data (Ellermann *et al.*, 1989). EPR spectroscopic analysis revealed that these enzyme preparations were essentially EPR silent, as were the cells from which the enzyme was purified. This indicates that the inactive methyl-coenzyme M reductase preparations contained F_{430} in the Ni(II) oxidation state.

A first breakthrough was the finding that the specific activity of methyl-coenzyme M reductase was much higher in extracts prepared from cells of *Methanobacterium thermoautotrophicum* reduced with 100 % H_2 prior to harvest. Such reduced cells and the methyl-coenzyme M reductase enriched from such cells exhibited a nickel-based EPR spectrum designated MCR_{red1} and the spin concentration of the signal correlated with the specific activity. The highest spin concentration reached was between 10 and 20 % and the highest specific activity was between 10 and 20 U per mg purified methyl-coenzyme M reductase (Rospert *et al.*, 1991; Bonacker *et al.*, 1993).

The final breakthrough was the finding that purified methyl-coenzyme M reductase in the MCR_{ox1} state (see below) can be activated to a specific activity of 100 U mg^{-1} by reduction with Ti(III) citrate at pH 10. The reduced enzyme exhibited an axial MCR_{red1} signal with a spin concentration near to 100 % (Goubeaud *et al.*, 1997).

$$MCR_{ox1}\ \text{(inactive)} \xrightarrow[\text{Ti(III); pH 10}]{} MCR_{red1}\ \text{(100 \% active)}$$

Methyl-coenzyme M reductase with a specific activity of 100 U mg^{-1} shows an EPR spectrum very similar and a characteristic UV/visible spectrum almost identical to that of non-protein-bound coenzyme F_{430} in the Ni(I) oxidation state (Goubeaud *et al.*, 1997). From this finding, it is concluded that the MCR_{red1} signal of methyl-coenzyme M reductase is derived from its Ni(I) reduced prosthetic group. From the correlation of the specific activity with the MCR_{red1} spin concentration it is deduced that the prosthetic group of methyl-coenzyme M reductase has to be in the Ni(I) oxidation state for the enzyme to be active.

EPR-signal-exhibiting forms of methyl-coenzyme M reductase: MCR_{red1}, MCR_{red2}, MCR_{ox1} and MCR_{ox2}

As already indicated, active methyl-coenzyme M reductase exhibits the EPR spectrum MCR_{red1}. The signal is induced in cells of *Methanobacterium thermoautotrophicum* growing on 80 % H_2/20 % CO_2 by switching to gassing with 100 % H_2. The axial signal builds up within 10 min. Upon further gassing with 100 % H_2, a second methyl-coenzyme M reductase derived signal appears, designated MCR_{red2}, which is rhombic rather than axial [Albracht *et al.*, 1988; for a Ni(I) macrocyclic complex exhibiting a rhombic EPR spectrum see Suh *et al.*, 1996]. Cell extracts of such reduced cells also show a high specific activity and also exhibit the MCR_{red2} signal. Upon addition of coenzyme M to the cell extracts, the MCR_{red2} signal increases; upon addition of methyl-coenzyme M, the MCR_{red2} signal is converted to the MCR_{red1} signal (Rospert *et al.*, 1991, 1992).

When gassing of growing *Methanobacterium thermoautotrophicum* cultures is switched from 80 % H_2/20 % CO_2 to 80 % N_2/20 % CO_2, a methyl-coenzyme M reductase derived EPR signal is induced which differs significantly from the MCR_{red1} and MCR_{red2} signals and which was designated MCR_{ox1} (Albracht *et al.*, 1986, 1988). This signal is also induced when the cultures of *Methanobacterium thermoautotrophicum* growing on 80 % H_2/20 % CO_2 are supplemented with Na_2S (50 mM) (Becker & Ragsdale, 1998). The methyl-coenzyme M reductase in the MCR_{ox1} form can be purified. The pure enzyme shows only very low specific activity but can be activated by reduction with Ti(III) citrate at pH 10 to the MCR_{red1} form (Goubeaud *et al.*, 1997) (see above).

When cultures of *Methanobacterium thermoautotrophicum* growing on 80 % H_2/20 % CO_2 are supplemented with Na_2SO_3 (10 mM) or $Na_2S_2O_4$ (10 mM), a methyl-coenzyme M reductase derived novel EPR signal is induced which is designated MCR_{ox2}. The enzyme in this form can be isolated but is inactive. It cannot be activated by reduction with Ti(III) (Becker & Ragsdale, 1998).

EPR-silent forms of methyl-coenzyme M reductase: MCR_{silent}, $MCR_{red1\text{-}silent}$, $MCR_{red2\text{-}silent}$, $MCR_{ox1\text{-}silent}$ and $MCR_{ox2\text{-}silent}$

Different EPR-silent forms of methyl-coenzyme M reductase have to be discriminated. These forms all have in common that they show only very little activity and are not susceptible to activation by reduction with Ti(III) at pH 10 to the MCR_{red1} form. (Residual activity may be due to the presence of traces of EPR active forms.)

The methyl-coenzyme M form referred to as MCR_{silent} is the one obtained by isolation from cells of *Meth-*

anobacterium thermoautotrophicum harvested without prior gassing with 100% H_2 or 20% CO_2/80% N_2. The forms designated $MCR_{red1\text{-}silent}$, $MCR_{red2\text{-}silent}$, $MCR_{ox1\text{-}silent}$ and $MCR_{ox2\text{-}silent}$ are generated spontaneously from the respective EPR active forms when stored under strictly anaerobic conditions at 4 °C: the spin concentration of the different EPR signals decreases by 50% within only a few hours. In the presence of traces of O_2, the MCR_{red1} and MCR_{red2} signals are instantaneously quenched (Rospert *et al.*, 1991, 1992) whereas the decay rate of the MCR_{ox} signals is similar under anaerobic and aerobic conditions (Goubeaud *et al.*, 1997; Becker & Ragsdale, 1998). The latter finding suggests that the MCR_{ox} forms of methyl-coenzyme M reductase could contain F_{430} in the Ni(III) oxidation state. Interestingly, the two MCR_{ox} forms exhibit a UV/visible spectrum more closely related to that of Ni(II) F_{430} than to Ni(I) F_{430} or Ni(III) F_{430} (Jaun, 1990, 1993).

Crystal structure of methyl-coenzyme M reductase in the $MCR_{ox1\text{-}silent}$ state

First crystals of methyl-coenzyme M reductase were obtained in 1991 by Rospert (1991) for the isoenzyme I from *Methanobacterium thermoautotrophicum* (Marburg). From this time on the enzyme from various methanogens and from various different preparations was crystallized and tested for defraction. Beginning 1996, the first good crystals were obtained from a highly purified preparation of methyl-coenzyme M reductase isoenzyme I in the MCR_{ox1} state from *Methanobacterium thermoautotrophicum* (Marburg strain) (Shima *et al.*, 1997). The crystal structure was solved in the $MCR_{ox1\text{-}silent}$ state to 1·4 Å resolution (1 Å = 0·1 nm) (Ermler *et al.*, 1997b; see also Ferry, 1997b; and Cammack, 1997). The primary structure of the enzyme had previously been resolved by Bokranz *et al.* (1988).

Methyl-coenzyme M reductase in the $MCR_{ox1\text{-}silent}$ state contains bound 2 mol coenzyme F_{430}, 2 mol reduced coenzyme M and 2 mol reduced coenzyme B as revealed by the X-ray structure.

Overall structure

It is characterized by a series of α helices arranged in a compact form with an ellipsoidal shape of about 120 by 85 by 80 Å. The subunits are mutually tightly associated, as indicated by extended interface areas, particularly between subunits α and α′ and subunits β and β′, and by the fact that, except for subunits γ and γ′, each subunit contacts all other subunits of the multisubunit complex (Ermler *et al.*, 1997b).

Two independent active sites

There are two binding sites, roughly 50 Å apart, for coenzyme F_{430}, coenzyme M and coenzyme B, forming two separated structurally identical active sites. In each binding site the three coenzymes are embedded inside a narrow channel, 30 Å long, and at the narrowest part 6 Å in diameter, extending from the protein surface deeply into the interior of the protein complex (see the diagram in Fig. 11). The channel and the coenzyme-binding sites are formed by residues of subunits α, α′, β and γ (and equivalently α′, α, β′ and γ′), indicating that one trimer is not sufficient to accomplish the enzymic reaction (Ermler *et al.*, 1997b).

Binding of coenzyme F_{430}

F_{430} is bound at the bottom of the 30-Å-long channel such that its tetrapyrrole plane points with its front face (reduced pyrrole rings A, B, C and D clockwise) (see Fig. 5) roughly towards the mouth of the channel, whereas its rear face points to the channel bottom. Thus only the front face is accessible to the substrate. Interestingly, in sulphite reductase the prosthetic group sirohaem, which as F_{430} is biosynthetically derived from dihydrosirohydrochlorin, is only accessible from the rear face (Crane *et al.*, 1995, 1997a, b). The tetrapyrrole ring of coenzyme F_{430} is bound in a rather flat conformation to the enzyme as predicted for the free coenzyme F_{430} in the hexagonally coordinated Ni(II) state (Färber *et al.*, 1991; Jaun, 1993). The Ni atom present as Ni(II) sits almost exactly in the tetrapyrrole plane and is coordinated to six ligands arranged in a nearly optimal octahedral configuration. The four equatorially located nitrogen atoms of the tetrapyrrole ring have distances to the nickel of 2·14 Å for ring A, 2·11 Å for ring B, 2·10 Å for ring C and 1·99 Å for ring D (Fig. 8). As the fifth ligand the side chain oxygen of $Gln^{\alpha'147}$ protrudes from a long loop to the rear face of F_{430} and approaches the Ni(II) atom to 2·3 Å (Fig. 9). The 1·45 Å electron density map demonstrates that the oxygen and not the nitrogen of the side chain $Gln^{\alpha'147}$ is the axial ligand (Ermler *et al.*, 1997b) as to be expected thermodynamically and as seen also in other proteins (Roach *et al.*, 1997). $Gln^{\alpha'147}$ is embedded in the protein matrix such that it will probably not be able to move away when F_{430} is reduced from the Ni(II) to the Ni(I) oxidation state in which the Ni(I) is preferentially tetraplanarily coordinated (Jaun, 1993; Telser *et al.*, 1997). By the enforced axial fifth ligand the nucleophilicity of the Ni(I) in F_{430} is predicted to be increased, facilitating the nucleophilic substitution of the methyl group of coenzyme M.

Binding of coenzyme M

In $MCR_{ox1\text{-}silent}$ the sixth coordination site of nickel, located in front of the tetrapyrrole ring plane of F_{430}, is occupied by the thiol group of coenzyme M, which is positioned almost parallel to the tetrapyrrole plane in contact with its front face (Fig. 9). The thiol group binds axially to the nickel (2·42 Å distance) and interacts with the hydroxyl group of $Tyr^{\alpha333}$ and $Tyr^{\beta367}$ and a water molecule that bridges coenzyme M and B. The ethyl moiety is embedded between the lactam ring of the Ni porphinoid and the phenyl ring of $Phe^{\alpha443}$. Coenzyme M is anchored to the polypeptide chain by its negatively charged sulphonate group, forming a salt bridge to the

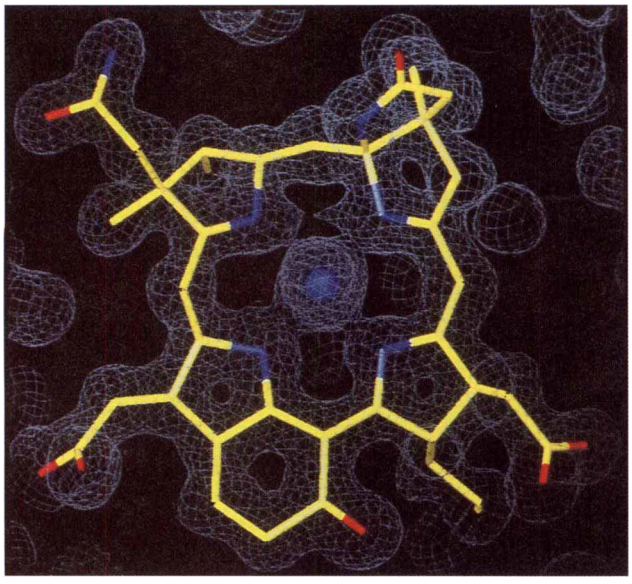

Fig. 8. Coenzyme F_{430} in the Ni(II) oxidation state as viewed by looking from outside through the 30-Å-long channel into the active site pocket of methyl-coenzyme M reductase ($MCR_{ox1\text{-}silent}$). The front face (reduced pyrrole rings A, B, C and D clockwise) is seen. Structure at 1·45 Å resolution.

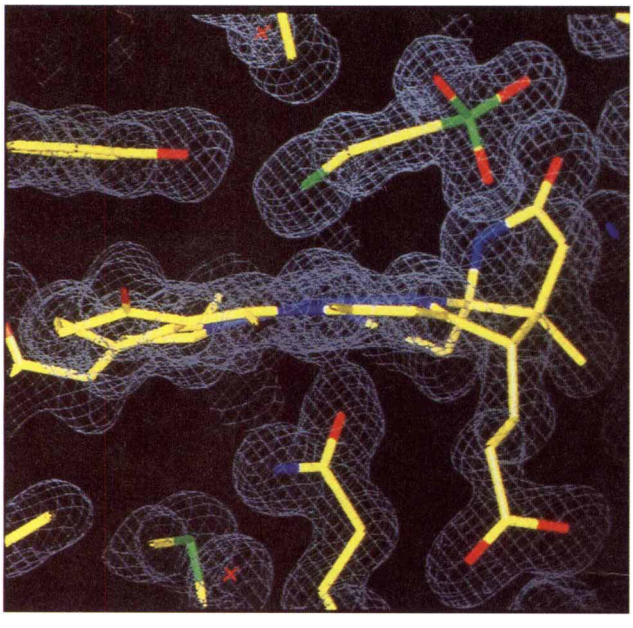

Fig. 9. Coenzyme M located in front of the tetrapyrrole ring plane of F_{430} with its thiol group bound to Ni(II) in the active site of methyl-coenzyme M reductase ($MCR_{ox1\text{-}silent}$). Structure at 1·45 Å resolution. The oxygen of $Gln^{\alpha'147}$ is seen to approach the Ni from the rear face of F_{430} to 2·3 Å.

guanidinium group of $Arg^{\gamma 120}$, a hydrogen bond to the peptide nitrogen of $Tyr^{\alpha 444}$ and a hydrogen bond to a water molecule connected to the peptide oxygen of $His^{\beta 364}$ (Ermler *et al.*, 1997b).

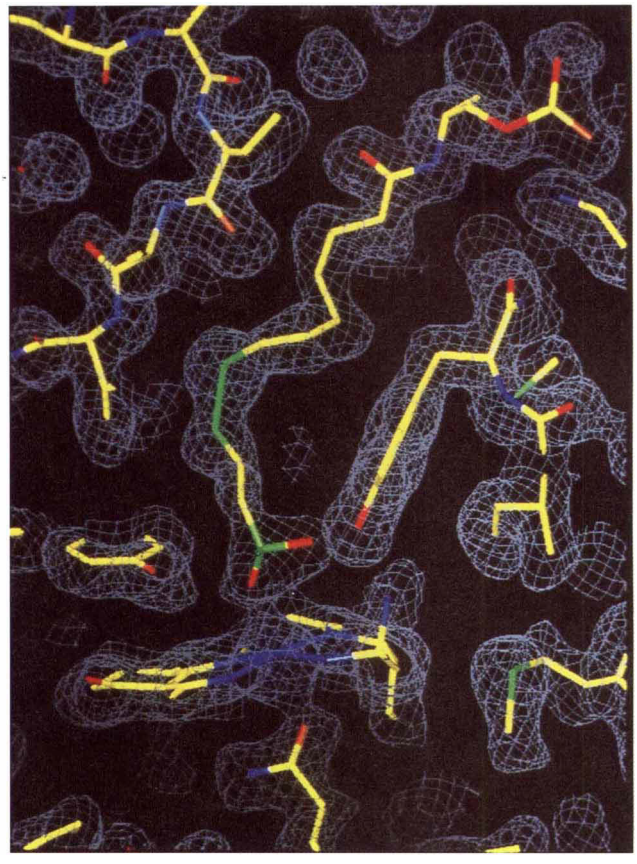

Fig. 10. Heterodisulphide of coenzyme M and coenzyme B bound via an oxygen of its sulphonate group to Ni(II) in the active site of methyl-coenzyme M reductase (MCR_{silent}). Structure at 1·75 Å resolution.

Binding of coenzyme B

With its elongated conformation, coenzyme B fits accurately into the most narrow segment of the channel formed by residues of subunits α, α' and β (see also the diagram in Fig. 11a). Coenzyme B is anchored to the protein mainly by salt bridges between the negatively charged L-threonine phosphate moiety and five positively charged amino acids. The heptanoyl arm is in van der Waal's contact with several hydrophobic residues. The thiol group of coenzyme B is positioned at a distance of 8·7 Å from the nickel of F_{430} and 6·2 Å from the coenzyme M thiol sulphur. It interacts with the side chain of nitrogen of $Asn^{\alpha 481}$, the main chain peptide nitrogen of $Val^{\alpha 482}$ and the bridging water molecule mentioned above. $Asn^{\alpha 481}$ is within hydrogen bond distance of the sulphur that is presumed to replace the backbone carbonyl oxygen of the modified $Gly^{\alpha 445}$ (see below).

Five modified amino acids in the active site region

The electron density map revealed five modified amino acids located in subunits α and α' at or very near the active site region, 1-*N*-methyl-$His^{\alpha 257}$, 4-methyl-$Arg^{\alpha 271}$, 2-methyl-$Gln^{\alpha 400}$, S-methyl-$Cys^{\alpha 452}$ and $Gly^{\alpha 445}$, where

the carbonyl oxygen appears to be substituted by sulphur (Ermler *et al.*, 1997b).

Exclusion of bulked water from the active site

When coenzyme B is bound to the enzyme the active site is shielded from bulk solvent. The sole water molecule found in the active site region of $MCR_{ox1\text{-}silent}$ between coenzyme M and coenzyme B should be displaced after binding of the more bulky methyl-coenzyme M. The active site is lined up by an annular arrangement of $Phe^{\alpha 330}$, $Tyr^{\alpha 333}$, $Phe^{\alpha 443}$, $Phe^{\beta 361}$ and $Tyr^{\beta 367}$ flanked further by hydrophobic and aromatic residues (Ermler *et al.*, 1997b). These amino acids are completely conserved in all methyl-coenzyme M reductases (Nölling *et al.*, 1996). Methane formation from methyl-coenzyme M and coenzyme B thus takes place in a greasy pocket and probably does not involve water molecules. These conditions would allow for radical intermediates. Solvent-inaccessible active sites coated by non-polar aromatic residues and attainable by a channel have been observed in several radical-based enzymes such as galactose oxidase (Ito *et al.*, 1991), prostaglandin H_2 synthase 1 (Picot *et al.*, 1994) and methylmalonyl-CoA mutase (Mancia *et al.*, 1996).

Crystal structure of methyl-coenzyme M reductase in the MCR_{silent}, $MCR_{red2\text{-}silent}$ and $MCR_{ox2\text{-}silent}$ states

The structure of the MCR_{silent} state was refined in the resolution range 2·0–10·0 Å with the use of the $MCR_{ox1\text{-}silent}$ structure for initial phase determination (Ermler *et al.*, 1997b). The two enzyme states exhibit nearly identical overall structures. The model of the MCR_{silent} structure is mainly distinguished from that of $MCR_{ox1\text{-}silent}$ by binding of the oxidized instead of the reduced forms of coenzyme M and coenzyme B (Fig. 10). A superposition of the structures reveals that the reduced coenzyme B in $MCR_{ox1\text{-}silent}$ and the coenzyme B moiety of the heterodisulphide in MCR_{silent} align perfectly except that the sulphur is turned slightly towards coenzyme M. In contrast to coenzyme B, coenzyme M has moved more than 4 Å away from its position in the $MCR_{ox1\text{-}silent}$ state. The thiol group is shifted perpendicular and the sulphonate group parallel to the tetrapyrrole plane of F_{430}, resulting in a 90° rotation of coenzyme M. In this position, one oxygen atom of the sulphonate is axially coordinated with the nickel and contacts the hydroxyl group of $Tyr^{\alpha 333}$. The distance between nickel and oxygen is 2·1 Å. The second oxygen atom is hydrogen bonded to the lactam ring of F_{430} and to the hydroxyl group of $Tyr^{\beta 367}$ and the third to a water molecule located at the former binding site of the sulphonate.

The structures of methyl-coenzyme M reductase in the $MCR_{red2\text{-}silent}$ and $MCR_{ox2\text{-}silent}$ states have, in the meantime, also been resolved but have not yet been completely refined. Available information indicates that in the $MCR_{red2\text{-}silent}$ state, the active site of methyl-coenzyme M reductase is probably devoid of both coenzyme M and coenzyme B. The 30-Å-long channel leading into the active site pocket is more open and the residues of the α-, α'-, β- and γ-subunits forming the channel are more flexible. In the $MCR_{ox2\text{-}silent}$ state, methyl-coenzyme M reductase appears to contain bound coenzyme B but not coenzyme M (W. Grabarse, unpublished results).

Catalytic mechanism proposed for methyl-coenzyme M reductase

The crystal structures display inactive states of methyl-coenzyme M reductase with coenzyme F_{430} in the Ni(II) oxidation state. Nevertheless, the arrangement of the coenzymes and the protein environment, combined with the catalytic properties of the enzyme and the chemical properties of free coenzyme F_{430}, allow for conclusions about the active site and the catalytic mechanism (Ermler *et al.*, 1997b).

First the structure of the enzyme–substrate complex and of the enzyme–product complex and their formation and dissociation, respectively, will be discussed. Then two alternative mechanisms for the conversion of the enzyme–substrate to the enzyme–product complex will be proposed.

Enzyme–substrate/product complex

In Fig. 11, diagrams of the enzyme–substrate complex and of the enzyme–product complex are given. The prosthetic group is shown in the Ni(I) oxidation state. This is deduced from the finding that methyl-coenzyme M reductase only exhibits activity when assayed in its Ni(I) F_{430} reduced form (either MCR_{red1} or MCR_{red2} state) (Rospert *et al.*, 1991, 1992; Goubeaud *et al.*, 1997).

The diagram in Fig. 11 shows that the prosthetic group of methyl-coenzyme M is accessible only through a narrow channel and only by methyl-coenzyme M (Ermler *et al.*, 1997b). Through this channel methyl-coenzyme M must enter before coenzyme B binds since upon coenzyme B binding the channel is completely locked. This is consistent with an ordered ternary complex kinetic mechanism displayed by the enzyme (Bonacker *et al.*, 1993).

Coenzyme B is able to penetrate the 30-Å-long channel only with its aliphatic arm and its arm is not long enough for its thiol group at the end to reach the Ni of F_{430}. A distance of 8·7 Å remains. A direct reaction of the coenzyme B thiol group with nickel, as has previously been proposed (Jaun, 1990; Berkessel, 1991), can therefore be excluded. Methyl-coenzyme M is, however, anchored in the active site to the protein matrix with its sulphonate group relative to the prosthetic group such that both the methyl group and the thioether sulphur could directly interact with the Ni(I). In the $MCR_{ox1\text{-}silent}$ structure, coenzyme M probably mimics the binding position of methyl-coenzyme M with respect to the binding mode of the sulphonate moiety but

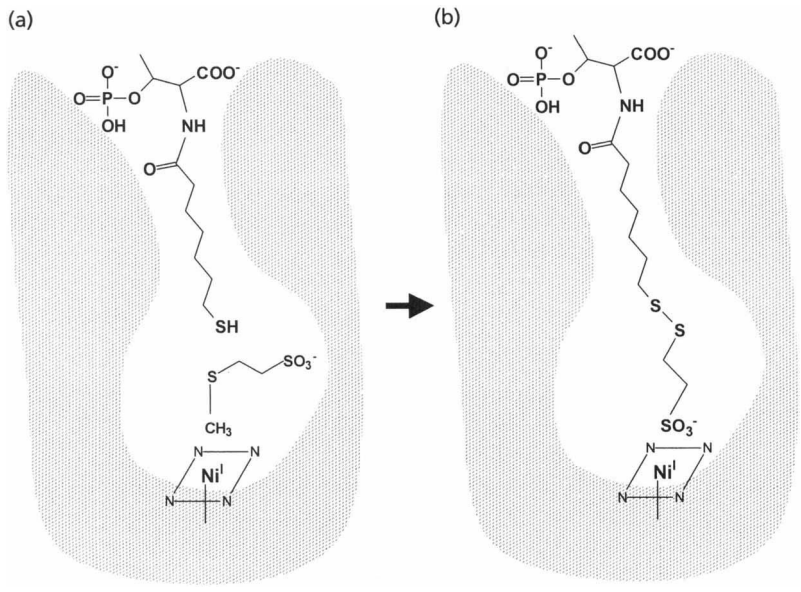

Fig. 11. Diagrams of the enzyme–substrate complex (a) and of the enzyme–product complex (b) of active methyl-coenzyme M reductase containing F_{430} in the Ni(I) oxidation state.

presumably not with respect to the binding mode of the thiol group. A Ni-S-CoM intermediate in the catalytic cycle is not attractive because of the long distance of 6·2 Å between the sulphurs of coenzyme M and coenzyme B. Model building studies indicate, however, that the two sulphurs of coenzyme M and coenzyme B come in van der Waal's contact when the methyl group of methyl-coenzyme M is placed in van der Waal's distance of the potentially attacking nickel (Fig. 11a) (Ermler *et al.*, 1997b). Therefore, a Ni-CH_3 intermediate proposed from free coenzyme F_{430} studies (Lin & Jaun, 1991; Jaun, 1993) appears to be compatible with the steric requirements of the active site.

From the structure of the enzyme–substrate complex shown in Fig. 11(a) it is evident that the H^+ required for methane formation from methyl-coenzyme M is lastly provided by coenzyme B. The $MCR_{ox1\text{-}silent}$ structure reveals an interaction between the thiol group of coenzyme B and two hydrogen donors, the amide and peptide nitrogen of $Asn^{\alpha 481}$ and $Val^{\alpha 482}$, which would facilitate the cleavage of the H^+ and permit the presence of a thiolate anion. However, the coenzyme B sulphur is probably too far away from the methyl group of methyl-coenzyme M for direct hydrogen transfer. $Tyr^{\alpha 333}$ and $Tyr^{\beta 367}$ are positioned in the active site such that they could mediate this transfer (Ermler *et al.*, 1997b).

The structure of the enzyme–product complex shown in Fig. 11(b) considers that the heterodisulphide cannot leave the enzyme when the sulphonate oxygen of the coenzyme M moiety binds to the Ni(II) atom of F_{430} as in the MCR_{silent} state. Coordination of the sulphonate oxygen to nickel is prevented when nickel is present as nucleophilic Ni(I) (Jaun, 1993; Telser *et al.*, 1997). The repulsion between Ni(I) and the sulphonate oxygen might even be used as a driving force to push the heterodisulphide out of the channel. It is not evident at present when, where and how the methane gets out (Ermler *et al.*, 1997b).

Both mechanisms of the conversion of the enzyme–substrate complex (Fig. 11a) to the enzyme–product complex (Fig. 11b) in the following to be discussed are based on the assumption that the methyl group of methyl-coenzyme M is transferred to Ni(I) of the prosthetic group before being reduced to methane and that the methyl group of methyl-coenzyme M has to be somehow activated in order that the transfer to Ni(I) F_{430} can proceed (Jaun, 1993).

Mechanism 1

It is assumed that methyl transfer from methyl-coenzyme M to Ni(I) yields CH_3-Ni(III) F_{430} involving protonation of methyl-coenzyme M by $Tyr^{\alpha 333}$ or $Tyr^{\beta 367}$ to the sulphonium cation in the transition state. [A precedent for such a protonation is found in the crystal structures of *ortho*-hydroxyphenyl- or *ortho*-hydroxybenzyl thioethers, giving rise to 5- and 6-membered rings, respectively, but also in the crystal structures of 3,4-dihydro-6-hydroxy-4,4,5,7,8-pentamethyl-2*H*-1-(benzothio)pyran (*d* S-H = 2·55 Å, O-H-S = 148 °) (Burton *et al.*, 1985) and 2,3,7,8-tetrahydroxythianthrene (*d* S-H = 2·53 Å, O-H-S = 167 °) (Mansel & Klar, 1992).] The H^+ would be replenished by the dissociation of coenzyme B.

$$CH_3\text{-S-CoM} + H^+ + \text{Ni(I)}\ F_{430} \rightarrow CH_3\text{-Ni(III)}\ F_{430} + \text{H-S-CoM}$$

$$\text{H-S-CoB} \rightarrow H^+ + {}^-\text{S-CoB}$$

The CH_3-Ni(III) F_{430} formed by methylation of Ni(I) F_{430} with methyl-coenzyme M is a strong one-electron oxidant and is therefore predicted to oxidize the protonated leaving group to the coenzyme M thiyl radical cation.

$$CH_3\text{-Ni(III)}\ F_{430} + \text{H-S-CoM} \rightarrow CH_3\text{-Ni(II)}\ F_{430} + \text{H-}^{\bullet}\text{S-CoM}^+$$

CH_3-Ni(II) F_{430} spontaneously protonolyses to CH_4 and Ni(II) F_{430}. The H^+ could be provided by the coenzyme M thiyl radical cation, which is more acidic than coenzyme M.

$$CH_3\text{-Ni(II)}\ F_{430} + H^+ \rightarrow CH_4 + \text{Ni(II)}\ F_{430}$$
$$H\text{-}^{\bullet}S\text{-CoM}^+ \rightarrow H^+ + {}^{\bullet}S\text{-CoM}$$

Protonolysis is essentially irreversible and thus probably pulls the preceding reactions and pushes the successive reactions. The coenzyme M thiyl radical could react with the thiolate of coenzyme B, generating a disulphide radical anion.

$$^{\bullet}S\text{-CoM} + {}^{-}S\text{-CoB} \rightarrow \text{CoM-S-}{}^{\bullet}S\text{-CoB}^-$$

A prerequisite is a loosening of the interaction between the sulphonate moiety of coenzyme M and the protein matrix, enabling a shift of coenzyme M towards coenzyme B. The disulphide radical anion has a redox potential (Lenz & Giese, 1997) negative enough for the reduction of Ni(II) F_{430} to Ni(I) F_{430}.

$$\text{Ni(II)}\ F_{430} + \text{CoM-S-}{}^{\bullet}S\text{-CoB}^- \rightarrow \text{Ni(I)}\ F_{430} + \text{CoM-S-S-CoB}$$

Electron transport from the disulphide anion radical to the nickel could be via the modified $Gly^{\alpha 445}$, a thioglycine, which interacts with the thiol group of coenzyme B via the side chain nitrogen of $Asn^{\alpha 481}$, which is in hydrogen bond interaction with both the coenzyme B sulphur and the presumed sulphur of the modified Gly^{445}. A thioketyl radical could be considered as intermediate (Buckel & Keese, 1995; Buckel, 1996). Reduction of a thioketone to the corresponding thioketyl with the disulphide radical anion should be thermodynamically feasible.

Mechanism 1 has several features in common with that proposed for ribonucleotide reductase (Lenz & Giese, 1997).

An argument against mechanism 1 is that it predicts that methyl-coenzyme M in the absence of coenzyme B should quench the MCR_{red1} EPR signal rather than stabilize it (Rospert *et al.*, 1992; Goubeaud *et al.*, 1997).

Mechanism 2

One can hypothesize alternative catalytic mechanisms that are also consistent with most of the findings. Only one alternative is discussed here. It considers that Ni(I) F_{430} with its unpaired electron is a radical and is most reactive in radical reactions (Jaun, 1993). Methylation of Ni(I) F_{430} with most methyl donors thus directly yields CH_3-Ni(II) F_{430} rather than CH_3-Ni(III) F_{430} (Lin & Jaun, 1991; Jaun, 1993). For methyl-coenzyme M to react with Ni(I) F_{430} in this manner, it has to be activated such that a homolytic cleavage of the S-methyl bond is favoured, e.g. by reaction of methyl-coenzyme M with the coenzyme B thiyl radical to the CoM-S(CH_3)-$^{\bullet}S$-CoB sulphuranyl radical (Jaun, 1990; Berkessel, 1991; Tada & Masuzawa, 1997).

$$CH_3\text{-S-CoM} + {}^{\bullet}S\text{-CoB} \rightarrow \text{CoM-S-}(CH_3)\text{-}{}^{\bullet}S\text{-CoB}$$

The catalytic cycle in the alternative mechanism is therefore assumed to start with the formation of the coenzyme B thiyl radical by one electron oxidation of coenzyme B.

$$H\text{-S-CoB} + X \rightarrow {}^{\bullet}S\text{-CoB} + XH$$

The electron acceptor X cannot be Ni(II) F_{430} as has been proposed since the enzyme is only active in its Ni(I) F_{430} reduced form (Goubeaud *et al.*, 1997). Therefore, there must be a second redox active group capable of oxidizing coenzyme B to the coenzyme B thiyl radical, and indeed there is evidence for two independent redox active groups in methyl-coenzyme M reductase (Rospert, 1991; Rospert *et al.*, 1992). This group could be the modified $Gly^{\alpha 445}$, a thioglycine, which interacts with the thiol group of coenzyme B via the side chain nitrogen of $Asn^{\alpha 481}$ and which could be susceptible to reduction to the thioketyl radical X^- (Buckel & Keese, 1995; Buckel, 1996). Probably the protonated thioketone would be reduced to the protonated thioketyl (XH) since this reaction is energetically more favourable as deduced from the thermodynamics of the reduction of ketones to ketyls in the protonated and unprotonated forms (Lenz & Giese, 1997).

The coenzyme B thiyl radical would react with methyl-coenzyme M, generating the above-mentioned sulphuranyl radical. From the sulphuranyl radical, the methyl radical would be transferred to Ni(I) F_{430}, generating the heterodisulphide and CH_3-Ni(II) F_{430}, which would protonolyse to Ni(II) F_{430} and CH_4.

$$\text{CoM-S}(CH_3)\text{-}{}^{\bullet}S\text{-CoM} + \text{Ni(I)}\ F_{430} \rightarrow \text{CoM-S-S-CoB} + CH_3\text{-Ni(II)}\ F_{430}$$
$$CH_3\text{-Ni(II)}\ F_{430} + XH \rightarrow \text{Ni(II)}\ F_{430} + CH_4 + X^-$$

The catalytic cycle would be closed by electron transfer from X^- to Ni(II) F_{430}.

$$X^- + \text{Ni(II)}\ F_{430} \rightarrow X + \text{Ni(I)}\ F_{430}$$

Via endergonic XH dissociation to $X^- + H^+$, the two last reactions could be thermodynamically tightly coupled.

The salient feature of mechanism 2 is that it involves transient methyl radical intermediates and does not involve CH_3-Ni(III) F_{430} as intermediate in the catalytic cycle. Transient methyl radical intermediates could possibly account for the presumed methylation of $His^{\alpha 257}$, $Arg^{\alpha 271}$, $Gln^{\alpha 400}$ and $Cys^{\alpha 452}$.

Future studies will have to show which of the proposed catalytic mechanisms is correct or whether both are wrong. Unfortunately, one can only disprove but not prove a catalytic mechanism (Stinson, 1995).

From hydrogenase to methyl-coenzyme M reductase

It has been a long way from the first demonstration of an enzyme, of hydrogenase, in methanogens in 1933 by Stephenson & Stickland (1933a) to the crystal structure of methyl-coenzyme M reductase in 1997 and there is still a good way to go until the biochemistry of methanogenesis is completely understood. Many ques-

tions remain to be answered. For methyl-coenzyme M reductase, the most pertinent questions are, what functions do the five amino acid modifications in the enzyme have and how are these modifications brought about? The answers to these questions could be the key to the understanding of the catalytic mechanism of the methane-forming enzyme. Fortunately, recently a genetic system for archaea of the genus *Methanosarcina* has become available (Metcalf *et al.*, 1997) now allowing these problems to be also tackled with genetic methods.

The work of my group on the biochemistry of methanogenesis started with growth experiments and the discovery that growth of methanogens is dependent on nickel (Schönheit *et al.*, 1979). This led to the finding that coenzyme F_{430} is a nickel porphinoid (Diekert *et al.*, 1980a, b), that carbon monoxide dehydrogenase from methanogens contains nickel (Hammel *et al.*, 1984) and that the hydrogenases first described by Marjory Stephenson (Stephenson & Stickland, 1931a) are nickel enzymes (Graf & Thauer, 1981; Albracht *et al.*, 1982; Albracht, 1994) (for recent reviews on nickel enzymes including methyl-coenzyme M reductase see Cammack & van Vliet, 1998; Maroney *et al.*, 1998). Later we found that methanogens also contain a metal-free hydrogenase (Zirngibl *et al.*, 1990; Thauer *et al.*, 1996) and just recently that methanogens contain a fourth type of hydrogenase (Künkel *et al.*, 1998) that shows sequence similarity to hydrogenase 3 (Sauter *et al.*, 1992) and 4 (Andrews *et al.*, 1997) of the formate hydrogenlyase from *E. coli*, an enzyme complex first studied in detail in the laboratory of Marjory Stephenson now almost 70 years ago (Stickland, 1929; Stephenson & Stickland, 1932, 1933b; Woods, 1936). With respect to my research, I, therefore, feel myself in the scientific tradition of Marjory Stephenson. And there is even a genealogical connection: I learned to grow methanogens from Greg Zeikus when he was in Marburg for a sabbatical (Zeikus *et al.*, 1977). He himself had obtained his training from Ralph Wolfe in Urbana (Zeikus & Wolfe, 1972), who discovered methyl-coenzyme M reductase (Wolfe, 1991). The first work of Ralph Wolfe on methanogens is together with Sidney Elsden (Knight *et al.*, 1966), who had been a student of Marjory Stephenson in the Cambridge Biochemistry Laboratory (Wolfe, 1991) where in 1933 the modern era of methanogenesis began (Wolfe, 1993). I am therefore very much indebted to the Society for General Microbiology for having chosen me to give the 1998 Marjory Stephenson Lecture.

Acknowledgements

This work was supported by the Max-Planck-Gesellschaft, the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie. I want to thank Sidney Elsden and Hans-Günter Schlegel for providing me with literature on M. Stephenson.

References

Abken, H.-J. & Deppenmeier, U. (1997). Purification and properties of an $F_{420}H_2$ dehydrogenase from *Methanosarcina mazei* Gö1. *FEMS Microbiol Lett* **154**, 231–237.

Abken, H.-J., Tietze, M., Brodersen, J., Bäumer, S., Beifuss, U. & Deppenmeier, U. (1998a). Isolation and characterization of methanophenazine and function of phenazines in membrane-bound electron transport of *Methanosarcina mazei* Gö1. *J Bacteriol* **180**, 2027–2032.

Abken, H.-J., Bäumer, S., Broderson, J., Murakami, E., Ragsdale, S. W., Gottschalk, G. & Deppenmeier, U. (1998b). Membrane-bound electron transport and H^+-translocation in *Methanosarcina mazei* Gö1. BIOspectrum Sonderausgabe, 38.

Afting, C., Hochheimer, A. & Thauer, R. K. (1998). Function of H_2-forming methylenetetrahydromethanopterin dehydrogenase from *Methanobacterium thermoautotrophicum* in coenzyme F_{420} reduction with H_2. *Arch Microbiol* **169**, 206–210.

Ahn, Y., Krzycki, J. A. & Floss, H. G. (1991). Steric course of the reduction of ethyl coenzyme M to ethane catalyzed by methyl coenzyme M reductase from *Methanosarcina barkeri*. *J Am Chem Soc* **113**, 4700–4701.

Albracht, S. P. J. (1994). Nickel hydrogenases: in search of the active site. *Biochim Biophys Acta* **1188**, 167–204.

Albracht, S. P. J., Graf, E.-G. & Thauer, R. K. (1982). The EPR properties of nickel in hydrogenase from *Methanobacterium thermoautotrophicum*. *FEBS Lett* **140**, 311–313.

Albracht, S. P. J., Ankel-Fuchs, D., van der Zwaan, J. W., Fontijn, R. D. & Thauer, R. K. (1986). A new EPR signal of nickel in *Methanobacterium thermoautotrophicum*. *Biochim Biophys Acta* **870**, 50–57.

Albracht, S. P. J., Ankel-Fuchs, D., Böcher, R., Ellermann, J., Moll, J., van der Zwaan, J. W. & Thauer, R. K. (1988). Five new EPR signals assigned to nickel in methyl-coenzyme M reductase from *Methanobacterium thermoautotrophicum*, strain Marburg. *Biochim Biophys Acta* **955**, 86–102.

Andrews, S. C., Berks, B. C., McClay, J., Ambler, A., Quail, M. A., Golby, P. & Guest, J. R. (1997). A 12-cistron *Escherichia coli* operon (*hyf*) encoding a putative proton-translocating formate hydrogenlyase system. *Microbiology* **143**, 3633–3647.

Ankel-Fuchs, D. & Thauer, R. K. (1986). Methane formation from methyl-coenzyme M in a system containing methyl-coenzyme M reductase, component B and reduced cobalamin. *Eur J Biochem* **156**, 171–177.

Asakawa, S., Sauer, K., Liesack, W. & Thauer, R. K. (1998). Tetramethylammonium:coenzyme M methyltransferase system from *Methanococcoides* species. *Arch Microbiol* (in press).

Balch, W. E. & Wolfe, R. S. (1979). Specificity and biological distribution of coenzyme M (2-mercaptoethanesulfonic acid). *J Bacteriol* **137**, 256–263.

Barker, H. A. (1956). *Bacterial Fermentations*. New York: Wiley.

Bäumer, S., Murakami, E., Brodersen, J., Gottschalk, G., Ragsdale, S. W. & Deppenmeier, U. (1998). The $F_{420}H_2$:heterodisulfide oxidoreductase system from *Methanosarcina* species. 2-Hydroxyphenazine mediates electron transfer from $F_{420}H_2$ dehydrogenase to heterodisulfide reductase. *FEBS Lett* **428**, 295–298.

Becher, B. & Müller, V. (1994). $\Delta\mu_{Na+}$ drives the synthesis of ATP via an $\Delta\mu_{Na+}$-translocating F_1F_0 ATP synthase in membrane vesicles of the archaeon *Methanosarcina mazei* strain Gö1. *J Bacteriol* **176**, 2543–2550.

Becher, B., Müller, V. & Gottschalk, G. (1992a). The methyltetrahydromethanopterin:coenzyme M methyltransferase of *Methanosarcina* strain Gö1 is a primary sodium pump. *FEMS Microbiol Lett* **91**, 239–244.

Becher, B., Müller, V. & Gottschalk, G. (1992b). N^5-methyltetrahydromethanopterin:coenzyme M methyltransferase of

Methanosarcina strain Göl is an Na^+ translocating membrane protein. *J Bacteriol* **174**, 7656–7660.

Becker, D. F. & Ragsdale, S. W. (1998). Activation of methyl-SCoM reductase to high specific activity after treatment of whole cells with sodium sulfide. *Biochemistry* **37**, 2639–2647.

Berk, H. & Thauer, R. K. (1997). Function of coenzyme F_{420}-dependent NADP reductase in methanogenic archaea containing an NADP-dependent alcohol dehydrogenase. *Arch Microbiol* **168**, 396–402.

Berk, H., Buckel, W., Thauer, R. K. & Frey, P. A. (1996). *Re*-face stereospecificity at C4 of NAD(P) for alcohol dehydrogenase from *Methanogenium organophilum* and for (*R*)-2-hydroxyglutarate dehydrogenase from *Acidaminococcus fermentans* as determined by ^{1}H-NMR spectroscopy. *FEBS Lett* **399**, 92–94.

Berkessel, A. (1991). Methyl-coenzyme M reductase: model studies on pentadentate nickel complexes and a hypothetical mechanism. *Bioorg Chem* **19**, 101–115.

Berkessel, A. & Thauer, R. K. (1995). On the mechanism of catalysis by a metal-free hydrogenase from methanogenic Archaea: enzymatic transformation of H_2 without a metal and its analogy to the chemistry of alkanes in superacidic solution. *Angew Chem Int Ed Engl* **34**, 2247–2250.

Bertram, P. A. & Thauer, R. K. (1994). Thermodynamics of the formylmethanofuran dehydrogenase reaction in *Methanobacterium thermoautotrophicum*. *Eur J Biochem* **226**, 811–818.

Bertram, P. A., Karrasch, M., Schmitz, R. A., Böcher, R., Albracht, S. P. J. & Thauer, R. K. (1994a). Formylmethanofuran dehydrogenases from methanogenic Archaea. Substrate specificity, EPR properties and reversible inactivation by cyanide of the molybdenum or tungsten iron–sulfur proteins. *Eur J Biochem* **220**, 477–484.

Bertram, P. A., Schmitz, R. A., Linder, D. & Thauer, R. K. (1994b). Tungstate can substitute for molybdate in sustaining growth of *Methanobacterium thermoautotrophicum*: identification and characterization of a tungsten isoenzyme of formylmethanofuran dehydrogenase. *Arch Microbiol* **161**, 220–228.

Blaut, M. (1994). Metabolism of methanogens. *Antonie Leeuwenhoek* **66**, 187–208.

Bobik, T. A. & Wolfe, R. S. (1989). An unusual thiol-driven fumarate reductase in Methanobacterium with the production of the heterodisulfide of coenzyme M and *N*-(7-mercaptoheptanoyl)threonine-O^3-phosphate. *J Biol Chem* **264**, 18714–18718.

Bobik, T. A., Olson, K. D., Noll, K. M. & Wolfe, R. S. (1987). Evidence that the heterodisulfide of coenzyme M and 7-mercaptoheptanoylthreonine phosphate is a product of the methylreductase reaction in *Methanobacterium*. *Biochem Biophys Res Commun* **149**, 455–460.

Bokranz, M. & Klein, A. (1987). Nucleotide sequence of the methyl-coenzyme M reductase gene cluster from *Methanosarcina barkeri*. *Nucleic Acids Res* **15**, 4350–4351.

Bokranz, M., Bäumner, G., Allmansberger, R., Ankel-Fuchs, D. & Klein, A. (1988). Cloning and characterization of the methyl coenzyme M reductase genes from *Methanobacterium thermoautotrophicum*. *J Bacteriol* **170**, 568–577.

Bonacker, L. G., Baudner, S. & Thauer, R. K. (1992). Differential expression of the two methyl-coenzyme M reductases in *Methanobacterium thermoautotrophicum* as determined immunochemically via isoenzyme-specific antisera. *Eur J Biochem* **206**, 87–92.

Bonacker, L. G., Baudner, S., Mörschel, E., Böcher, R. & Thauer, R. K. (1993). Properties of the two isoenzymes of methyl-coenzyme M reductase in *Methanobacterium thermoautotrophicum*. *Eur J Biochem* **217**, 587–595.

Boone, D. R., Whitman, W. B. & Rouvière, P. (1993). Diversity and taxonomy of methanogens. In *Methanogenesis*, pp. 35–80. Edited by J. G. Ferry. New York & London: Chapman & Hall.

Braks, I. J., Hoppert, M., Roge, S. & Mayer, F. (1994). Structural aspects and immunolocalization of the F_{420}-reducing and non-F_{420}-reducing hydrogenases from *Methanobacterium thermoautotrophicum* Marburg. *J Bacteriol* **176**, 7677–7687.

Brenner, M. C., Zhang, H. & Scott, R. A. (1993). Nature of the low activity of S-methyl-coenzyme-M reductase as determined by active-site titrations. *J Biol Chem* **268**, 18491–18495.

Buckel, W. (1996). Unusual dehydrations in anaerobic bacteria: considering ketyls (radical anions) as reactive intermediates in enzymatic reactions. *FEBS Lett* **389**, 20–24.

Buckel, W. & Keese, R. (1995). One electron redox reactions of CoASH esters in anaerobic bacteria – a mechanistic proposal. *Angew Chem Int Ed Engl* **34**, 1502–1506.

Bult, C. J., White, O., Olsen, G. J. & 37 other authors (1996). Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. *Science* **273**, 1058–1073.

Burke, S. A. & Krzycki, J. A. (1995). Involvement of the "A" isozyme of methyltransferase II and the 29-kilodalton corrinoid protein in methanogenesis from monomethylamine. *J Bacteriol* **177**, 4410–4416.

Burke, S. A. & Krzycki, J. A. (1997). Reconstitution of monomethylamine:coenzyme M methyl transfer with a corrinoid protein and two methyltransferases purified from *Methanosarcina barkeri*. *J Biol Chem* **272**, 16570–16577.

Burke, S. A., Lo, S. L. & Krzycki, J. A. (1998). Clustered genes encoding the methyltransferases of methanogenesis from monomethylamine. *J Bacteriol* **180**, 3432–3440.

Burton, G. W., Dobu, T., Gabe, E. J., Hughes, I., Lee, F. L., Prasad, I. & Ingold, K. U. (1985). Autoxidation of biological molecules. 4. Maximizing the antioxidant activity of phenols. *J Am Chem Soc* **107**, 7053–7065.

Cammack, R. (1997). The enzyme at the end of the food chain. *Nature* **390**, 443–444.

Cammack, R. & van Vliet, P. (1998). Catalysis by nickel in biological systems. In *Bioinorganic Catalysis*, 2nd edn. Edited by J. Reedijk & E. Bouwman. New York: Marcel Dekker (in press).

Chen, W. & Konisky, J. (1993). Characterization of a membrane-associated ATPase from *Methanococcus voltae*, a methanogenic member of the Archaea. *J Bacteriol* **175**, 5677–5682.

Chistoserdova, L., Vorholt, J. A., Thauer, R. K. & Lidstrom, M. E. (1998). C_1 transfer enzymes and coenzymes linking methylotrophic bacteria and methanogenic archaea. *Science* **281**, 99–102.

Conrad, R. (1996). Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O and NO). *Microbiol Rev* **60**, 609–640.

Cram, D. S., Sherf, B. A., Libby, R. T., Mattaliano, R. J., Ramachandran, K. L. & Reeve, J. N. (1987). Structure and expression of the genes, *mcrBDCGA*, which encode the subunits of component C of methyl coenzyme M reductase in *Methanococcus vannielii*. *Proc Natl Acad Sci USA* **84**, 3992–3996.

Crane, B. R., Siegel, L. M. & Getzoff, E. D. (1995). Sulfite reductase structure at 1·6 Å: evolution and catalysis for reduction of inorganic anions. *Science* **270**, 59–67.

Crane, B. R., Siegel, L. M. & Getzoff, E. D. (1997a). Structures of the siroheme- and Fe-4S-4-containing active center of sulfite reductase in different states of oxidation: heme activation via

reduction-gated exogenous ligand exchange. *Biochemistry* **36**, 12101–12119.

Crane, B. R., Siegel, L. M. & Getzoff, E. D. (1997b). Probing the catalytic mechanism of sulfite reductase by X-ray crystallography: structures of the *Escherichia coli* hemoprotein in complex with substrates, inhibitors, intermediates, and products. *Biochemistry* **36**, 12120–12137.

Daas, P. J. H., Gerrits, K. A. A., Keltjens, J. T., van der Drift, C. & Vogels, G. D. (1993). Involvement of an activation protein in the methanol:2-mercaptoethanesulfonic acid methyltransferase reaction in *Methanosarcina barkeri*. *J Bacteriol* **175**, 1278–1283.

Daas, P. J. H., Hagen, W. R., Keltjens, J. T. & Vogels, G. D. (1994). Characterization and determination of the redox properties of the 2[4Fe–4S] ferredoxin from *Methanosarcina barkeri* strain MS. *FEBS Lett* **356**, 342–344.

Daas, P. J. H., Keltjens, J. T., Hagen, W. R. & van der Drift, C. (1995). The electrochemistry of 5-hydroxybenzimidazolylcobamide. *Arch Biochem Biophys* **319**, 244–249.

Daas, P. J. H., Hagen, W. R., Keltjens, J. T., van der Drift, C. & Vogels, G. D. (1996a). Activation mechanism of methanol:5-hydroxybenzimidazolylcobamide methyltransferase from *Methanosarcina barkeri*. *J Biol Chem* **271**, 22346–22351.

Daas, P. J. H., Wassenaar, R. W., Willemsen, P., Theunissen, R. J., Keltjens, J. T., van der Drift, C. & Vogels, G. D. (1996b). Purification and properties of an enzyme involved in the ATP-dependent activation of the methanol:2-mercaptoethanesulfonic acid methyltransferase reaction in *Methanosarcina barkeri*. *J Biol Chem* **271**, 22339–22345.

Daniels, L. (1993). Biochemistry of methanogenesis. In *The Biochemistry of Archaea (Archaebacteria)*, pp. 41–112. Edited by M. Kates, D. J. Kushner & A. T. Matheson. New York: Elsevier.

Deppenmeier, U. (1995). Different structure and expression of the operons encoding the membrane-bound hydrogenases from *Methanosarcina mazei* Gö1. *Arch Microbiol* **164**, 370–376.

Deppenmeier, U., Blaut, M., Mahlmann, A. & Gottschalk, G. (1990a). Reduced coenzyme F_{420}:heterodisulfide oxidoreductase, a proton-translocating redox system in methanogenic bacteria. *Proc Natl Acad Sci USA* **87**, 9449–9453.

Deppenmeier, U., Blaut, M., Mahlmann, A. & Gottschalk, G. (1990b). Membrane-bound $F_{420}H_2$-dependent heterodisulfide reductase in methanogenic bacterium strain Gö1 and *Methanolobus tindarius*. *FEBS Lett* **261**, 199–203.

Deppenmeier, U., Blaut, M. & Gottschalk, G. (1991). H_2:heterodisulfide oxidoreductase, a second energy-conserving system in the methanogenic strain Gö1. *Arch Microbiol* **155**, 272–277.

Deppenmeier, U., Blaut, M., Lentes, S., Herzberg, C. & Gottschalk, G. (1995). Analysis of the *vhoGAC* and *vhtGAC* operons from *Methanosarcina mazei* strain Gö1, both encoding a membrane-bound hydrogenase and a cytochrome *b*. *Eur J Biochem* **227**, 261–269.

Deppenmeier, U., Müller, V. & Gottschalk, G. (1996). Pathways of energy conservation in methanogenic archaea. *Arch Microbiol* **165**, 149–163.

Diekert, G., Klee, B. & Thauer, R. K. (1980a). Nickel, a component of factor F_{430} from *Methanobacterium thermoautotrophicum*. *Arch Microbiol* **124**, 103–106.

Diekert, G., Jaenchen, R. & Thauer, R. K. (1980b). Biosynthetic evidence for a nickel tetrapyrrole structure of factor F_{430} from *Methanobacterium thermoautotrophicum*. *FEBS Lett* **119**, 118–120.

Diekert, G., Konheiser, U., Piechulla, K. & Thauer, R. K. (1981). Nickel requirement and factor F_{430} content of methanogenic bacteria. *J Bacteriol* **148**, 459–464.

Eggen, R. I. L., Geerling, A. C. M., Boshoven, A. B. P. & de Vos, W. M. (1991a). Cloning, sequence analysis, and functional expression of the acetyl coenzyme A synthetase gene from *Methanothrix soehngenii* in *Escherichia coli*. *J Bacteriol* **173**, 6383–6389.

Eggen, R. I. L., Geerling, A. C. M., Jetten, M. S. M. & de Vos, W. M. (1991b). Cloning, expression, and sequence analysis of the genes for carbon monoxide dehydrogenase of *Methanothrix soehngenii*. *J Biol Chem* **266**, 6883–6887.

Eggen, R. I. L., van Kranenburg, R., Vriesema, A. J. M., Geerling, A. C. M., Verhagen, M. F. J. M., Hagen, W. R. & de Vos, W. M. (1996). Carbon monoxide dehydrogenase from *Methanosarcina frisia* Gö1. Characterization of the enzyme and the regulated expression of two operon-like cdh gene clusters. *J Biol Chem* **271**, 14256–14263.

Ellefson, W. L., Whitman, W. B. & Wolfe, R. S. (1982). Nickel-containing factor F_{430}: chromophore of the methylreductase of *Methanobacterium*. *Proc Natl Acad Sci USA* **79**, 3707–3710.

Ellermann, J., Hedderich, R., Böcher, R. & Thauer, R. K. (1988). The final step in methane formation: investigations with highly purified methyl-CoM reductase (component C) from *Methanobacterium thermoautotrophicum* (strain Marburg). *Eur J Biochem* **172**, 669–677.

Ellermann, J., Rospert, S., Thauer, R. K., Bokranz, M., Klein, A., Voges, M. & Berkessel, A. (1989). Methyl-coenzyme-M reductase from *Methanobacterium thermoautotrophicum* (strain Marburg): purity, activity and novel inhibitors. *Eur J Biochem* **184**, 63–68.

Elsden, S. R. (1981). Hydrogenase 1931–1981. *Trends Biochem Sci* **6**, 251–253.

Elsden, S. R. & Pirie, N. W. (1949). Obituary notice – Marjory Stephenson, 1885–1948. *J Gen Microbiol* **3**, 328–339.

Enßle, M., Zirngibl, C., Linder, D. & Thauer, R. K. (1991). Coenzyme F_{420} dependent N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenase in methanol grown *Methanosarcina barkeri*. *Arch Microbiol* **155**, 483–490.

Ermler, U., Merckel, M. C., Thauer, R. K. & Shima, S. (1997a). Formylmethanofuran:tetrahydromethanopterin formyltransferase from *Methanopyrus kandleri* – new insights into salt-dependence and thermostability. *Structure* **5**, 635–646.

Ermler, U., Grabarse, W., Shima, S., Goubeaud, M. & Thauer, R. K. (1997b). Crystal structure of methyl-coenzyme M reductase: the key enzyme of biological methane formation. *Science* **278**, 1457–1462.

Escalante-Semerena, J. C., Rinehart, K. L., Jr & Wolfe, R. S. (1984). Tetrahydromethanopterin, a carbon carrier in methanogenesis. *J Biol Chem* **259**, 9447–9455.

Eschenmoser, A. (1988). Vitamin B_{12}: Experimente zur Frage nach dem Ursprung seiner molekularen Struktur. *Angew Chemie* **100**, 5–40.

Färber, G., Keller, W., Kratky, C., Jaun, B., Pfaltz, A., Spinner, C., Kobelt, A. & Eschenmoser, A. (1991). Coenzyme F430 from methanogenic bacteria: complete assignment of configuration based on an X-ray analysis of 12,13-diepi-F430 pentamethyl ester and on NMR spectroscopy. *Helv Chim Acta* **74**, 697–716.

Ferguson, D. J., Jr & Krzycki, J. A. (1997). Reconstitution of trimethylamine-dependent coenzyme M methylation with the trimethylamine corrinoid protein and the isozymes of methyltransferase II from *Methanosarcina barkeri*. *J Bacteriol* **179**, 846–852.

Ferguson, D. J., Jr, Krzycki, J. A. & Grahame, D. A. (1996). Specific

roles of methylcobamide:coenzyme M methyltransferase isozymes in metabolism of methanol and methylamines in *Methanosarcina barkeri*. *J Biol Chem* **271**, 5189–5194.

Ferry, J. G. (1993a). *Methanogenesis: Ecology, Physiology, Biochemistry & Genetics*. New York & London: Chapman & Hall.

Ferry, J. G. (1993b). Fermentation of acetate. In *Methanogenesis*, pp. 304–334. Edited by J. G. Ferry. New York & London: Chapman & Hall.

Ferry, J. G. (1995). CO dehydrogenase. *Annu Rev Microbiol* **49**, 305–333.

Ferry, J. G. (1997a). Enzymology of the fermentation of acetate to methane by *Methanosarcina thermophila*. *BioFactors* **6**, 25–35.

Ferry, J. G. (1997b). Methane: small molecule, big impact. *Science* **278**, 1413–1414.

Fischer, R. & Thauer, R. K. (1989). Methyltetrahydromethanopterin as an intermediate in methanogenesis from acetate in *Methanosarcina barkeri*. *Arch Microbiol* **151**, 459–465.

Fischer, R. & Thauer, R. K. (1990). Ferredoxin-dependent methane formation from acetate in cell extracts of *Methanosarcina barkeri* (strain MS). *FEBS Lett* **269**, 368–372.

Fischer, R., Gärtner, P., Yeliseev, A. & Thauer, R. K. (1992). N^5-Methyltetrahydromethanopterin:coenzyme M methyltransferase in methanogenic archaebacteria is a membrane protein. *Arch Microbiol* **158**, 208–217.

Friedmann, H. C., Klein, A. & Thauer, R. K. (1991). Biochemistry of coenzyme F430, a nickel porphinoid involved in methanogenesis. In *Biosynthesis of Tetrapyrroles. New Comprehensive Biochemistry*, pp. 139–154. Edited by P. M. Jordan. Amsterdam: Elsevier.

Gärtner, P., Ecker, A., Fischer, R., Linder, D., Fuchs, G. & Thauer, R. K. (1993). Purification and properties of N^5-methyltetrahydromethanopterin:coenzyme M methyltransferase from *Methanobacterium thermoautotrophicum*. *Eur J Biochem* **213**, 537–545.

Gärtner, P., Weiss, D. S., Harms, U. & Thauer, R. K. (1994). N^5-methyltetrahydromethanopterin:coenzyme M methyltransferase from *Methanobacterium thermoautotrophicum*. Catalytic mechanism and sodium ion dependence. *Eur J Biochem* **226**, 465–472.

Gorris, L. G. M. & van der Drift, C. (1994). Cofactor contents of methanogenic bacteria reviewed. *BioFactors* **4**, 139–145.

Goubeaud, M., Schreiner, G. & Thauer, R. K. (1997). Purified methyl-coenzyme M reductase is activated when the enzyme-bound coenzyme F_{430} is reduced to the nickel(I) oxidation state by titanium(III) citrate. *Eur J Biochem* **243**, 110–114.

Graf, E. G. & Thauer, R. K. (1981). Hydrogenase from *Methanobacterium thermoautotrophicum*, a nickel-containing enzyme. *FEBS Lett* **136**, 165–169.

Grahame, D. A. (1989). Different isozymes of methylcobalamin:2-mercaptoethanesulfonate methyltransferase predominate in methanol- *versus* acetate-grown *Methanosarcina barkeri*. *J Biol Chem* **264**, 12890–12894.

Grahame, D. A. (1993). Substrate and cofactor reactivity of a carbon monoxide dehydrogenase–corrinoid enzyme complex: stepwise reduction of iron–sulfur and corrinoid centers, the corrinoid $Co^{2+/1+}$ redox midpoint potential, and overall synthesis of acetyl-CoA. *Biochemistry* **32**, 10786–10793.

Grahame, D. A. & DeMoll, E. (1995). Substrate and accessory protein requirements and thermodynamics of acetyl-CoA synthesis and cleavage in *Methanosarcina barkeri*. *Biochemistry* **34**, 4617–4624.

Grahame, D. A. & DeMoll, E. (1996). Partial reactions catalyzed by protein components of the acetyl-CoA decarbonylase synthase enzyme complex from *Methanosarcina barkeri*. *J Biol Chem* **271**, 8352–8358.

Grahame, D. A. & Stadtman, T. C. (1993). Redox enzymes of methanogens: physicochemical properties of selected, purified oxidoreductases. In *Methanogenesis*, pp. 334–359. Edited by J. G. Ferry. New York & London: Chapman & Hall.

Grahame, D. A., Khangulov, S. & DeMoll, E. (1996). Reactivity of a paramagnetic enzyme-CO adduct in acetyl-CoA synthesis and cleavage. *Biochemistry* **35**, 593–600.

Gunsalus, R. P. & Wolfe, R. S. (1978). Chromophoric factors F_{342} and F_{430} of *Methanobacterium thermoautotrophicum*. *FEMS Microbiol Lett* **3**, 191–193.

Gunsalus, R. P. & Wolfe, R. S. (1980). Methyl coenzyme M reductase from *Methanobacterium thermoautotrophicum*. Resolution and properties of the components. *J Biol Chem* **255**, 1891–1895.

Gunsalus, R. P., Romesser, J. A. & Wolfe, R. S. (1978). Preparation of coenzyme M analogues and their activity in the methyl coenzyme M reductase system of *Methanobacterium thermoautotrophicum*. *Biochemistry* **17**, 2374–2377.

Haase, P., Deppenmeier, U., Blaut, M. & Gottschalk, G. (1992). Purification and characterization of $F_{420}H_2$-dehydrogenase from *Methanolobus tindarius*. *Eur J Biochem* **203**, 527–531.

Hammel, K. E., Cornwell, K. L., Diekert, G. B. & Thauer, R. K. (1984). Evidence for a nickel-containing carbon monoxide dehydrogenase in *Methanobrevibacter arboriphilicus*. *J Bacteriol* **157**, 975–978.

Harms, U. & Thauer, R. K. (1996a). The corrinoid-containing 23-kDa subunit MtrA of the energy-conserving N^5-methyltetrahydromethanopterin:coenzyme M methyltransferase complex from *Methanobacterium thermoautotrophicum*. EPR spectroscopic evidence for a histidine residue as a cobalt ligand of the cobamide. *Eur J Biochem* **241**, 149–154.

Harms, U. & Thauer, R. K. (1996b). Methylcobalamin:coenzyme M methyltransferase isoenzymes MtaA and MtbA from *Methanosarcina barkeri*. Cloning, sequencing and differential transcription of the encoding genes, and functional overexpression of the *mtaA* gene in *Escherichia coli*. *Eur J Biochem* **235**, 653–659.

Harms, U. & Thauer, R. K. (1997). Identification of the active site histidine in the corrinoid protein MtrA of the energy-conserving methyltransferase complex from *Methanobacterium thermoautotrophicum*. *Eur J Biochem* **250**, 783–788.

Harms, U. & Thauer, R. K. (1998). EPR spectroscopic evidence that in the energy-conserving methyltransferase complex from methanogenic Archaea a histidine residue is ligated to the cobamide-cobalt. In *Vitamin B_{12} and B_{12}-Proteins*, pp. 157–165. Edited by B. Kräutler, D. Arigoni & B. T. Golding. Lectures presented at the 4th European Symposium on Vitamin B_{12} and B_{12}-Proteins. Weinheim: Wiley-VCH.

Harms, U., Weiss, D., Gärtner, P., Linder, D. & Thauer, R. K. (1995). The energy conserving N^5-methyltetrahydromethanopterin:coenzyme M methyltransferase complex from *Methanobacterium thermoautotrophicum* is composed of eight different subunits. *Eur J Biochem* **228**, 640–648.

Hartmann, G. C., Klein, A. R., Linder, M. & Thauer, R. K. (1996a). Purification, properties and primary structure of H_2-forming N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenase from *Methanococcus thermolithotrophicus*. *Arch Microbiol* **165**, 187–193.

Hartmann, G. C., Santamaria, E., Fernández, V. M. & Thauer, R. K. (1996b). Studies on the catalytic mechanism of H_2-forming methylenetetrahydromethanopterin dehydrogenase: *para-ortho*

H_2 conversion rates in H_2O and D_2O. *J Biol Inorg Chem* **1**, 446–450.

Hartzell, P. L. & Wolfe, R. S. (1986). Requirement of the nickel tetrapyrrole F_{430} for *in vitro* methanogenesis: reconstitution of methylreductase component C from its dissociated subunits. *Proc Natl Acad Sci USA* **83**, 6726–6730.

Hartzell, P. L., Donnelly, M. I. & Wolfe, R. S. (1987). Incorporation of coenzyme M into component C of methyl-coenzyme M methylreductase during *in vitro* methanogenesis. *J Biol Chem* **262**, 5581–5586.

Hedderich, R. & Thauer, R. K. (1988). *Methanobacterium thermoautotrophicum* contains a soluble enzyme system that specifically catalyzes the reduction of the heterodisulfide of coenzyme M and 7-mercaptoheptanoylthreonine phosphate with H_2. *FEBS Lett* **234**, 223–227.

Hedderich, R., Berkessel, A. & Thauer, R. K. (1990). Purification and properties of heterodisulfide reductase from *Methanobacterium thermoautotrophicum* (strain Marburg). *Eur J Biochem* **193**, 255–261.

Hedderich, R., Albracht, S. P. J., Linder, D., Koch, J. & Thauer, R. K. (1992). Isolation and characterization of polyferredoxin from *Methanobacterium thermoautotrophicum*. The *mvhB* gene product of the methylviologen-reducing hydrogenase operon. *FEBS Lett* **298**, 65–68.

Hedderich, R., Koch, J., Linder, D. & Thauer, R. K. (1994). The heterodisulfide reductase from *Methanobacterium thermoautotrophicum* contains sequence motifs characteristic of pyridine-nucleotide-dependent thioredoxin reductases. *Eur J Biochem* **225**, 253–261.

Heiden, S., Hedderich, R., Setzke, E. & Thauer, R. K. (1993). Purification of a cytochrome *b* containing H_2:heterodisulfide oxidoreductase complex from membranes of *Methanosarcina barkeri*. *Eur J Biochem* **213**, 529–535.

Heiden, S., Hedderich, R., Setzke, E. & Thauer, R. K. (1994). Purification of a two-subunit cytochrome-*b*-containing heterodisulfide reductase from methanol-grown *Methanosarcina barkeri*. *Eur J Biochem* **221**, 855–861.

Heim, S., Künkel, A., Thauer, R. K. & Hedderich, R. (1998). Thiol:fumarate reductase (Tfr) from *Methanobacterium thermoautotrophicum*: identification of the catalytic sites for fumarate reduction and thiol oxidation. *Eur J Biochem* **253**, 292–299.

Hochheimer, A., Schmitz, R. A., Thauer, R. K. & Hedderich, R. (1995). The tungsten formylmethanofuran dehydrogenase from *Methanobacterium thermoautotrophicum* contains sequence motifs characteristic for enzymes containing molybdopterin dinucleotide. *Eur J Biochem* **234**, 910–920.

Hochheimer, A., Linder, D., Thauer, R. K. & Hedderich, R. (1996). The molybdenum formylmethanofuran dehydrogenase operon and the tungsten formylmethanofuran dehydrogenase operon from *Methanobacterium thermoautotrophicum*: structures and transcriptional regulation. *Eur J Biochem* **242**, 156–162.

Holliger, C., Pierik, A. J., Reijerse, E. J. & Hagen, W. R. (1993). A spectroelectrochemical study of factor F_{430} nickel(II/I) from methanogenic bacteria in aqueous solution. *J Am Chem Soc* **115**, 5651–5656.

Holm, L. & Sander, C. (1997). An evolutionary treasure: unification of a broad set of amidohydrolases related to urease. *Proteins Struct Funct Genet* **28**, 72–82.

Howell, D. M., Harich, K., Xu, H. & White, R. H. (1998). The α-keto acid chain elongation reactions involved in the biosynthesis of 7-mercaptoheptanoylthreonine-phosphate in methanogenic archaea. *Biochemistry* (in press).

Hungerer, C., Weiss, D. S., Thauer, R. K. & Jahn, D. (1996). The *hemA* gene encoding glutamyl-tRNA reductase from the archaeon *Methanobacterium thermoautotrophicum* strain Marburg. *Bioorg Med Chem* **4**, 1089–1095.

Inatomi, K. I. (1996). ATP-dependent H^+-pump activity in inverted vesicles of *Methanosarcina mazei* Gö1 and characterization of membrane ATPase. *J Bacteriol* **178**, 2424–2426.

Ito, N., Phillips, S. E. V., Stevens, C., Ogel, Z. B., McPherson, M. J., Keen, J. N., Yadav, K. D. S. & Knowles, P. F. (1991). Novel thioether bond revealed by a 1·7 Å crystal structure of galactose oxidase. *Nature* **350**, 87–90.

Jäger, E.-G. & Rudolph, M. (1997). Cyclic voltammetric and impedance spectrometric investigations on addition/elimination reactions of Lewis bases accompanying the electrode reactions of a nickel chelate complex with a structural resemblance to the coenzyme F430. *J Electroanal Chem* **434**, 1–18.

Jaun, B. (1990). Coenzyme F430 from methanogenic bacteria: oxidation of F430 pentamethyl ester to the Ni(III) form. *Helv Chim Acta* **73**, 2209–2217.

Jaun, B. (1993). Methane formation by methanogenic bacteria: redox chemistry of coenzyme F430. In *Metal Ions in Biological Systems, vol 29, Properties of Metal Alkyl Derivatives*, pp. 287–337. Edited by H. Sigel & A. Sigel. New York: Marcel Dekker.

Jaun, B. & Pfaltz, A. (1986). Coenzyme F430 from methanogenic bacteria: reversible one-electron reduction of F430 pentamethyl ester to the nickel(I) form. *J Chem Soc Chem Commun*, 1327–1329.

Jaun, B. & Pfaltz, A. (1988). Coenzyme F430 from methanogenic bacteria: methane formation by reductive carbon-sulphur bond cleavage of methyl sulphonium ions catalysed by F430 pentamethyl ester. *J Chem Soc Chem Commun*, 293–294.

Kaesler, B. & Schönheit, P. (1989a). The role of sodium ions in methanogenesis. Formaldehyde oxidation to CO_2 and $2H_2$ in methanogenic bacteria is coupled with primary electrogenic Na^+ translocation at a stoichiometry of 2–3 Na^+/CO_2. *Eur J Biochem* **184**, 223–232.

Kaesler, B. & Schönheit, P. (1989b). The sodium cycle in methanogenesis. CO_2 reduction to the formaldehyde level in methanogenic bacteria is driven by a primary electrochemical potential of Na^+ generated by formaldehyde reduction to CH_4. *Eur J Biochem* **186**, 309–316.

Kamp, A. F., la Rivière, J. W. M. & Verhoeven, W. (1959). *Albert Jan Kluyver, His Life and Work*. Amsterdam: North-Holland Publishing.

Kandler, O. & König, H. (1998). Cell wall polymers in Archaea (Archaebacteria). *Cell Mol Life Sci* **54**, 305–308.

Keltjens, J. T. & Vogels, G. D. (1993). Conversion of methanol and methylamines to methane and carbon dioxide. In *Methanogenesis*, pp. 253–303. Edited by J. G. Ferry. New York & London: Chapman & Hall.

Kemner, J. M. & Zeikus, J. G. (1994a). Purification and characterization of membrane-bound hydrogenase from *Methanosarcina barkeri* MS. *Arch Microbiol* **161**, 47–54.

Kemner, J. M. & Zeikus, J. G. (1994b). Regulation and function of ferredoxin-linked versus cytochrome *b*-linked hydrogenase in electron transfer and energy metabolism of *Methanosarcina barkeri* MS. *Arch Microbiol* **162**, 26–32.

Klein, A. R. & Thauer, R. K. (1995). *Re*-face specificity at C14a of methylenetetrahydromethanopterin and *Si*-face specificity at C5 of coenzyme F_{420} for coenzyme F_{420}-dependent methylene-

tetrahydromethanopterin dehydrogenase from methanogenic Archaea. *Eur J Biochem* **227**, 169–174.

Klein, A. R. & Thauer, R. K. (1997). Overexpression of the coenzyme F_{420}-dependent N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenase gene from the hyperthermophilic *Methanopyrus kandleri*. *Eur J Biochem* **245**, 386–391.

Klein, A. R., Breitung, J., Linder, D., Stetter, K. O. & Thauer, R. K. (1993a). N^5,N^{10}-Methenyltetrahydromethanopterin cyclohydrolase from the extremely thermophilic sulfate reducing *Archaeoglobus fulgidus*: comparison of its properties with those of the cyclohydrolase from the extremely thermophilic *Methanopyrus kandleri*. *Arch Microbiol* **159**, 213–219.

Klein, A. R., Koch, J., Stetter, K. O. & Thauer, R. K. (1993b). Two N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenases in the extreme thermophile *Methanopyrus kandleri*: characterization of the coenzyme F_{420}-dependent enzyme. *Arch Microbiol* **160**, 186–192.

Klein, A. R., Fernandez, V. M. & Thauer, R. K. (1995a). H_2-forming N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenase: mechanism of H_2 formation analyzed using hydrogen isotopes. *FEBS Lett* **368**, 203–206.

Klein, A. R., Hartmann, G. C. & Thauer, R. K. (1995b). Hydrogen isotope effects in the reactions catalyzed by H_2-forming N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenase from methanogenic Archaea. *Eur J Biochem* **233**, 372–376.

Klein, A. R., Berk, H., Purwantini, E., Daniels, L. & Thauer, R. K. (1996). *Si*-face stereospecificity at C5 of coenzyme F_{420} for F_{420}-dependent glucose-6-phosphate dehydrogenase from *Mycobacterium smegmatis* and F_{420}-dependent alcohol dehydrogenase from *Methanoculleus thermophilicus*. *Eur J Biochem* **239**, 93–97.

Klenk, H.-P., Clayton, R. A., Tomb, J.-F. & 48 other authors (1997). The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. *Nature* **390**, 364–370.

Knight, M., Wolfe, R. S. & Elsden, S. R. (1966). The synthesis of amino acids by *Methanobacterium omelianskii*. *Biochem J* **99**, 76–86.

Kräutler, B., Arigoni, D. & Golding, B. T. (1998). *Vitamin B_{12} and B_{12}-Proteins*. Weinheim: Wiley-VCH.

Kuhner, C. H., Lindenbach, B. D. & Wolfe, R. S. (1993). Component A2 of methylcoenzyme M reductase system from *Methanobacterium thermoautotrophicum* ΔH: nucleotide sequence and functional expression by *Escherichia coli*. *J Bacteriol* **175**, 3195–3203.

Kumazawa, Y., Fujiwara, T., Fukumori, Y., Koga, Y. & Yamanaka, T. (1994). Cytochrome *bc* purified from the methanogen *Methanosarcina barkeri*. *Curr Microbiol* **29**, 53–56.

Künkel, A., Vaupel, M., Heim, S., Thauer, R. K. & Hedderich, R. (1997). Heterodisulfide reductase from methanol-grown cells of *Methanosarcina barkeri* is not a flavoenzyme. *Eur J Biochem* **244**, 226–234.

Künkel, A., Vorholt, J. A., Thauer, R. K. & Hedderich, R. (1998). An *E. coli* hydrogenase 3 type hydrogenase in methanogenic archaea. *Eur J Biochem* **252**, 467–476.

Kunow, J., Schwörer, B., Setzke, E. & Thauer, R. K. (1993). *Si*-face stereospecificity at C5 of coenzyme F_{420} for F_{420}-dependent N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenase, F_{420}-dependent N^5,N^{10}-methylenetetrahydromethanopterin reductase and $F_{420}H_2$:dimethylnaphthoquinone oxidoreductase. *Eur J Biochem* **214**, 641–646.

Kunow, J., Linder, D., Stetter, K. O. & Thauer, R. K. (1994). $F_{420}H_2$:quinone oxidoreductase from *Archaeoglobus fulgidus*: characterization of a membrane-bound multisubunit complex containing FAD and iron–sulfur clusters. *Eur J Biochem* **223**, 503–511.

Kunow, J., Linder, D. & Thauer, R. K. (1995). Pyruvate:ferredoxin oxidoreductase from the sulfate-reducing *Archaeoglobus fulgidus*: molecular composition, catalytic properties and sequence alignments. *Arch Microbiol* **163**, 21–28.

Kunow, J., Shima, S., Vorholt, J. A. & Thauer, R. K. (1996). Primary structure and properties of the formyltransferase from the mesophilic *Methanosarcina barkeri*: comparison with the enzymes from thermophilic and hyperthermophilic methanogens. *Arch Microbiol* **165**, 97–105.

Latimer, M. T. & Ferry, J. G. (1993). Cloning, sequence analysis, and hyperexpression of the genes encoding phosphotransacetylase and acetate kinase from *Methanosarcina thermophila*. *J Bacteriol* **175**, 6822–6829.

LeClerc, G. M. & Grahame, D. A. (1996). Methylcobamide:coenzyme M methyltransferase isozymes from *Methanosarcina barkeri*: physicochemical characterization, cloning, sequence analysis and heterologous gene expression. *J Biol Chem* **271**, 18725–18731.

Lehmacher, A. (1994). Cloning, sequencing and transcript analysis of the gene encoding formylmethanofuran:tetrahydromethanopterin formyltransferase from the hyperthermophilic *Methanothermus fervidus*. *Mol Gen Genet* **242**, 73–80.

Lehmacher, A. & Klenk, H. P. (1994). Characterization and phylogeny of *mcr*II, a gene cluster encoding an isoenzyme of methyl coenzyme M reductase from hyperthermophilic *Methanothermus fervidus*. *Mol Gen Genet* **243**, 198–206.

Lenz, R. & Giese, B. (1997). Studies on the mechanism of ribonucleotide reductases. *J Am Chem Soc* **119**, 2784–2794.

Lexa, D. & Saveant, J.-M. (1983). The electrochemistry of vitamin B_{12}. *Acc Chem Res* **16**, 235–243.

Lienard, T. & Gottschalk, G. (1998). Cloning, sequencing and expression of the genes encoding the sodium translocating N^5-methyltetrahydromethanopterin:coenzyme M methyltransferase of the methylotrophic archaeon *Methanosarcina mazei* Gö1. *FEBS Lett* **425**, 204–208.

Lienard, T., Becher, B., Marschall, M., Bowien, S. & Gottschalk, G. (1996). Sodium ion translocation by N^5-methyltetrahydromethanopterin:coenzyme M methyltransferase from *Methanosarcina mazei* Gö1 reconstituted in ether lipid liposomes. *Eur J Biochem* **239**, 857–864.

Lin, S.-K. & Jaun, B. (1991). Coenzyme F430 from methanogenic bacteria: detection of a paramagnetic methylnickel(II) derivative of the pentamethyl ester by ^{2}H-NMR spectroscopy. *Helv Chim Acta* **74**, 1725–1738.

Lin, S.-K. & Jaun, B. (1992). Coenzyme F430 from methanogenic bacteria: mechanistic studies on the reductive cleavage of sulfonium ions catalyzed by F430 pentamethyl ester. *Helv Chem Acta* **75**, 1478–1490.

Lovley, D. R., White, R. H. & Ferry, J. G. (1984). Identification of methyl coenzyme M as an intermediate in methanogenesis from acetate in *Methanosarcina* spp. *J Bacteriol* **160**, 521–525.

Lu, W.-P., Jablonski, P. E., Rasche, M., Ferry, J. G. & Ragsdale, S. W. (1994). Characterization of the metal centers of the Ni/Fe-S component of the carbon-monoxide dehydrogenase enzyme complex from *Methanosarcina thermophila*. *J Biol Chem* **269**, 9736–9742.

Lu, W.-P., Becher, B., Gottschalk, G. & Ragsdale, S. W. (1995). Electron paramagnetic resonance spectroscopic and electrochemical characterization of the partially purified N^5-methyl-

tetrahydromethanopterin:coenzyme M methyltransferase from *Methanosarcina mazei* Gö1. *J Bacteriol* **177**, 2245–2250.

Ma, K. & Thauer, R. K. (1990). N^5,N^{10}-Methylenetetrahydromethanopterin reductase from *Methanosarcina barkeri*. *FEMS Microbiol Lett* **70**, 119–124.

Mancia, F., Keep, N. H., Nakagawa, A., Leadlay, P. F., McSweeney, S., Rasmussen, B., Bösecke, P., Diat, O. & Evans, P. R. (1996). How coenzyme B_{12} radicals are generated: the crystal structure of methylmalonyl-coenzyme A mutase at 2 Å resolution. *Structure* **4**, 339–350.

Mansel, V. & Klar, G. (1992). Organometalloidal compounds with *o*-phenylene substituents. Part 25. Crystal and molecular structures of 2,3,7,8-tetrahydroxythianthrene. *J Chem Res (Suppl.)* 158–159.

Maroney, M. J., Davidson, G., Allan, C. B. & Figlar, J. (1998). The structure and function of nickel sites in metalloproteins. *Struct Bonding* **92**, 1–66.

Martin, W. & Müller, M. (1998). The hydrogen hypothesis for the first eukaryote. *Nature* **392**, 37–41.

Mason, J. (1992). The admission of the first women to the Royal Society of London. *Notes Rec R Soc Lond* **46**, 279–300.

Mason, J. (1996). Marjory Stephenson 1885–1948. In *Cambridge Women: Twelve Portraits*, pp. 113–135. Cambridge: Cambridge University Press.

Maupin-Furlow, J. & Ferry, J. G. (1996a). Analysis of the CO dehydrogenase/acetyl-coenzyme A synthase operon of *Methanosarcina thermophila*. *J Bacteriol* **178**, 6849–6856.

Maupin-Furlow, J. & Ferry, J. G. (1996b). Characterization of the *cdhD* and *cdhE* genes encoding subunits of the corrinoid/iron–sulfur enzyme of the CO dehydrogenase complex from *Methanosarcina thermophila*. *J Bacteriol* **178**, 340–346.

Metcalf, W. W., Zhang, J. K., Apolinario, E., Sowers, K. R. & Wolfe, R. S. (1997). A genetic system for archaea of the genus *Methanosarcina*: liposome-mediated transformation and construction of shuttle vectors. *Proc Natl Acad Sci USA* **94**, 2626–2631.

Morgan, R. M., Pihl, T. D., Nölling, J. & Reeve, J. N. (1997). Hydrogen regulation of growth, growth yields, and methane gene transcription in *Methanobacterium thermoautotrophicum* ΔH. *J Bacteriol* **179**, 889–898.

Mukhopadhyay, B., Purwantini, E., Pihl, T. D., Reeve, J. N. & Daniels, L. (1995). Cloning, sequencing, and transcriptional analysis of the coenzyme F_{420}-dependent methylene-5,6,7,8-tetrahydromethanopterin dehydrogenase gene from *Methanobacterium thermoautotrophicum* strain Marburg and functional expression in *Escherichia coli*. *J Biol Chem* **270**, 2827–2832.

Müller, V. & Gottschalk, G. (1994). The sodium ion cycle in acetogenic and methanogenic bacteria: generation and utilization of a primary electrochemical sodium ion gradient. In *Acetogenesis*, pp. 127–156. Edited by H. L. Drake. New York & London: Chapman & Hall.

Noll, K. M. & Wolfe, R. S. (1986). Component C of the methylcoenzyme M methylreductase system contains bound 7-mercaptoheptanoyl phosphate (HS-HTP). *Biochem Biophys Res Commun* **139**, 889–895.

Noll, K. M., Rinehart, K. L., Jr, Tanner, R. S. & Wolfe, R. S. (1986). Structure of component B (7-mercaptoheptanoylthreonine phosphate) of the methyl-coenzyme M methylreductase system of *Methanobacterium thermoautotrophicum*. *Proc Natl Acad Sci USA* **83**, 4238–4242.

Nölling, J. & Reeve, J. N. (1997). Growth- and substrate-dependent transcription of the formate dehydrogenase (*fdhCAB*) operon in *Methanobacterium thermoformicicum* Z-245. *J Bacteriol* **179**, 899–908.

Nölling, J., Pihl, T. D. & Reeve, J. N. (1995a). Cloning, sequencing, and growth phase-dependent transcription of the coenzyme F_{420}-dependent N^5,N^{10}-methylenetetrahydromethanopterin reductase-encoding genes from *Methanobacterium thermoautotrophicum* ΔH and *Methanopyrus kandleri*. *J Bacteriol* **177**, 7238–7244.

Nölling, J., Pihl, T. D., Vriesema, A. & Reeve, J. N. (1995b). Organization and growth phase-dependent transcription of methane genes in two regions of the *Methanobacterium thermoautotrophicum* genome. *J Bacteriol* **177**, 2460–2468.

Nölling, J., Ishii, M., Koch, J., Pihl, T. D., Reeve, J. N., Thauer, R. K. & Hedderich, R. (1995c). Characterization of a 45-kDa flavoprotein and evidence for a rubredoxin, two proteins that could participate in electron transport from H_2 to CO_2 in methanogenesis in *Methanobacterium thermoautotrophicum*. *Eur J Biochem* **231**, 628–638.

Nölling, J., Elfner, A., Palmer, J. R., Steigerwald, V. J., Pihl, T. D., Lake, J. A. & Reeve, J. N. (1996). Phylogeny of *Methanopyrus kandleri* based on methyl-coenzyme M reductase operons. *Int J Syst Bacteriol* **46**, 1170–1173.

Olson, K. D., Chmurkowska-Cichowlas, L., McMahon, C. W. & Wolfe, R. S. (1992). Structural modifications and kinetic studies of the substrates involved in the final step of methane formation in *Methanobacterium thermoautotrophicum*. *J Bacteriol* **174**, 1007–1012.

Pandiyan, T., Bernés, S. & de Bazúa, C. D. (1997). Structure, spectra and redox studies of nickel(II) bis(benzimidazolyle-2-ylmethyl)amines with coenzyme M reductase. *Polyhedron* **16**, 2819–2826.

Paul, L. & Krzycki, J. A. (1996). Sequence and transcript analysis of a novel *Methanosarcina barkeri* methyltransferase II homolog and its associated corrinoid protein homologous to methionine synthase. *J Bacteriol* **178**, 6599–6607.

Peer, C. W., Painter, M. H., Rasche, M. E. & Ferry, J. G. (1994). Characterization of a CO:heterodisulfide oxidoreductase system from acetate-grown *Methanosarcina thermophila*. *J Bacteriol* **176**, 6974–6979.

Peinemann, S., Hedderich, R., Blaut, M., Thauer, R. K. & Gottschalk, G. (1990). ATP synthesis coupled to electron transfer from H_2 to the heterodisulfide of 2-mercaptoethanesulfonate and 7-mercaptoheptanoylthreonine phosphate in vesicle preparations of the methanogenic bacterium strain Gö1. *FEBS Lett* **263**, 57–60.

Pennings, J. L. A., de Wijs, J. L. J., Keltjens, J. T. & van der Drift, C. (1997). Medium-reductant directed expression of methyl coenzyme M reductase isoenzymes in *Methanobacterium thermoautotrophicum* (strain ΔH). *FEBS Lett* **410**, 235–237.

Pfaltz, A., Jaun, B., Fässler, A., Eschenmoser, A., Jaenchen, R., Gilles, H. H., Diekert, G. & Thauer, R. K. (1982). Zur Kenntnis des Faktors F430 aus methanogenen Bakterien: Struktur des porphinoiden Ligandsystems. *Helv Chim Acta* **65**, 828–865.

Picot, D., Loll, P. J. & Garavito, R. M. (1994). The X-ray crystal structure of the membrane protein prostaglandin H_2 synthase-1. *Nature* **367**, 243–249.

Pihl, T. D., Sharma, S. & Reeve, J. N. (1994). Growth phase-dependent transcription of the genes that encode the two methyl coenzyme M reductase isoenzymes and N^5-methyltetrahydromethanopterin:coenzyme M methyltransferase in *Methanobacterium thermoautotrophicum* ΔH. *J Bacteriol* **176**, 6384–6391.

Postgate, J. (1995). *Society for General Microbiology: Fifty Years On*. Special Publication of the Society for General Microbiology.

Reeve, J. N., Beckler, G. S., Cram, D. S. & 7 other authors (1989).

A hydrogenase-linked gene in *Methanobacterium thermoautotrophicum* strain ΔH encodes a polyferredoxin. *Proc Natl Acad Sci USA* **86**, 3031–3035.

Reeve, J. N., Nölling, J., Morgan, R. M. & Smith, D. R. (1997). Methanogenesis: genes, genomes, and who's on first. *J Bacteriol* **179**, 5975–5986.

Roach, P. L., Clifton, I. J., Hensgens, C. M. H., Shibata, N., Schofield, C. J., Hajdu, J. & Baldwin, J. E. (1997). Structure of isopenicillin *N* synthase complexed with substrate and mechanism of penicillin formation. *Nature* **387**, 827–830.

Robertson, M. (1949). Marjory Stephenson (1885–1948). *Obituary Notices of Fellows of the Royal Society* **6**, 563–575.

Rospert, S. (1991). *ESR-Eigenschaften der Methyl-CoM-Reduktase aus Methanobacterium thermoautotrophicum und ihre Korrelation zur Enzymtätigkeit.* Dissertation, Philipps-Universität Marburg.

Rospert, S., Linder, D., Ellermann, J. & Thauer, R. K. (1990). Two genetically distinct methyl-coenzyme M reductases in *Methanobacterium thermoautotrophicum* strain Marburg and ΔH. *Eur J Biochem* **194**, 871–877.

Rospert, S., Böcher, R., Albracht, S. P. J. & Thauer, R. K. (1991). Methyl-coenzyme M reductase preparations with high specific activity from H_2-preincubated cells of *Methanobacterium thermoautotrophicum*. *FEBS Lett* **291**, 371–375.

Rospert, S., Voges, M., Berkessel, A., Albracht, S. P. J. & Thauer, R. K. (1992). Substrate-analogue-induced changes in the nickel EPR spectrum of active methyl-coenzyme-M reductase from *Methanobacterium thermoautotrophicum*. *Eur J Biochem* **210**, 101–107.

Ruppert, C., Wimmers, S., Lemker, T. & Müller, V. (1998). The A_1A_0 ATPase from *Methanosarcina mazei*: cloning of the 5′ end of the *aha* operon encoding the membrane domain and expression of the proteolipid in a membrane-bound form in *Escherichia coli*. *J Bacteriol* **180**, 3448–3452.

Sauer, F. D. (1991). Inhibition of methylcoenzyme M methylreductase by a uridine 5′-diphospho-*N*-acetylglucosamine derivative. *Biochem Biophys Res Commun* **174**, 619–624.

Sauer, F. D., Blackwell, B. A., Kramer, J. K. G. & Marsden, B. J. (1990). Structure of a novel cofactor containing *N*-(7-mercaptoheptanoyl)-*O*-3-phosphothreonine. *Biochemistry* **29**, 7593–7600.

Sauer, K. & Thauer, R. K. (1997). Methanol:coenzyme M methyltransferase from *Methanosarcina barkeri*. Zinc dependence and thermodynamics of the methanol:cob(I)alamin methyltransferase reaction. *Eur J Biochem* **249**, 280–285.

Sauer, K. & Thauer, R. K. (1998). Methanol:coenzyme M methyltransferase from *Methanosarcina barkeri*: identification of the active-site histidine in the corrinoid-harboring subunit MtaC by site-directed mutagenesis. *Eur J Biochem* **253**, 698–705.

Sauer, K., Harms, U. & Thauer, R. K. (1997). Methanol:coenzyme M methyltransferase from *Methanosarcina barkeri*: purification, properties and encoding genes of the corrinoid protein MT1. *Eur J Biochem* **243**, 670–677.

Sauter, M., Böhm, R. & Böck, A. (1992). Mutational analysis of the operon (*hyc*) determining hydrogenase 3 formation in *Escherichia coli*. *Mol Microbiol* **6**, 1523–1532.

Schauder, R. & Kröger, A. (1993). Bacterial sulphur respiration. *Arch Microbiol* **159**, 491–497.

Schleucher, J., Griesinger, C., Schwörer, B. & Thauer, R. K. (1994). H_2-forming N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenase from *Methanobacterium thermoautotrophicum* catalyzes a stereoselective hydride transfer as determined by two-dimensional NMR spectroscopy. *Biochemistry* **33**, 3986–3993.

Schleucher, J., Schwörer, B., Thauer, R. K. & Griesinger, C. (1995). Elucidation of the stereochemical course of chemical reactions by magnetic labeling. *J Am Chem Soc* **117**, 2941–2942.

Schmid, C. L., Kempf, C., Taubert, A., Neuburger, M., Zehnder, M., Kaden, T. A., Bujno, K. & Bilewicz, R. (1996). Metal complexes with macrocyclic ligands. Part XLII. Tetraazamacrocyclic nickel(III) complexes with a methylthio or a methoxy pendant chain as model for cofactor F430. *Helv Chim Acta* **79**, 1011–1020.

Schmid, C. L., Neuburger, M., Zehnder, M., Kaden, T. A., Bujno, K. & Bilewicz, R. (1997). Metal complexes with macrocyclic ligands. Part XLIII. Tetraazamacrocyclic nickel(II) and copper(II) complexes with aliphatic methylthio- and methoxy-substituted pendant chains. *Helv Chim Acta* **80**, 241–252.

Schmitz, R. A., Bertram, P. A. & Thauer, R. K. (1994). Tungstate does not support synthesis of active formylmethanofuran dehydrogenase in *Methanosarcina barkeri*. *Arch Microbiol* **161**, 528–530.

Schönheit, P. & Beimborn, D. B. (1985). Presence of a Na^+/H^+ antiporter in *Methanobacterium thermoautotrophicum* and its role in Na^+ dependent methanogenesis. *Arch Microbiol* **142**, 354–361.

Schönheit, P., Moll, J. & Thauer, R. K. (1979). Nickel, cobalt, and molybdenum requirement for growth of *Methanobacterium thermoautotrophicum*. *Arch Microbiol* **123**, 105–107.

Schönheit, P., Moll, J. & Thauer, R. K. (1980). Growth parameters (K_2, μ_{max}, Y_S) of *Methanobacterium thermoautotrophicum*. *Arch Microbiol* **127**, 59–65.

Schwörer, B., Breitung, J., Klein, A. R., Stetter, K. O. & Thauer, R. K. (1993a). Formylmethanofuran:tetrahydromethanopterin formyltransferase and N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenase from the sulfate-reducing *Archaeoglobus fulgidus*: similarities with the enzymes from methanogenic Archaea. *Arch Microbiol* **159**, 225–232.

Schwörer, B., Fernandez, V. M., Zirngibl, C. & Thauer, R. K. (1993b). H_2-forming N^5,N^{10}-methylenetetrahydromethanopterin dehydrogenase from *Methanobacterium thermoautotrophicum*. Studies of the catalytic mechanism of the H_2 formation with hydrogen isotopes. *Eur J Biochem* **212**, 255–261.

Setzke, E., Hedderich, R., Heiden, S. & Thauer, R. K. (1994). H_2:heterodisulfide oxidoreductase complex from *Methanobacterium thermoautotrophicum*: composition and properties. *Eur J Biochem* **220**, 139–148.

Shapiro, S. & Wolfe, R. S. (1980). Methyl-coenzyme M, an intermediate in methanogenic dissimilation of C_1 compounds by *Methanosarcina barkeri*. *J Bacteriol* **141**, 728–734.

Sherf, B. A. & Reeve, J. N. (1990). Identification of the *mcrD* gene product and its association with component C of methyl coenzyme M reductase in *Methanococcus vannielii*. *J Bacteriol* **172**, 1828–1833.

Shima, S., Weiss, D. S. & Thauer, R. K. (1995). Formylmethanofuran:tetrahydromethanopterin formyltransferase (Ftr) from the hyperthermophilic *Methanopyrus kandleri*: cloning, sequencing and functional expression of the *ftr* gene and one step purification of the enzyme overproduced in *Escherichia coli*. *Eur J Biochem* **230**, 906–913.

Shima, S., Thauer, R. K., Michel, H. & Ermler, U. (1996). Crystallization and preliminary X-ray diffraction studies of a formylmethanofuran:tetrahydromethanopterin formyltransferase from *Methanopyrus kandleri*. *Proteins Struct Funct Genet* **26**, 118–120.

Shima, S., Goubeaud, M., Vinzenz, D., Thauer, R. K. & Ermler, U. (1997). Crystallization and preliminary X-ray diffraction studies

of methyl-coenzyme M reductase from *Methanobacterium thermoautotrophicum*. *J Biochem* **121**, 829–830.

Singh-Wissmann, K. & Ferry, J. G. (1995). Transcriptional regulation of the phosphotransacetylase-encoding and acetate kinase-encoding genes (*pta* and *ack*) from *Methanosarcina thermophila*. *J Bacteriol* **177**, 1699–1702.

Smigan, P., Majernik, A. & Greksak, M. (1994). Na^+-driven ATP synthesis in *Methanobacterium thermoautotrophicum* and its differentiation from H^+-driven ATP synthesis by rhodamine 6G. *FEBS Lett* **347**, 190–194.

Smith, D. R., Doucette-Stamm, L. A., Deloughery, C. & 34 other authors (1997). Complete genome sequence of *Methanobacterium thermoautotrophicum* ΔH: functional analysis and comparative genomics. *J Bacteriol* **179**, 7135–7155.

Sorgenfrei, O., Müller, S., Pfeiffer, M., Sniezko, I. & Klein, A. (1997a). The [NiFe] hydrogenases of *Methanococcus voltae*: genes, enzymes and regulation. *Arch Microbiol* **167**, 189–195.

Sorgenfrei, O., Duin, E. C., Klein, A. & Albracht, S. P. J. (1997b). Changes in the electronic structure around Ni in oxidized and reduced selenium-containing hydrogenases from *Methanococcus voltae*. *Eur J Biochem* **247**, 681–687.

Sowers, K. R., Thai, T. T. & Gunsalus, R. P. (1993). Transcriptional regulation of the carbon monoxide dehydrogenase gene (*cdhA*) in *Methanosarcina thermophila*. *J Biol Chem* **268**, 23172–23178.

Sparling, R., Blaut, M. & Gottschalk, G. (1993). Bioenergetic studies of *Methanosphaera stadtmanae*, an obligate H_2-methanol utilising methanogen. *Can J Microbiol* **39**, 742–748.

Springer, E., Sachs, M. S., Woese, C. R. & Boone, D. R. (1995). Partial gene sequences for the A subunit on methyl-coenzyme M reductase (*mcrI*) as a phylogenetic tool for the family *Methanosarcinaceae*. *Int J Syst Bacteriol* **45**, 554–559.

Sprott, G. D. & Beveridge, T. J. (1993). Microscopy. In *Methanogenesis*, pp. 81–127. Edited by J. G. Ferry. New York & London: Chapman & Hall.

Stadtman, T. C. (1967). Methane fermentation. *Annu Rev Microbiol* **21**, 121–142.

Stephenson, M. (1932). The chemistry of the bacteria. *Annu Rev Biochem* **1**, 637–654.

Stephenson, M. (1933). The chemistry of bacteria. *Annu Rev Biochem* **2**, 485–502.

Stephenson, M. (1934). The chemistry of bacteria. *Annu Rev Biochem* **3**, 519–534.

Stephenson, M. (1935). The chemistry of bacteria. *Annu Rev Biochem* **4**, 593–614.

Stephenson, M. (1948). Obituary Notice. Frederick Gowland Hopkins, 1861–1947. *Biochem J* **42**, 161–169.

Stephenson, M. (1949). *Bacterial Metabolism*, 3rd edn. London: Longmans, Green and Co.

Stephenson, M. & Stickland, L. H. (1931a). XXVII. Hydrogenase: a bacterial enzyme activating molecular hydrogen. I. The properties of the enzyme. *Biochem J* **25**, 205–214.

Stephenson, M. & Stickland, L. H. (1931b). XXVIII. Hydrogenase. II. The reduction of sulphate to sulphide by molecular hydrogen. *Biochem J* **25**, 215–220.

Stephenson, M. & Stickland, L. H. (1932). LXXXVIII. Hydrogenlyases. Bacterial enzymes liberating molecular hydrogen. *Biochem J* **26**, 712–725.

Stephenson, M. & Stickland, L. H. (1933a). CCVII. Hydrogenase. III. The bacterial formation of methane by the reduction of one-carbon compounds by molecular hydrogen. *Biochem J* **27**, 1517–1527.

Stephenson, M. & Stickland, L. H. (1933b). CCVIII. Hydrogenlyases. III. Further experiments on the formation of formic hydrogenlyase by *Bact. coli*. *Biochem J* **27**, 1528–1532.

Stickland, L. H. (1929). CXXXI. The bacterial decomposition of formic acid. *Biochem J* **23**, 1187–1198.

Stinson, S. (1995). Hydrogenase enzyme shows unique mechanism. *Chem Eng News October* 30, 7.

Stolzenberg, A. M. & Zhang, Z. (1997). F430 model chemistry. An investigation of nickel complexes as catalysts for the reduction of alkyl halides and methyl coenzyme-M sodium borohydride. *Inorg Chem* **36**, 593–600.

Stroup, D. & Reeve, J. N. (1993a). Identification of the *mcrC* gene product in *Methanococcus vannielii*. *FEMS Microbiol Lett* **111**, 129–134.

Stroup, D. & Reeve, J. N. (1993b). Association of the *mcrD* gene product with methyl-coenzyme M reductase in *Methanococcus vannielii*. *Biochim Biophys Acta* **1203**, 175–183.

Stupperich, E., Juza, A., Hoppert, M. & Mayer, F. (1993). Cloning, sequencing, and immunological characterization of the corrinoid-containing subunit of the N^5-methyltetrahydromethanopterin: coenzyme M methyltransferase from *Methanobacterium thermoautotrophicum*. *Eur J Biochem* **217**, 116–121.

Suh, M. P., Oh, K. Y., Lee, J. W. & Bae, Y. Y. (1996). Acetamide coordination in a nickel(I) macrocyclic complex: synthesis, properties, and X-ray crystal structure of a five-coordinate nickel(I) iminol complex of 1,3,6,8,12,15-hexaazatricyclo[13.3.1.$1^{8,12}$]icosane. *J Am Chem Soc* **118**, 777–783.

Tada, M. & Masuzawa, Y. (1997). Biomimetic methane generation and disulfide formation by catalysis with a nickel complex. *Chem Commun* **22**, 2161–2162.

Tallant, T. C. & Krzycki, J. A. (1996). Coenzyme M methylase activity of the 480-kilodalton corrinoid protein from *Methanosarcina barkeri*. *J Bacteriol* **175**, 1295–1301.

Tallant, T. C. & Krzycki, J. A. (1997). Methylthiol:coenzyme M methyltransferase from *Methanosarcina barkeri*, an enzyme of methanogenesis from dimethylsulfide and methylmercaptopropionate. *J Bacteriol* **179**, 6902–6911.

Tanaka, K. (1994). Anaerobic degradation of tetramethylammonium by a newly isolated marine methanogen. *J Ferment Bioeng* **78**, 386–388.

Taylor, C. D. & Wolfe, R. S. (1974a). Structure and methylation of coenzyme M ($HSCH_2CH_2SO_3$). *J Biol Chem* **249**, 4879–4885.

Taylor, C. D. & Wolfe, R. S. (1974b). A simplified assay for coenzyme M ($HSCH_2CH_2SO_3$). *J Biol Chem* **249**, 4886–4890.

Te Brömmelstroet, B. W., Hensgens, C. M. H., Geerts, W. J., Keltjens, J. T., van der Drift, C. & Vogels, G. D. (1990). Purification and properties of 5,10-methenyltetrahydromethanopterin cyclohydrolase from *Methanosarcina barkeri*. *J Bacteriol* **172**, 564–571.

Te Brömmelstroet, B. W., Geerts, W. J., Keltjens, J. T., van der Drift, C. & Vogels, G. D. (1991). Purification and properties of 5,10-methylenetetrahydromethanopterin dehydrogenase and 5,10-methylenetetrahydromethanopterin reductase, two coenzyme F_{420}-dependent enzymes, from *Methanosarcina barkeri*. *Biochim Biophys Acta* **1079**, 293–302.

Telser, J. (1998). Nickel in F_{430}. In *Bioinorganic Chemistry: Trace Element Evolution from Anaerobes to Aerobes*, vol. 91, pp. 31–63. Edited by R. J. P. Williams. Heidelberg: Springer.

Telser, J., Fann, Y.-C., Renner, M. W., Fajer, J., Wang, S. K., Zhang, H., Scott, R. A. & Hoffman, B. M. (1997). Investigation by EPR and ENDOR spectroscopy of the Ni(I) form of cofactor F_{430} of *Methanobacterium thermoautotrophicum* and of Ni(I) octaethylisobacteriochlorin. *J Am Chem Soc* **119**, 733–743.

Tersteegen, A., Linder, D., Thauer, R. K. & Hedderich, R. (1997). Structures and functions of four anabolic 2-oxoacid oxidoreductases in *Methanobacterium thermoautotrophicum*. *Eur J Biochem* **244**, 862–868.

Thauer, R. K. (1990). Energy metabolism of methanogenic bacteria. *Biochim Biophys Acta* **1018**, 256–259.

Thauer, R. K. (1997). Biodiversity and unity in biochemistry. *Antonie Leeuwenhoek* **71**, 21–32.

Thauer, R. K. & Bonacker, L. G. (1994). Biosynthesis of coenzyme F_{430}, a nickel porphinoid involved in methanogenesis. In *The Biosynthesis of the Tetrapyrrole Pigments*, pp. 210–227. Edited by D. J. Chadwick & K. Ackrill. Ciba Foundation Symposium 180. Chichester: Wiley.

Thauer, R. K. & Kunow, J. (1995). Sulfate reducing Archaea. In *Sulfate Reducing Bacteria*, pp. 33–48. Edited by L. L. Barton. New York: Plenum.

Thauer, R. K., Jungermann, K. & Decker, K. (1977). Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol Rev* **41**, 100–180.

Thauer, R. K., Hedderich, R. & Fischer, R. (1993). Biochemistry. Reactions and enzymes involved in methanogenesis from CO_2 and H_2. In *Methanogenesis*, pp. 209–252. Edited by J. G. Ferry. New York & London: Chapman & Hall.

Thauer, R. K., Klein, A. R. & Hartmann, G. C. (1996). Reactions with molecular hydrogen in microorganisms: evidence for a purely organic hydrogenation catalyst. *Chem Rev* **96**, 3031–3042.

Thompson, H., Tersteegen, A., Thauer, R. K. & Hedderich, R. (1998). Two malate dehydrogenases in *Methanobacterium thermoautotrophicum*. *Arch Microbiol* **170**, 38–42.

Vaupel, M. (1993). *Untersuchungen mit Methanobacterium thermoformicicum und Methanobacterium formicicum zur Rolle von H_2-bildender N^5,N^{10}-Methylentetrahydromethanopterin-Dehydrogenase in methanogenen Archaea.* Diploma thesis, Philipps-Universität Marburg.

Vaupel, M. & Thauer, R. K. (1995). Coenzyme F_{420}-dependent N^5,N^{10}-methylenetetrahydromethanopterin reductase (Mer) from *Methanobacterium thermoautotrophicum* strain Marburg. Cloning, sequencing, transcriptional analysis and functional expression in *Escherichia coli* of the *mer* gene. *Eur J Biochem* **231**, 773–778.

Vaupel, M. & Thauer, R. K. (1998). Two F_{420}-reducing hydrogenases in *Methanosarcina barkeri*. *Arch Microbiol* **169**, 201–205.

Vaupel, M., Dietz, H., Linder, D. & Thauer, R. K. (1996). Primary structure of cyclohydrolase (Mch) from *Methanobacterium thermoautotrophicum* (strain Marburg) and functional expression of the *mch* gene in *Escherichia coli*. *Eur J Biochem* **236**, 294–300.

Vaupel, M., Vorholt, J. A. & Thauer, R. K. (1998). Overproduction and one-step purification of the N^5,N^{10}-methenyltetrahydromethanopterin cyclohydrolase (Mch) from the hyperthermophilic *Methanopyrus kandleri*. *Extremophiles* **2**, 15–22.

Vermeij, P., Detmers, F. J. M., Broers, F. J. M., Keltjens, J. T. & van der Drift, C. (1994). Purification and characterization of coenzyme F_{390} synthetase from *Methanobacterium thermoautotrophicum* (strain ΔH). *Eur J Biochem* **226**, 185–191.

Vermeij, P., Vinke, E., Keltjens, J. T. & van der Drift, C. (1995). Purification and properties of coenzyme F_{390} hydrolase from *Methanobacterium thermoautotrophicum* (strain Marburg). *Eur J Biochem* **234**, 592–597.

Vermeij, P., van der Steen, R. J. T., Keltjens, J. T., Vogels, G. D. & Leisinger, T. (1996). Coenzyme F_{390} synthetase from *Methanobacterium thermoautotrophicum* Marburg belongs to the superfamily of adenylate-forming enzymes. *J Bacteriol* **178**, 505–510.

Vogel, G. (1998). Did the first complex cell eat hydrogen? *Science* **279**, 1633–1634.

Vorholt, J. A. & Thauer, R. K. (1997). The active species of "CO_2" utilized by formylmethanofuran dehydrogenase from methanogenic Archaea. *Eur J Biochem* **248**, 919–924.

Vorholt, J., Kunow, J., Stetter, K. O. & Thauer, R. K. (1995). Enzymes and coenzymes of the carbon monoxide dehydrogenase pathway for autotrophic CO_2 fixation in *Archaeoglobus lithotrophicus* and the lack of carbon monoxide dehydrogenase in the heterotrophic *A. profundus*. *Arch Microbiol* **163**, 112–118.

Vorholt, J. A., Vaupel, M. & Thauer, R. K. (1996). A polyferredoxin with eight [4Fe–4S] clusters as a subunit of molybdenum formylmethanofuran dehydrogenase from *Methanosarcina barkeri*. *Eur J Biochem* **236**, 309–317.

Vorholt, J. A., Hafenbradl, D., Stetter, K. O. & Thauer, R. K. (1997a). Pathways of autotrophic CO_2 fixation and of dissimilatory nitrate reduction to N_2O in *Ferroglobus placidus*. *Arch Microbiol* **167**, 19–23.

Vorholt, J. A., Vaupel, M. & Thauer, R. K. (1997b). A selenium-dependent and selenium-independent formylmethanofuran dehydrogenase and their transcriptional regulation in the hyperthermophilic *Methanopyrus kandleri*. *Mol Microbiol* **23**, 1033–1042.

Wackett, L. P., Honek, J. F., Begley, T. P., Wallace, V., Orme-Johnson, W.-H. & Walsh, C. T. (1987). Substrate analogues as mechanistic probes of methyl-S-coenzyme M reductase. *Biochemistry* **26**, 6012–6018.

Wassenaar, R. W., Daas, P. J. H., Geerts, W. J., Keltjens, J. T. & van der Drift, C. (1996). Involvement of methyltransferase-activating protein and methyltransferase 2 isoenzyme II in methylamine:coenzyme M methyltransferase reactions in *Methanosarcina barkeri* Fusaro. *J Bacteriol* **178**, 6937–6944.

Wassenaar, R. W., Keltjens, J. T., van der Drift, C. & Vogels, G. D. (1998). Purification and characterization of dimethylamine:5-hydroxybenzimidazolyl-cobamide methyltransferase from *Methanosarcina barkeri* Fusaro. *Eur J Biochem* **253**, 692–697.

Wasserfallen, A. (1994). Formylmethanofuran synthesis by formylmethanofuran dehydrogenase from *Methanobacterium thermoautotrophicum* Marburg. *Biochem Biophys Res Commun* **199**, 1256–1261.

Wasserfallen, A., Huber, K. & Leisinger, T. (1995). Purification and structural characterization of a flavoprotein induced by iron limitation in *Methanobacterium thermoautotrophicum* Marburg. *J Bacteriol* **177**, 2436–2441.

Weiss, D. S. & Thauer, R. K. (1993). Methanogenesis and the unity of biochemistry. *Cell* **72**, 819–822.

Weiss, D. S., Gärtner, P. & Thauer, R. K. (1994). The energetics and sodium-ion dependence of N^5-methyltetrahydromethanopterin: coenzyme M methyltransferase studied with cob(I)alamin as methyl acceptor and methylcob(III)alamin as methyl donor. *Eur J Biochem* **226**, 799–809.

White, R. H. (1994). Biosynthesis of (7-mercaptoheptanoyl)-threonine phosphate. *Biochemistry* **33**, 7077–7081.

White, R. H. (1998). Methanopterin biosynthesis: methylation of the biosynthetic intermediates. *Biochim Biophys Acta* **1380**, 257–267.

White, R. H. & Zhou, D. (1993). Biosynthesis of the coenzymes in methanogens. In *Methanogenesis*, pp. 409–444. Edited by J. G. Ferry. New York & London: Chapman & Hall.

Whitman, W. B. & Wolfe, R. S. (1980). Presence of nickel in factor F_{430} from *Methanobacterium bryantii*. *Biochem Biophys Res Commun* **92**, 1196–1201.

Widdel, F. & Frimmer, U. (1995). Purification of enzymes involved in alcohol utilization by methanogenic archaea. In *Archaea – A Laboratory Manual, Methanogens*, pp. 263–268. Edited by K. R. Sowers & H. J. Schreier. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Wilms, R., Freiberg, C., Wegerle, E., Meier, I., Mayer, F. & Müller, V. (1996). Subunit structure and organization of the genes of the A_1A_0 ATPase from the archaeon *Methanosarcina mazei* Gö1. *J Biol Chem* **271**, 18843–18852.

Wolfe, R. S. (1991). My kind of biology. *Annu Rev Microbiol* **45**, 1–35.

Wolfe, R. S. (1993). An historical overview of methanogenesis. In *Methanogenesis*, pp. 1–32. Edited by J. G. Ferry. New York & London: Chapman & Hall.

Wolfe, R. S. (1996). 1776–1996: Alessandro Volta's combustible air. *ASM News* **62**, 529–534.

Won, H., Olson, K. D., Summers, M. F. & Wolfe, R. S. (1993). F430-dependent biocatalysis in methanogenic archaebacteria. *Comments Inorg Chem* **15**, 1–26.

Woods, D. D. (1936). LXXVII. Hydrogenlyases. IV. The synthesis of formic acid by bacteria. *Biochem J* **30**, 515–527.

Woods, D. D. (1950). Obituary Notice. Marjory Stephenson (1885–1948). *Biochem J* **46**, 377.

Yeliseev, A., Gärtner, P., Harms, U., Linder, D. & Thauer, R. K. (1993). Function of methylcobalamin:coenzyme M methyltransferase isoenzyme II in *Methanosarcina barkeri*. *Arch Microbiol* **159**, 530–536.

Zeikus, J. G. & Wolfe, R. S. (1972). *Methanobacterium thermoautotrophicum* sp. n., an anaerobic autotrophic, extreme thermophile. *J Bacteriol* **109**, 707–713.

Zeikus, J. G., Fuchs, G., Kenealy, W. & Thauer, R. K. (1977). Oxidoreductases involved in cell carbon synthesis of *Methanobacterium thermoautotrophicum*. *J Bacteriol* **132**, 604–613.

Zinder, S. H. (1993). Physiological ecology of methanogens. In *Methanogenesis*, pp. 128–206. Edited by J. G. Ferry. New York & London: Chapman & Hall.

Zirngibl, C., Hedderich, R. & Thauer, R. K. (1990). N^5,N^{10}-Methylenetetrahydromethanopterin dehydrogenase from *Methanobacterium thermoautotrophicum* has hydrogenase activity. *FEBS Lett* **261**, 112–116.