The *eutD* Gene of *Salmonella enterica* Encodes a Protein with Phosphotransacetylase Enzyme Activity

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Ethanolamine is used by *Salmonella enterica* serovar Typhi-
murium LT2 (hereafter referred to as *S. enterica*) as a source of
carbon, nitrogen, and energy (8, 21, 22). In principle, the bio-
chemistry of the conversion of ethanolamine to the central
metabolite acetyl-coenzyme A (Ac-CoA) is relatively simple
(Fig. 1A). The first step in the catabolism of ethanolamine is
alyzed by the well-characterized ethanolamine ammonia-
lyase, a coenzyme B12-dependent enzyme (2, 3, 5, 9, 10, 24).
The products of the ethanolamine ammonia-lyase-catalyzed
reaction are ammonia and acetaldehyde, whose oxidation to
acetate may be coupled to the synthesis of Ac-CoA (1). There
are two possible fates for Ac-CoA in this bacterium. When
the cell is not starved for energy, Ac-CoA enters the glyoxylate
bypass of the tricarboxylic acid cycle to fuel the energy gener-
ation system and serves as a building block for many interme-
diary and secondary metabolism pathways (16). Under condi-
tions of energy limitation, Ac-CoA is converted to acetyl-
phosphate (Ac-P) and ultimately to acetate by the action of the
phosphotransacetylase (Pta [EC 2.3.1.8]) and acetate kinase
(Ack [EC 2.7.2.1]) enzymes. Under these conditions, acetate is
excreted and later recaptured (17). In this pathway, the con-
version of Ac-P to acetate is coupled to substrate-level phos-
phorylation of ADP to yield ATP. The EutE protein has been
proposed to be the enzyme responsible for converting acetal-
dehyde to Ac-CoA; however, the EutE protein has not been
isolated or studied in detail. Ethanolamine catabolic functions
in *S. enterica* are encoded by the 17-gene *eut* operon (15, 25).
Many of the annotated functions of the *eut* genes are based
exclusively on their homology to proteins of known function.
For example, the predicted primary amino acid sequence of
the EutD protein is 37% identical and 56% similar to that of
the catalytic domain of the Pta enzyme of *S. enterica* and many
other prokaryotes (11). However, no experimental support for
this annotation has been reported. In this paper, we provide in
vivo and in vitro evidence that shows that the EutD protein is
a bona fide Pta enzyme.

The EutD protein compensates for the lack of Pta activity
during growth on acetate. To investigate whether the EutD
protein had Pta activity, the *eut* gene was cloned under the
control of an arabinose-inducible promoter and was intro-
duced into a *pta acs* strain unable to grow on acetate. The *acs*
gene encodes a high-affinity Ac-CoA synthetase enzyme (EC
6.2.1.1) that activates acetate to Ac-CoA when acetate is
present at a low concentration in the environment (17, 18).
Because the Ack/Pta and Ack systems are the only ones *S.
enterica* has for the conversion of acetate to Ac-CoA, inactiva-
tion of both of these systems renders a strain unable to use
acetate as a carbon and energy source. Arabinose-induced
expression of *eutD* in the *pta acs* strain (JE7245) restored growth on acetate to a rate comparable
to that measured for the wild-type strain (Fig. 2) and to the
activity associated with the Pta enzyme of the methanogenic
archaeon *Methanosarcina thermophila* (Fig. 2).

**EutD converts Ac-P to Ac-CoA in the presence of free CoA.**
Incubation of the H2-EutD enzyme with Ac-P and CoA yielded
Ac-CoA. Reverse-phase high-pressure liquid chromatography
was used to separate components of the reaction mixture on
the basis of their hydrophobicity characteristics. The chro-
matogram of the reaction mixture showed a compound eluting
104 min after injection (Fig. 3A). This compound was identi-
fied as Ac-CoA on the basis of its retention time and its mass
spectrum (Fig. 3B). The mass spectrum of the unknown com-
 pound was identical to that obtained with authentic, commer-
cially available Ac-CoA. For the sake of simplicity, only the
signals diagnostic of the molecular ion are labeled in Fig. 3B.
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signals diagnostic of the molecular ion are labeled in Fig. 3B.
The latter were observed with mass/charge ratios (m/z) of 808.6
(M – 1H), 810.6 (M + 1H), and 846.4 (M – 2H + K), where
M is the mass of the molecular ion, H is a proton, and K is a
potassium ion. These data confirmed that EutD had Pta activity.

**Isolation and initial characterization of the EutD Pta activ-
ity.** Basic kinetic parameters of the Pta activity were obtained
with homogeneous, N-terminally tagged H2-EutD protein pu-
FIG. 1. Biochemical steps for the conversion of ethanolamine and 1,2-propanediol to the corresponding acyl-CoA derivative. (A) Ethanolamine is converted to Ac-CoA in two steps. EutD and Ack then convert Ac-CoA to acetate, which is excreted. (B) Similarly, 1,2-propanediol is converted from Pro-CoA to propionate by Pta and Ack. Propionate is eventually excreted.

FIG. 2. EutD can restore growth of a pta acs strain on 50 mM acetate. Growth kinetics was analyzed using a 96-well microtiter plate (Becton Dickinson) and a computer-controlled Ultra microplate reader (Bio-Tek Instruments) equipped with KC4 software. The temperature of the incubation chamber was set at 37°C. Strains were grown on a minimal medium (4) supplemented with MgSO₄ (1 mM), L-methionine (0.5 mM), and acetate (50 mM). pBAD30, cloning vector; pET15b, pEUTD2.

EutD Pta activity is a key difference between ethanolamine and 1,2-propanediol catabolism in S. enterica. Despite the fact that ethanolamine and 1,2-propanediol catabolisms in S. enterica occur via very similar biochemical reactions (Fig. 1) (6, 15), no evidence has been reported for the existence of a structural or functional homolog of Pta encoded by the propanediol utilization (pdu) operon. The catabolism of both compounds requires the assembly of a carboxysome-like structure, with increasing evidence supporting the hypothesis that both compounds are catabolized inside the carboxysome-like

rified using Ni-affinity chromatography. H₅-EutD protein was overproduced in Escherichia coli strain BL21(ADE3) harboring plasmid pEUTD8 (pET15b eutD™) after induction with isopropyl-β-D-thiogalactopyranoside (250 μM). Cells were broken open using a French press (Spectronic Unicam) at 1.034 × 10⁵ kPa. Cell debris was pelleted by centrifugation at 39,191 g in an Avanti J-25 centrifuge (Beckman-Coulter) for 40 min at 4°C with a J25.50 rotor, and the clarified cell extract was loaded onto a 1-ml His-Bind nitritolactric acid affinity chromatography column (Novagen) which was developed per the manufacturer’s instructions. Fractions containing H₅-EutD protein were dialyzed overnight at 4°C with 1 liter of 50 mM Tris-Cl buffer (pH 7.2) containing 2 mM dithiothreitol, 10 mM EDTA, and 20 mM KCl. EutD protein was dialyzed using the buffer described above without EDTA and with 10% (vol/vol) glycerol as cryoprotectant. H₅-EutD protein (>95% homogeneous) was stored at −80°C until used. Assay conditions and detection of product formation were as described previously (20) except that the final volume of the reaction mixture was 1 ml, the reaction was started by the addition of substrate (i.e., potassium lithium Ac-P [1 μmol]), and the reaction was performed at 30°C. The purity and the concentration of the H₅-EutD protein were established as previously described (7, 19, 23) (data not shown). Pseudo-first-order kinetics yielded apparent Kₘ values of 46 μM for free CoA and 129 μM for Ac-P. The kₘ cat value calculated per micromole of EutD monomer was 1.927 s⁻¹, and the catalytic efficiency (kₘ cat/Kₘ) values for free CoA and Ac-P were 4.2 × 10⁴ and 1.5 × 10⁴, respectively. A pH activity profile for the H₅-EutD enzyme showed maximal activity at pH 7.2, with an approximately 90% loss of activity measured at pH 6.5 and 25% of the activity lost at pH 7.8 (data not shown). A thermal stability analysis showed that >50% of the enzyme’s activity was lost after a 5-min incubation at 25°C, with >90% of the activity lost at >45°C (data not shown).

EutD Pta activity is a key difference between ethanolamine and 1,2-propanediol catabolism in S. enterica. Despite the fact that ethanolamine and 1,2-propanediol catabolisms in S. enterica occur via very similar biochemical reactions (Fig. 1) (6, 15), no evidence has been reported for the existence of a structural or functional homolog of Pta encoded by the propanediol utilization (pdu) operon. The catabolism of both compounds requires the assembly of a carboxysome-like structure, with increasing evidence supporting the hypothesis that both compounds are catabolized inside the carboxysome-like
structure (12, 13, 15, 25). Why does ethanolamine catabolism require EutD in addition to the housekeeping Pta enzyme? Why is an additional Pta activity not required for 1,2-propanediol catabolism? Answers to these questions will shed light on the physiological restrictions confronted by S. enterica during the catabolism of these compounds.

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