Electrochemical Cells Conducting Mitochondorial Oxidative Phosphorylation and NADH Oxidation

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ABSTRACT

An artificial mitochondoria has been devised without using whole cells or organelles and cell-free enzymes. It has been successful due to an electrically conductive titanium nitroxicarbide base ceramics. Each of ATP phosphorylation and NADH oxidation has been conducted electrolytically with an external voltage supply in each electrochemical cell using an electrode made of that material. Moreover, each of them has been carried out galvanically while consuming O_2 and generating electricity in each electrochemical cell using the electrode. From experimental results, a chemical mechanism of mitochondorial oxidative phosphorylation coupled to NADH oxidation has been explained completely concerning the P:O ratio. Generally, phosphorylation coupled to NADH oxidation at the P:O ratio n is achieved by combining two galvanic NADH oxidation cells of this study, and the galvanic and electrolytic phosphorylation cells of this study by n.

Mitochondorial oxidative phosphorylation and NADH oxidation are generally known to be coupled by reaction [1] (1). However, there has been no chemical method

$$NADH + H^+ + 1/2\ O_2 + n\text{ADP} + n\text{H}_3\text{PO}_4 \rightarrow \text{NAD}^+ + n\text{ATP} + m\text{H}_2\text{O} \quad [1]$$

which has achieved mitochondorial oxidative phosphorylation or NADH oxidation in aqueous solutions without using whole cells or organelles and cell-free enzymes. The previously mentioned chemical method, if found, will be an artificial mitochondoria. Moreover, it will be very helpful for both producing ATP and clarifying the chemical mechanisms of biological mitochondoria.

This study was aimed at devising an artificial mitochondoria and at estimating the chemical mechanisms of biological mitochondoria.

Two electrochemical methods, which were effective in the aqueous solutions, have been found by this study. An electrolytic method carried out ATP phosphorylation and NADH oxidation in each electrolytic cell. A galvanic method carried them out in each galvanic cell. The electrolytic method consumed electricity for phosphorylation or NADH oxidation. The galvanic method consumed O_2 to generate electricity for phosphorylation or NADH oxidation. These methods were successful due to an electrode made of electrically conductive titanium nitroxicarbide base ceramics.

Experimental

Each of the ADP phosphorylations and NADH oxidations is checked by the electrochemical and nonelectrochemical methods.

Nonelectrochemical method.—Nonelectrochemical ATP phosphorylation and NADH oxidation were carried out separately by two experimental ways. One of them was to leave sample solutions for each of the phosphorylations and NADH oxidations without stirring. Another way was to stir the solutions. An electrically conductive ceramic board was immersed in half of the samples for each of the phosphorylations and NADH oxidations, and not immersed in the remainder. One-half of the board was in the air. Forty milliliters of each sample solution was contained in a 50 ml glass beaker. Every sample solution was exposed to the atmosphere. Any whole cells or organelles and cell-free enzymes were not used by the nonelectrochemical method. The ceramic board used was titanium nitroxicarbide base ceramics (Toshiba Tungaloy, Japan. Type-MK70X, resistivity: $4 \times 10^{-2}\ \Omega \cdot$ cm, density: 5.06 g/cm^3, size: $50 \times 15 \times 4$ mm). Sample solutions were a 8 weight percent (w/o) KCl aqueous solution containing 1-3 m*M* ADP (Sigma) and 2-6 m*M* acetyl phosphate (Sigma) for the phosphorylation, and an 8 w/o KCl aqueous solution containing 1.1 m*M* NADH (Sigma) for NADH oxidation. Temperature in the solutions was room temperature, 24°-27°C, without stirring, and elevated to 30°-34°C by heat liberated from a magnetic stirrer with stirring.

Electrochemical method.—The electrochemical method consisted of the electrolytic and galvanic methods.

The electrolytic method separately carried out phosphorylation and NADH oxidation. This method used an electrolytic cell shown in Fig. 1A. External voltage was supplied to each cell for phosphorylation and NADH oxidation. Iron wire was used as a cathode for the electrolytic method. The purity of it was 99.998%, and the diameter was 0.5 mm. There was no specific reason why iron wire was chosen as the cathode. The electrically conductive substances like carbon platinum could be used instead of iron wire. To investigate the effect of impressed voltage on phosphorylation and NADH oxidation by the electrolytic method, the voltage was kept constant within the 0.75-1.50V range for phosphorylation, and within the 0.50-1.0V range for NADH oxidation. The voltage was supplied to the anode by a voltage supplier in comparison with the saturated calomel electrode immersed in the catholyte. The voltage was controlled by a potentiostat.

The galvanic method separately carried out phosphorylation and NADH oxidation. This method used a galvanic cell shown in Fig. 1B. O_2 gas was supplied at 100 ml/min on the surface of cathode (platinum plate: $50 \times 10 \times 1$ mm) during phosphorylation and NADH oxidation by the galvanic method. The flow rate of O_2 gas was adjusted by a flowmeter. A resistance of 1.0Ω was added to the outer circuit of each galvanic cell for electric discharge.

To investigate the effect of the anolytes *p*H on the electrolytic phosphorylation and NADH oxidation by the electrolytic and galvanic methods, the *p*H was controlled by 0.1*N* HCl and 0.2*N* K_2CO_3 before the experiment. However, it decreased step by step during the experiment due to reaction products and in-flow of hydrochloric acid from the cathode room through a salt bridge. Therefore, the *p*H was checked continuously by a glass-electrode *p*H meter during the experiment.

The salt bridge contained 16 w/o KCl aqueous solution, and connected the anolytes and catholytes of every cell. Both ends of the salt bridge, which were immersed in the anolyte and catholyte, were made from two porous aluminum tubes with their ends sealed on one side. The other part, except the aluminum tubes, was made from glass. The salt bridge was used similarly for every cell.

The same electrically conductive ceramic board, used by the nonelectrochemical method, was applied to anode of every cell. This ceramic electrode was not eroded during phosphorylation and NADH oxidation by the electrolytic and galvanic methods so far as the naked eye could see. Therefore, it was used repeatedly after washing by 3*N* HCl and pure water. Carbon, platinum, and an electrically conductive alumina-base ceramic were applied to the anode of the electrolytic and galvanic cells. However, they were not effective for phosphorylation and NADH oxidation by any electrochemical methods.

Anolytes of the cells were 40 ml of an 8 w/o KCl aqueous solution containing ADP and acetyl phosphate for phosphorylation, and 40 ml of an 8 w/o KCl aqueous solution

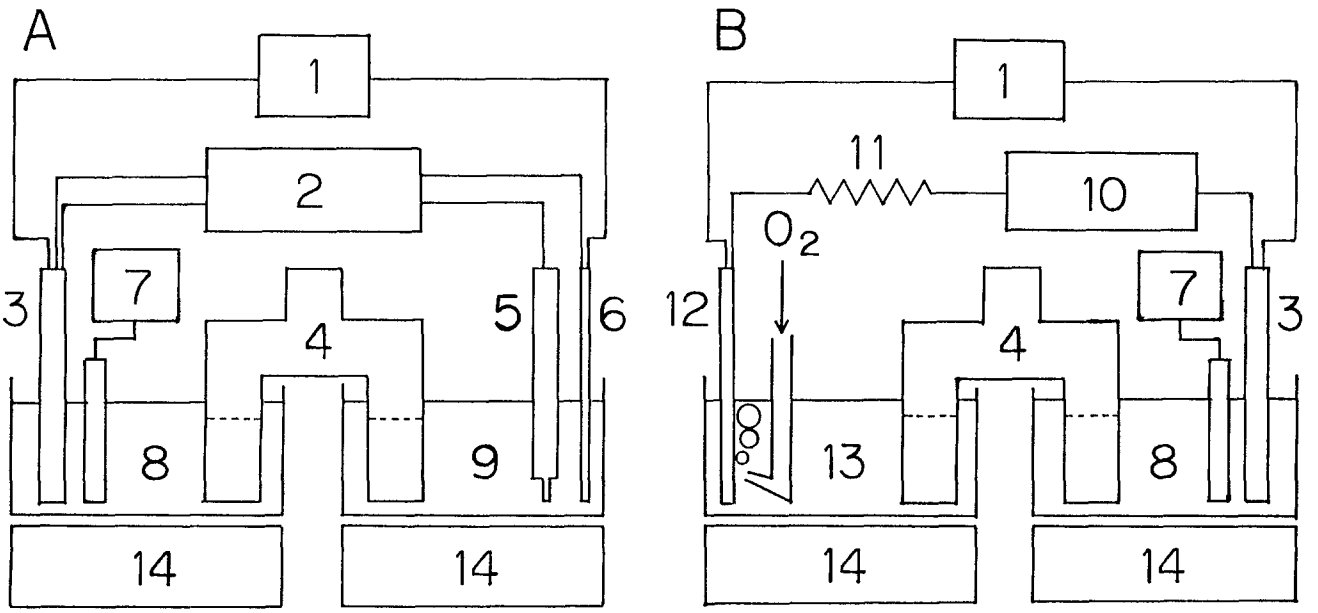

Fig. 1. Electrolytic cell (A) and galvanic cell (B). 1, voltameter; 2, a set of voltage supplier, potentiostat, and coulometer; 3, anode of titanium nitroxicarbide base ceramics; 4, salt bridge fitted two porous aluminum tubes sealed on one side containing 16 w/o KCl aqueous solution; 5, the saturated calomel electrode; 6, cathode of iron wire; 7, pH meter using a glass electrode; 8, 8 w/o KCl aqueous solution (40 ml) containing 1-3 mM ADP (Sigma) and 2-6 mM acetyl phosphate (Sigma) for ADP phosphorylation, or 8 w/o KCl aqueous solution containing 1.1 mM NADH (Sigma) for NADH oxidation; 9, 0.1*N* HCl (40 ml); 10, coulometer; 11, resistance (1.0Ω); 12, cathode of a platinum plate; 13, 2.8*N* HCl (40 ml); 14, magnetic stirrer.

containing NADH for NADH oxidation. Concentrations of each solute were equivalent to those of the sample solutions for the nonelectrochemical method.

Catholytes of the cells were 40 ml of 0.1*N* HCl for the electrolytic method, and 40 ml of 2.8*N* HCl for the galvanic method. There was no specific reason why 0.1*N* and 2.8*N* HCl were chosen as the catholytes. Aqueous solutions of the electrolytes such as KCl or NaCl could be used instead of the HCl solution for the electrolytic cell. For the galvanic cell, acidic solutions should be chosen preferably in conjunction with the cathodic reaction of $2H^+ + 1/2\ O_2 + 2e \rightarrow H_2O$.

All the anolytes and catholytes were exposed to the atmosphere during the experiment, and stirred by each magnetic stirrer. The temperature was similar in all the anolytes and catholytes, and elevated to 30°-34°C by heat liberated from the stirrer.

Voltage was measured by a voltmeter fitted with 1000 MΩ input impedance for both the electrolytic and galvanic methods.

Progress of phosphorylation and NADH oxidation was observed coulometrically and chemically. Coulometric measurement was carried out by a coulometer. Chemical measurement was carried out by liquid chromatography (Shimadzu, Japan. Type LC-6A, column: ZORBAX NH_2 (du Pont), mobile phase: 30 w/o acetonitrile-4 w/o $NH_4H_2PO_4$ mixed aqueous solution adjusted to pH 3.0, flow rate: 1 cm^3/min, Temp.: 23°C, detector: UV, abs.: 257 nm for ADP and ATP, and 260 nm for NAD^+ and NADH, pressure: below 200 bars).

Results and Discussion

Comparison of the electrochemical method with the nonelectrochemical method.—Figure 2 shows a comparison of the electrochemical method with the nonelectrochemical method on ADP phosphorylation and NADH oxidation. Concentrations of ATP and NAD^+ in each solution were compared at the beginning and the end of each experiment.

Figure 2 leads to the following: both phosphorylation and NADH oxidation take place with both the electrochemical and nonelectrochemical methods, and their velocities with the electrochemical method are much faster than the velocities with the nonelectrochemical method.

Phosphorylation takes place simply with mixing ADP and acetyl phosphate in 8 w/o KCl aqueous solution without using whole cells or organelles and cell-free enzymes. The phosphorylation velocity with the nonelectrochemical method is faster in the stirred solutions than in the solutions at rest. O_2 in the air may promote phosphorylation. An electrically conductive titanium nitroxicarbide base ceramic board promotes phosphorylation, whose velocity

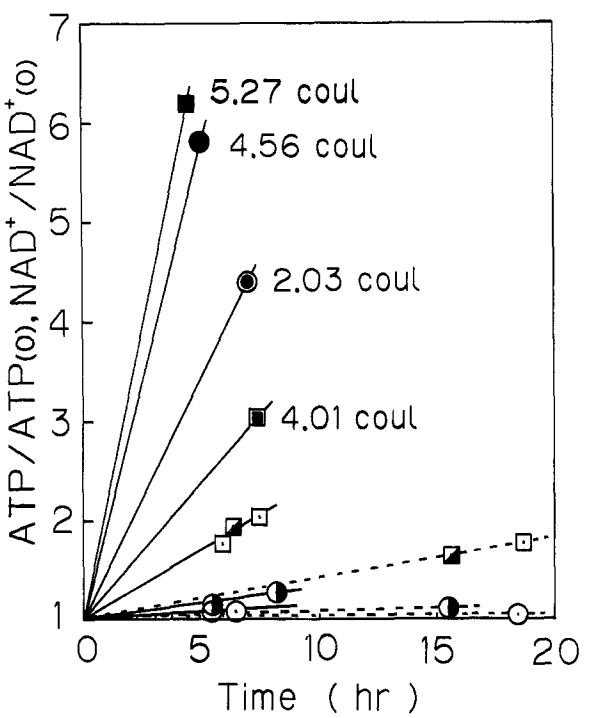

Fig. 2. Comparison of electrochemical method with nonelectrochemical method. ●: ADP phosphorylation by electrochemical method(electrolytic), ◉: ADP phosphorylation by electrochemical method(galvanic), ◑: ADP phosphorylation with titanium nitroxicarbide base ceramic board by nonelectrochemical method, ○: ADP phosphorylation without the ceramic board by nonelectrochemical method, ■: NADH oxidation by electrochemical method(electrolytic), ⊡: NADH oxidation by electrochemical method(galvanic), ◪: NADH oxidation with the ceramic board by nonelectrochemical method, and □: NADH oxidation without the ceramic board by nonelectrochemical method. The solid lines represent ADP phosphorylation or NADH oxidation with stirring, and the dotted lines represent them without stirring.

is faster in the solutions immersing the board than in the solutions wihtout immersing it. The ceramic board catalyzes the phosphorylation.

NADH oxidation also takes place simply with dissolving NADH into 8 w/o KCl aqueous solution without using whole cell or organelles and cell-free enzymes. The oxidation velocity with the nonelectrochemical method is faster in the stirred solutions than in the solutions at rest. O_2 in the air may promote the oxidation. However, the ceramic board does not catalyze the oxidation with the nonelectrochemical method.

With both the electrolytic and galvanic methods, phosphorylation and NADH oxidation take place. As previously described, their velocities are much faster with both the electrolytic and galvanic methods than with the nonelectrochemical method. Electric current flowed in each outer circuit of the electrolytic and galvanic cells for phosphorylation and NADH oxidation, and the quantity of electricity was measured during each of the phosphorylations and NADH oxidations as shown by the legend of Fig. 2. Therefore, the phosphorylation and NADH oxidation took place as their anodic reactions with both the electrolytic and galvanic cells. With the anodic reaction for phosphorylation, ADP or acetyl phosphate or both in the anolyte liberate their electrons to each anode of the electrolytic and galvanic cells. With the anodic reaction for NADH oxidation, NADH in the anolyte similarly liberates its electrons to each anode of the cells.

In conclusion, phosphorylation and NADH oxidation are electrochemical and oxidative, so far as they are carried out by the electrochemical method. Further details of the electrochemical method will be described below.

Electrochemical method (electrolytic).—Figure 3 shows the typical liquid chromatograms of phosphorylation and NADH oxidation. Figure 3A is a case in which 2.2 μmol ATP was produced from 103 μmol ADP and 220 μmol acetyl phosphate in an 8 w/o KCl aqueous solution at *p*H 5.7 at 34°C with 4.57C supplied for 5.83h at 0.78V. In this case, the anolyte's *p*H changed from 5.7 to 4.5 in the end. The effect of the *p*H on phosphorylation could not be confirmed clearly. Figure 3B is a case in which 9.4 μmol NAD^+ was produced from 68 μmol NADH in an 8 w/o KCl aqueous solution at *p*H 6.0 at 30°C with 5.27C supplied for 4.50h at 0.5V. The anolyte's *p*H changed from 6.0 to 3.9 in the end. Effect of the *p*H on NADH oxidation could not be confirmed clearly.

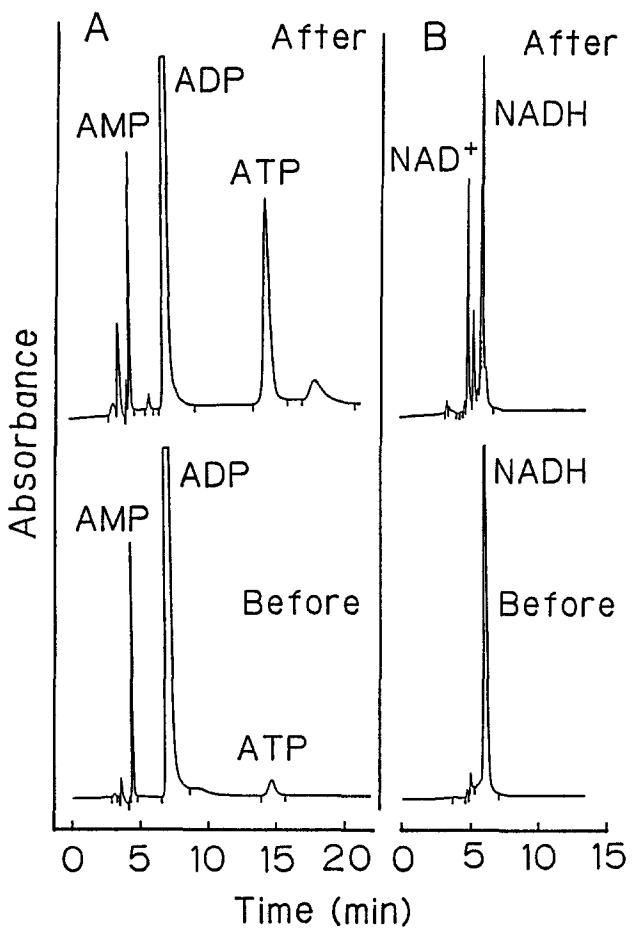

Fig. 3. Liquid chromatograms of the anolytes before/after (A) ADP phosphorylation and (B) NADH oxidation by the electrolytic method.

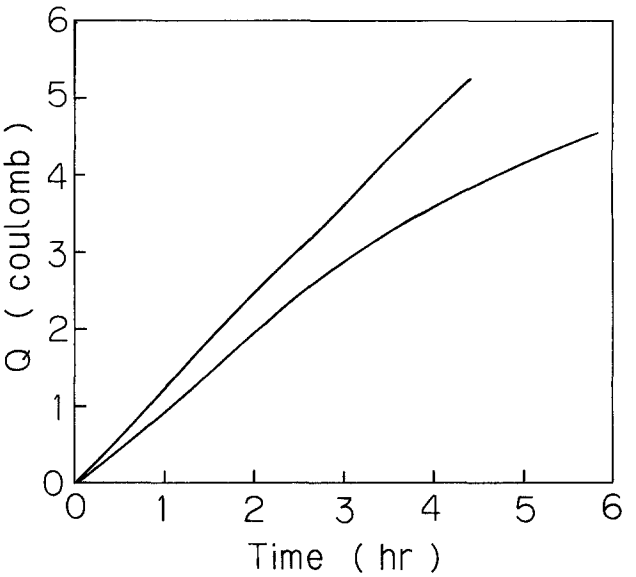

Fig. 4. Quantity of electricity (Q) vs. time during (A) ADP phosphorylation and (B) NADH oxidation by the electrolytic method.

Figure 4 shows the quantity of electricity as a function of time concerning phosphorylation and NADH oxidation. The electrolytic capability of the anode might remain unchanged during NADH oxidation. On the other hand, the capability decreased during phosphorylation because products, including by-products, might cover the anode during phosphorylation. The liquid chromatograms in Fig. 3 show that products other than ATP might be produced during the electrolytic phosphorylation.

Phosphorylation started at about 0.61V impressed to the anode in comparison with the saturated calomel electrode.

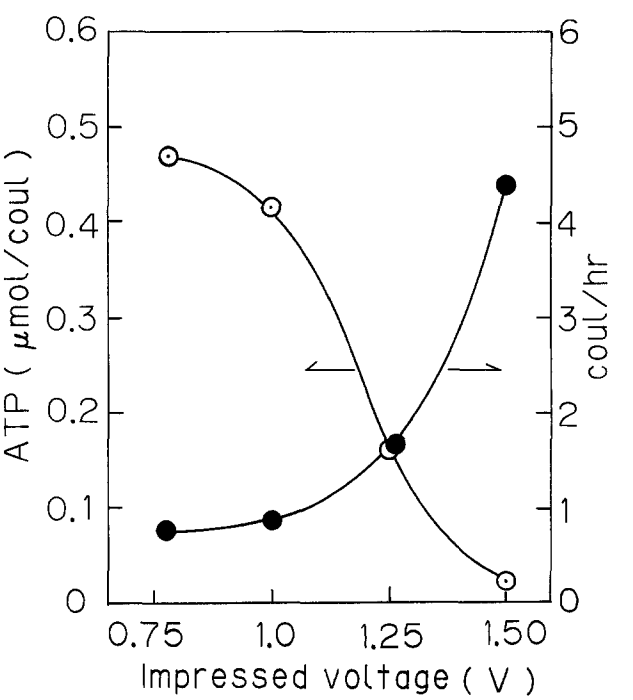

Fig. 5. ATP produced per coulomb and quantity of electricity (Q) per hour vs. impressed voltage for the electrolytic method.

NADH oxidation started at about 0.41V impressed to the anode in comparison with the saturated calomel electrode. Both phosphorylation and NADH oxidation depend upon the impressed voltage for the electrolytic method. Figure 5 shows ATP produced per coulomb and the quantity of electricity per hour as a function of the impressed voltage. The quantity of electricity was determined assuming a linear relationship between the quantity of electricity and reaction time. Figure 5 leads to the following: (*i*) ATP produced per coulomb decreased steeply with increasing voltage, (*ii*) ATP is produced efficiently and selectively below 1.0V rather than above the voltage, (*iii*) the quantity of electricity increased abruptly above 1.25V, and, therefore, (*iv*) the by-products may increase with increasing voltage.

Figure 6 shows NAD^+ per coulomb and quantity of electricity per hour as a function of the impressed voltage. The quantity of electricity was determined from a relationship between quantity of electricity and reaction time in Fig. 4. Figure 6 leads to the following: (*i*) NAD^+ produced per coulomb decreased linearly with increasing voltage, (*ii*) NADH is oxidized efficiently and selectively at lower voltages, (*iii*) the quantity of electricity increased linearly with increasing voltage up to 0.9V, and then decreased at 1.0V, and (*iv*) by-products may increase with increasing voltage. A break of the quantity of electricity at 0.9V in Fig. 6 was similar on repeated experiments. The basis for this has not been defined yet.

Electrochemical method (galvanic).—Each electrochemical reaction on the surface of cathode and anode is shown as follows: $1/2\ O_2 + 2H^+ + 2e \rightarrow H_2O$ on the surface of the cathode for both phosphorylation and NADH oxidation, and $ADP + H_3PO_4 \rightarrow ATP + H_2O + 2e$ on the surface of the anode for phosphorylation, or $NADH \rightarrow NAD^+ + H^+ + 2e$ on the surface of the anode for NADH oxidation. Therefore, the galvanic cells of this study are two kinds of fuel cells conducting phosphorylation and NADH oxidation while consuming O_2 to generate electricity.

Figures 7 and 8 show generated electricities and cell voltages as a function of time during the galvanic phosphorylation and NADH oxidation, respectively. The *p*H of the anolyte changed from 5.0 in the beginning of the phosphorylation to 3.5 in the end, and from 5.0 in the beginning of NADH oxidation to 3.0 in the end. Figure 7 leads to the following: (*i*) cell voltage decreased step by step with the passage of time except for an abrupt drop and elevation in the voltage in the beginning of phosphorylation; (*ii*) the voltage decreased with decreasing *p*H in the anolyte; (*iii*) generated electricity increased linearly with the passage of time during phosphorylation, though it changed irregularly with the abrupt elevation in the voltage; and (*iv*) the generated electricity was independent of decreasing *p*H in the anolyte. The abrupt drop in the voltage might be caused by formation of an electrochemical double layer on the surfaces of the anode, or cathode, or both. The abrupt elevation might be caused by a sudden change of stirrer's rotation number or a sudden change of O_2 flow rate. However, the specific cause could hardly be detected.

Figure 8 leads to the following : (*i*) cell voltage decreased gradually with the passage of time except for an abrupt

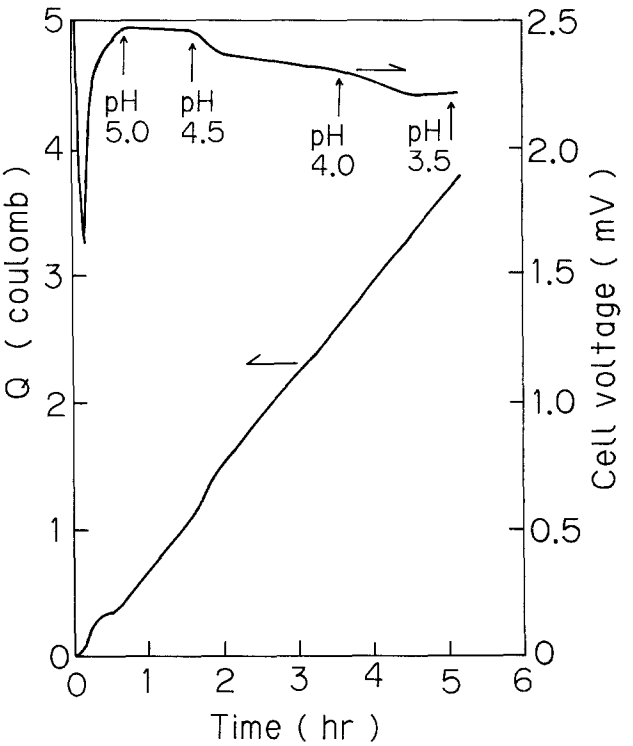

Fig. 7. Generated electricity (Q) and cell voltage *vs.* time for ADP phosphorylation by the galvanic method.

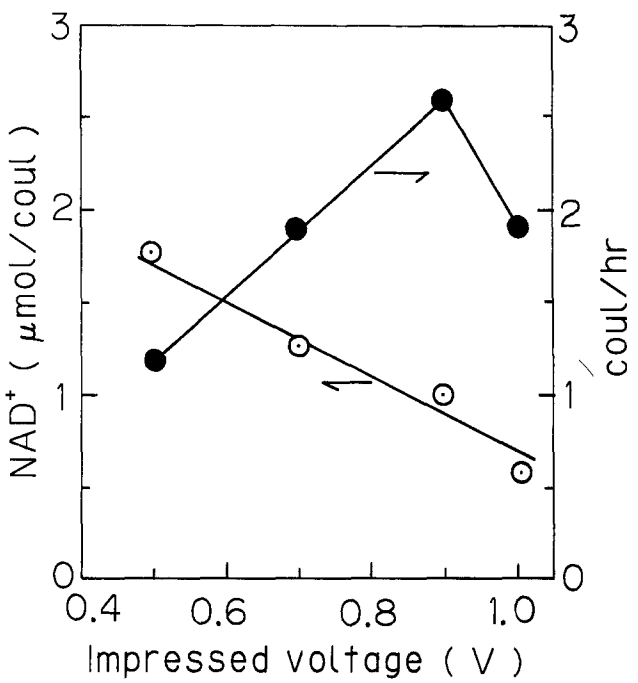

Fig. 6. NAD^+ produced per coulomb and quantity of electricity (Q) per hour *vs.* impressed voltage for the electrolytic method.

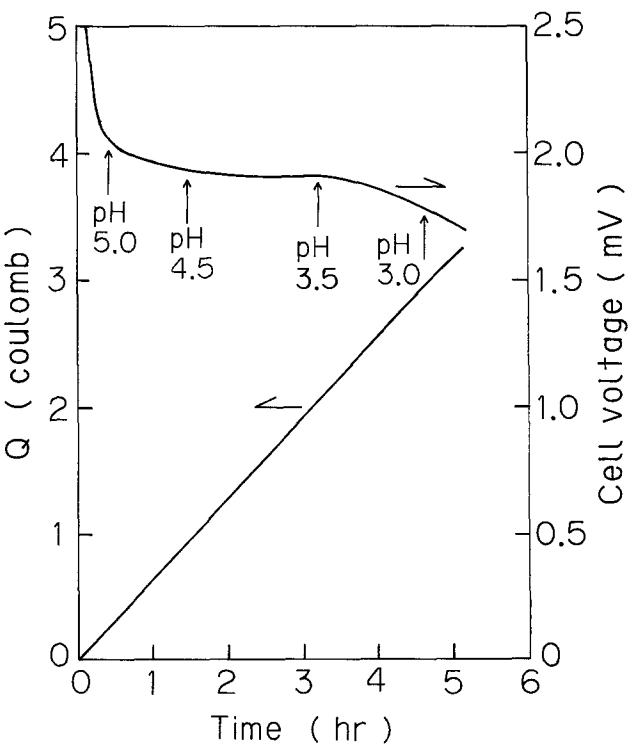

Fig. 8. Generated electricity (Q) and cell voltage *vs.* time for NADH oxidation by the galvanic method.

Table I. ADP phosphorylation and NADH oxidation carried out separately at 30°C by the galvanic method

Solution pH	Reaction time (h)	Initial ADP or NADH (μmol)	Produced ATP or NAD^+ (μmol)	Generated electricity (C)
(Phosphorylation)				
<3.0	3	196	0	2.0
3.0-4.0	4	197	0.52	2.2
4.0-5.0	4	192	2.3	3.1
5.0-6.0	3	201	1.6	1.9
6.0-7.0	3	199	0.71	0.77
>7.0	3	199	0	0.94
(NADH oxidation)				
1.3-4.0	8	65	1.0	5.64
4.0-5.0	2.5	68	4.3	1.56
1.5-7.0	7	69	5.2	4.00
1.6-8.0	7	66	5.5	4.00
7.7-10.9	6	66	1.0	3.08

drop in the voltage in the beginning of NADH oxidation; (*ii*) the voltage decreased with decreasing *p*H in the anolyte; (*iii*) the generated electricity increased linearly with the passage of time; and (*iv*) the generated electricity was independent of decreasing *p*H in the anolyte. The abrupt voltage drop might be caused by formation of an electrochemical double layer on the surfaces of the electrodes because cell voltage will depend upon an electrochemical double layer on the surfaces of the electrodes.

The effect of the anolyte's *p*H on phosphorylation and NADH oxidation were investigated. The results are shown in Table I. On phosphorylation, ATP was not produced in the anolytes above *p*H 7.0 and below *p*H 3.0, though electricity was generated in those *p*H ranges. Products other than ATP might be produced in the anolytes above *p*H 7.0 and below *p*H 3.0. The by-products produced were estimated by analyzing the liquid chromatograms on phosphorylation but could not be identified. The phosphorylation velocity was fastest in the anolytes within the 4.0-5.0 *p*H range, and decreased as the *p*H was apart from the *p*H range. ATP produced per coulomb increased with increasing *p*H up to *p*H 7.0. Therefore, the purest ATP will be produced in the anolytes within the 6.0-7.0 *p*H range. On NADH oxidation, NAD^+s produced per hour and per coulomb increased in the anolytes within the 4.0-5.0 *p*H range. Generated electricity per hour increased with decreasing *p*H and reached the maximum in the anolytes within the 1.3-4.0 *p*H range. Products other than NAD^+ might be produced in the anolytes below *p*H 4.0. The by-products produced were estimated by analyzing the liquid chromatograms on the galvanic NADH oxidation but could not be identified.

Mechanisms of mitochondorial oxidative phosphorylation coupled to NADH oxidation.—Biological and biochemical studies already clarified that mitochondorial oxidative phosphorylation was coupled to NADH oxidation in biological mitochondoria as shown by reaction [1]. However, no study has clarified or estimated that both phosphorylation and NADH oxidation were carried out not only galvanically but electrolytically. This electrochemical study has shown that both phosphorylation and NADH oxidation were carried out electrolytically as well as galvanically in each electrochemical cell.

The galvanic and electrolytic phosphorylations and NADH oxidations of this study are shown by four couples of the electrochemical reactions as follows

(I) galvanic cell of NADH oxidation

$$\text{anode reaction: } NADH \rightarrow NAD^+ + H^+ + 2e \quad [2]$$

$$\text{cathode reaction: } 2H^+ + 1/2\ O_2 + 2e \rightarrow H_2O \quad [3]$$

and the overall reaction

$$NADH + H^+ + 1/2\ O_2 \rightarrow NAD^+ + H_2O \quad [4]$$

(II) electrolytic cell of NADH oxidation

$$\text{anode reaction: } NADH \rightarrow NAD^+ + H^+ + 2e \quad [2]$$

$$\text{cathode reaction: } H_2O + 2e \rightarrow 2H^+ + 1/2\ O_2 \quad [5]$$

and the overall reaction

$$NADH + H_2O \rightarrow NAD^+ 3H^+ 1/2\ O_2 \quad [6]$$

(III) galvanic cell of phosphorylation

$$\text{anode reaction: } ADP + H_3PO_4 \rightarrow ATP + H_2O + 2e \quad [7]$$

$$\text{cathode reaction: } 2H^+ + 1/2\ O_2 + 2e \rightarrow H_2O \quad [3]$$

and the overall reaction

$$ADP + H_3PO_4 + 2H^+ + 1/2\ O_2 \rightarrow ATP + 2H_2O \quad [8]$$

(IV) electrolytic cell of phosphorylation

$$\text{anode reaction: } ADP + H_3PO_4 \rightarrow ATP + H_2O + 2e \quad [7]$$

$$\text{cathode reaction: } H_2O + 2e \rightarrow 2H^+ + 1/2\ O_2 \quad [5]$$

and the overall reaction

$$ADP + H_3PO_4 \rightarrow ATP + 2H^+ + 1/2\ O_2 \quad [9]$$

An artificial mitochondoria carrying out phosphorylation coupled to NADH oxidation is composed by combinations among the electrochemical cells I, II, III, and IV.

Former studies agreed generally that the P:O ratios might be 2 to 3 on phosphorylation coupled to NADH oxidation in biological mitochondoria, as shown by reactions [10] and [11] (1-8)

$$NADH + H^+ + 1/2\ O_2 + 2ADP + 2H_3\ PO_4 \rightarrow NAD^+ + 2ATP + 3H_2O \quad [10]$$

$$NADH + H^+ + 1/2\ O_2 + 3ADP + 3H_3PO_4 \rightarrow NAD^+ + 3ATP + 4H_2O \quad [11]$$

These reactions are easily achieved by the combinations among the electrochemical cells of this study. Reaction [10] is achieved by combining electrochemical cells I, III, and IV by one, *i.e.*, reaction [4] + reaction [8] + reaction [9]. Reaction [11] is achieved by combining two of cell I, and the cells III and IV by three, *i.e.*, reaction [4] + (3/2)(reaction [8] + reaction [9]). Phosphorylation coupled to NADH oxidation at the P:O ratio 1 is achieved as reaction [12] by combining two of cell I, and the cells III and IV by one, *i.e.*, reaction (4) + (1/2)(reaction [8] + reaction [9])

$$NADH + H^+ + 1/2\ O_2 + ADP + H_3PO_4 \rightarrow NAD^+ + ATP + 2H_2O \quad [12]$$

As is obvious from these demonstrations, former experimental results using biological mitochondoria have been explained exactly by this study. Furthermore, phosphorylation coupled to NADH oxidation at P:O ratios more than 4 are predicted by using the results of this study. For example, a case at the P:O ratio 4 will be carried out as reaction [13], and is achieved by combining two of cell I, and the cells III and IV by four, *i.e*, reaction [4] + 2(reaction [8] + reaction [9])

$$NADH + H^+ + 1/2\ O_2 + 4ADP + 4H_3PO_4 \rightarrow NAD^+ + 4ATP + 5H_2O \quad [13]$$

Generally, phosphorylation coupled to NADH oxidation at

the P:O ratio n is achieved by combining two of cell I, and the cells III and IV by n, *i.e.*, 2 (cell I) + n(cell III + cell IV), or reaction [4] + (n/2) (reaction [8] + reaction [9]). The electrochemical cells I, III, and IV appear to be essential for phosphorylation coupled to NADH oxidation. However, it is not known whether electrochemical cell II of this study is essential for phosphorylation coupled to NADH oxidation.

It is difficult functionally to specify a biological tissue of mitochondoria corresponding to each of the electrochemical cells. For example, b-c_1 complex of biological mitochondoria can hardly be specified in any electrochemical cells of this study. However, the anode of the galvanic cell for NADH oxidation appears to function similarly to the NADH dehydrogenase complex of the mitochondoria in promoting NADH oxidation. The anodes of the galvanic and electrolytic cells for ADP phosphorylation appear to function similarly to ATP synthetase of the mitochondoria in promoting ADP phosphorylation. The cathode of every galvanic cell of this study appears to function similarly to cytochrome oxidase complex of the mitochondoria in promoting reaction [3]. The lead wire of every electrochemical cell of this study appears to function similarly to ubiquinone or cytochrome c of the mitochondoria because of electron transport. Therefore, phosphorylation coupled to NADH oxidation in biological mitochondoria may be carried out by biologically galvanic and electrolytic cells, which must arrange in the inner membrane of the mitochonodira.

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Kinetics of the Electro-Oxidation of Sulfite Catalyzed by Copper Ion

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ABSTRACT

Kinetic studies have been performed on the reaction of an intermediate Cu(II) species in the copper-catalyzed electro-oxidation of sulfite using the potential step chronoamperometry, spectroelectrochemical method, and rotating ring-disk electrode technique. It was confirmed that the kinetics of the reaction of the Cu(II) species was of the second order with respect to the Cu(II) species. The rate constant of the reaction was measured by different methods, and the obtained values were in good agreement. The kinetic results are consistent with the proposed mechanism involving a binuclear Cu(II) complex.

Copper ion is known as a catalyst in some oxidation-reduction reactions and electrochemical reactions (1-5). In previous papers (3-5) we have reported that copper ion catalyzes the electro-oxidation of cyanide to cyanogen

$$2CN^- \rightarrow (CN)_2 + 2e^- \quad [1]$$

The electro-oxidation of sulfite is also catalyzed by copper ion in which dithionate is the major product (6)

$$2SO_3^{2-} \rightarrow S_2O_6^{2-} + 2e^- \quad [2]$$

The above reactions can be regarded as oxidative dimerization reactions expressed in a general form

$$2X^{n-} \rightarrow X_2^{(2n-2)-} + 2e^- \quad [3]$$

We have proposed a catalytic mechanism which involves a copper(II)/copper(I) redox couple and a binuclear copper(II) complex of the type $Cu^{II}(X^{n-})_2Cu^{II}$. It seems worthwhile to verify the mechanism since such a mechanism might be operative in other reactions of the type Eq. [3].

The electro-oxidation of sulfite in the presence of copper ion in neutral to weakly alkaline solutions is written in a simplified scheme (6)

$$Cu(I) \rightarrow Cu(II) + e^- \quad [4]$$

$$Cu(II) + SO_3^{2-} \rightarrow Cu(I) + 1/2\ S_2O_6^{2-} \quad [5]$$

Thus a sulfito copper(I) complex is oxidized to a copper(II) species at the electrode, and the latter diffuses away into the bulk of solution and reacts with sulfite ion according to Eq. [5]. The steady-state current-potential relationship has been interpreted on the assumptions that the electron transfer reaction Eq. [4] is at quasi-equilibrium and that reaction [5] is a second-order reaction with respect to the Cu(II) species. Formation of a binuclear Cu(II) complex is assumed to explain the second-order kinetics of reaction [5]. In order to confirm the above kinetics and to elucidate details of the mechanism a kinetic study is needed.

Electrochemical reactions involving catalytic processes have been investigated by polarography, potential step chronoamperometry, potential step chronocoulometry, spectroelectrochemical methods, and rotating disk and ring-disk electrode techniques (7-16). Most of the work so far reported is applied to first-order reactions, since relevant diffusion-kinetic equations are linear and can be solved analytically. Numerical methods have been used in cases of second-order catalytic processes (12, 16).

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