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Phorphorylative Electron Transport Chains Lacking a Cytochrome bc_1 Complex

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Abstract

Electron transport-coupled phosphorylation with fumarate as terminal acceptor in *Wolinella succinogenes* yields less than 1 ATP/2 electrons. The $\Delta\tilde{\mu}_H$ generated by the electron transport is 0.18 V and the H^+/electron ratio is 1. The electron transport chain is made up of two dehydrogenases (hydrogenase and formate dehydrogenase) that catalyze the reduction of menaquinone, and fumarate reductase which catalyzes the oxidation of menaquinol. *C*-type cytochromes are not involved. The phosphorylative electron transport with sulfur as terminal acceptor in *W. succinogenes* or *Desulfuromonas acetoxidans* does not involve known quinones. The ATP yields should be even smaller than those with fumarate. Succinate oxidation by sulfur, which is a catabolic reaction in *D. acetoxidans*, is accomplished by reversed electron transport.

Key Words: *Wolinella succinogens*; electron transport; hydrogenase; format dehydrogenase; menaquinone; fumarate reductase; anaerobic respiration.

Introduction

The basic mechanism of electron transport-coupled phosphorylation in anaerobic bacteria appears to be the same as that in mitochondria or aerobic bacteria (Thauer *et al.*, 1977; Haddock and Jones, 1977; Kröger, 1978; Bragg, 1980; Ferguson and Sorgato, 1982; Ingledew and Poole, 1984). The overall reaction is brought about by two different enzymic systems, the electron transport chain and the ATP synthase. These enzyme systems are integrated in a unit membrane which is basically impermeable to protons and other solutes. The ATP synthase within the membrane appears to be spatially separated from the enzymes which make up the electron transport chain. The ATP synthases isolated so far from phototrophic, aerobic, facultative, or anaerobic bacteria capable of electron transport-coupled phosphorylation

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show remarkable similarities in structure and function to those of mitochondria or chloroplasts (Downie *et al.*, 1979; Shavit, 1980; Maloney, 1982; Futai and Kanazawa, 1983; Senior and Wise, 1983; van Walraven *et al.*, 1983; Bokranz *et al.*, 1985, 1986). These ATP synthases seem to differ from the functional ATPases of purely fermentative bacteria which gain ATP merely from substrate level phosphorylation (Clarke *et al.*, 1979).

The electron transport chains of the chemotophic aerobic or anaerobic bacteria appear to differ considerably from those of mitochondria. This refers to the electron transport reactions catalyzed as well as to the constituents of the electron transport chains. While mitochondria perform respiration merely with oxygen, certain bacteria can use various other terminal acceptors including fumarate and elemental sulfur instead of oxygen [Thauer *et al.*, 1977; Thauer and Morris, 1984; Kröger, 1977, 1978). The cytochrome bc_1 complex, which is an essential constituent of the electron transport chains of mitochondria (Rich, 1984; von Jagow *et al.*, 1986), chloroplasts, certain phototrophic bacteria (Hauska *et al.*, 1983) and of *Paracoccus denitrificans* (Berry and Trumpower, 1985), seems to be missing in anaerobic and most of the aerobic chemotrophic bacteria. Even quinones do not appear to be obligatory components of all bacterial electron transport chains. Recently it was discovered that certain phosphorylative electron transport chains operate at redox potentials far below those of Q or MK (Macy *et al.*, 1986; Paulsen *et al.*, 1986).

This work is devoted to phosphorylative electron transport reactions of anaerobic bacteria (anaerobic respiration) that operate at very negative redox potentials. The free energies of the redox reactions allow the synthesis of maximally 1 ATP per 2 electrons or less. The anaerobic respiration of *Escherichia coli* has been extensively reviewed recently (Ingledew and Poole, 1984). The *E. coli* system has the advantage that some of the enzymes are known with respect to subunit composition and amino acid sequence from genetic studies. In spite of this advantage, knowledge of the mechanism of electron transport and energy conservation is still very limited.

Wolinella succinogenes

Growth of *W.* (formerly *Vibrio*) *succinogenes* can be sustained catabolically by reactions (a)–(d) (Bronder *et al.*, 1982; Macy *et al.*, 1986).

$$H_2 + \text{fumarate} \longrightarrow \text{succinate} \qquad \text{(a)}$$

$$\Delta G_0' = -86\,\text{kJ/mol}$$

$$\text{formate} + \text{fumarate} + H^+ \longrightarrow CO_2 + \text{succinate} \qquad \text{(b)}$$

$$\Delta G_0' = -90\,\text{kJ/mol}$$

$$H_2S + \text{fumarate} \longrightarrow S + \text{succinate} \quad \text{(c)}$$

$$\Delta G_0' = -53\,\text{kJ/mol}$$

$$\text{formate} + S + H^+ \longrightarrow CO_2 + H_2S \quad \text{(d)}$$

$$\Delta G_0' = -37\,\text{kJ/mol}$$

(The $\Delta G_0'$ values of reactions (b), (c), and (d) refer to CO_2 and H_2S in the gaseous states.)

The ATP yields (n_{ATP}/n_e) referring to equilibrium conditions were calculated from the $\Delta G'$ values of reactions (a)–(d), valid under growth conditions, and the assumed phosphorylation potential ΔG_P (44 kJ/mol ATP) of the growing bacteria (Kröger and Winkler, 1981) using Eq. (1).

$$n_{ATP} \cdot \Delta G_P = n_e \cdot \Delta G'/2 \quad (1)$$

As seen from Table I, the n_{ATP}/n_e ratio at equilibrium is 1 with reaction (a) and (b) and 0.5 with reaction (c) and (d). Earlier measurements of the P/e ratios of reactions (a) and (b) after extrapolation gave values of approximately 0.5 (Kröger and Winkler, 1981). However, more recent experiments suggest that the ratio is 0.33 (Mell *et al.*, to be published). The P/e ratios of reactions (c) and (d) have not yet been measured.

According to the Mitchell theory, it is assumed that the free energy of reactions (a)–(d) is transferred to the phosphorylation reaction by means of the $\Delta\tilde{\mu}_H$ across the bacterial membrane. The H^+/ATP ratio is assumed to be 3 ($n_H/n_{ATP} = 3$). With Eq. (2) these assumptions require that $\Delta\tilde{\mu}_H \geqslant 0.15$ V.

$$n_H \cdot \Delta\tilde{\mu}_H \cdot F = n_{ATP} \cdot \Delta G_P \quad (2)$$

Direct measurements of thiocyanate distribution across the membrane of inverted vesicles catalyzing reaction (a) gave a value of 0.18 V (Mell, 1985).

Table I. ATP Yields and H^+/e Ratios of Growing *W. succinogenes*[a]

Reaction	$\Delta G'$	n_{ATP}/n_e	P/e	n_H/n_e	H^+/e
(a) $H_2 \longrightarrow$ fumarate	−86[b]	1	0.33	2.5	1
(b) Formate $\longrightarrow$ fumarate	−90[c]	1	0.33	2.6	–
(c) $H_2S \longrightarrow$ fumarate	−47[d]	0.5	–	1.4	–
(d) Formate $\longrightarrow$ S	−43[e]	0.5	–	1.2	–

[a] The n_{ATP}/n_e and n_H/n_e ratios were calculated using Eqs. (1) and (3), respectively. The P/e and H^+/e ratios were measured as described in the text.
[b] $\Delta G_0'$.
[c] Concentrations of formate, fumarate, and succinate 0.1 M, $P_{CO_2} = 0.1$ bar.
[d] Equal concentrations of fumarate and succinate, $P_{H_2S} = 0.1$ bar.
[e] Formate concentration 0.1 M, $P_{CO_2} = P_{H_2S} = 0.1$ bar.

$$n_H \cdot \Delta\tilde{\mu}_H = n_e \cdot \Delta G'/2F \quad (3)$$

Using this value, the maximum n_H/n_e ratios (at equilibrium) are calculated according to Eq. (3) (Table I). The ratios are between 2 and 3 with reactions (a) and (b) and between 1 and 2 with reactions (c) and (d). Direct measurement of the H^+/e ratio with inverted vesicles reducing fumarate by H_2 [reaction (a)] in the absence of a $\Delta\tilde{\mu}_H$ gave a value of approximately 1 (Mell, 1985). The H^+/e ratio measured with bacteria catalyzing reaction (b) was smaller than 1 (Kröger, 1975; Mell, 1985). The H^+/e ratios associated with reactions (c) and (d) have not yet been measured. It is expected that these ratios are distinctly below 1.

Fumarate Respiration

The system catalyzing fumarate respiration with H_2 [reaction (a)] or formate [reaction (b)] has been investigated in great detail (Kröger, 1978; Kröger and Unden, 1985; Unden and Kröger, 1986). The electron transport chain consists of three different enzymes which are integrated in the bacterial membrane and which react with the MK present in the membrane (Kröger and Innerhofer, 1976a, b). The enzymes have been isolated, characterized (Kröger *et al.*, 1979; Unden *et al.*, 1980; Albracht *et al.*, 1981; Unden and Kröger, 1981; Unden *et al.*, 1982, 1984), and incorporated into liposomes (Unden and Kröger, 1982; Unden *et al.*, 1983; Unden and Kröger, 1983; Graf *et al.*, 1985; Unden and Kröger, 1986). Reaction (a) was catalyzed by liposomes containing hydrogenase, fumarate reductase, and vitamin K_1. With formate dehydrogenase, fumarate reductase, and vitamin K_1 incorporated, the liposomes catalyze reaction (b). The quinone-reactive site and the substrate site of fumarate reductase are localized on different subunits (Unden and Kröger, 1981). MK reacts with the cytochrome *b* ($E_m = -20$ mV) which forms the anchor of the enzyme in the membrane (Unden *et al.*, 1983). The residual part of the enzyme protrudes into the aqueous phase. Similarly, the reaction of formate dehydrogenase with MK is dependent of a low-potential cytochrome *b* ($E_m = -200$ mV), which is probably situated in the membrane (Unden and Kröger, 1983). The subunits (homodimer) carrying the formate site protrude into the aqueous phase (Unden *et al.*, 1983).

The substrate site of formate dehydrogenase is oriented toward the bacterial periplasm, while that of fumarate reductase faces the cytoplasm (Fig. 1) (Kröger *et al.*, 1980). Hydrogenase is accessible to nonpermeant redox dyes which are applied externally (Kröger and Winkler, 1981). Considering the nature of the redox carriers of the dehydrogenases and of fumarate reductase, it is likely that only the electrons of the donor substrates are transported to fumarate. This vectorial electron transport leads to the

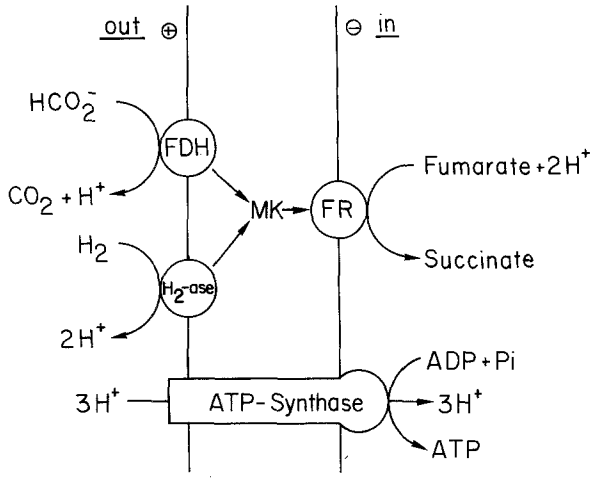

Fig. 1. Electron transport and hypothetical mechanism of $\Delta\tilde{\mu}_H$ generation ($1H^+/e$) in *W. succinogenes* catalyzing reaction (a) or (b).

generation of a $\Delta\tilde{\mu}_H$ without proton transport, provided that the proton exchange with the substrates is accomplished at the substrate sites or at least on the corresponding sides of the membrane. The H^+/e ratio predicted by this mechanism would be 1, in agreement with the experimental result (Table I). Additional proton translocation by MK as illustrated in Fig. 2 ($H^+/e = 2$) would not be required, but cannot be excluded with certainty. According to this mechanism (Fig. 2) the quinone-reactive site of fumarate reductase (cytochrome *b*) should be situated on the outer side of the bacterial

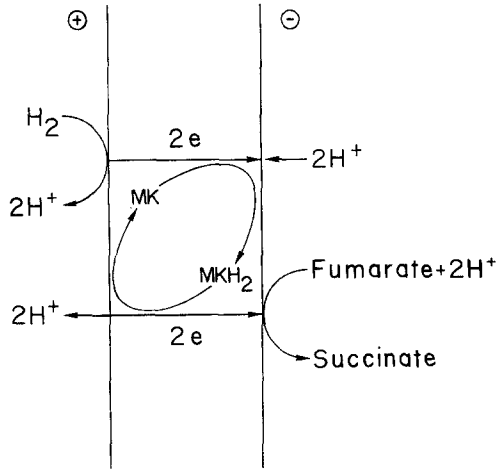

Fig. 2. Possible mechanism of $\Delta\tilde{\mu}_H$ generation ($2H^+/e$) in *W. succinogenes* catalyzing reaction (a).

membrane. Therefore, the oxidation of the reduced MK-analogue $DMNH_2$ by fumarate should generate a $\Delta\tilde{\mu}_H$ (positive outside). In fact the bacteria catalyze the rapid oxidation of $DMNH_2$ by fumarate. However, the reaction is not associated with the generation of a $\Delta\tilde{\mu}_H$ (Mell, 1985). This argues against the validity of the mechanism of Fig. 2.

The cytoplasmic membrane of *W. succinogenes* contains an ATP synthase which has been isolated, characterized, and incorporated into liposomes (Bokranz *et al.*, 1985, 1986). On application of a $\Delta\tilde{\mu}_H$ of sufficient magnitude, these liposomes catalyze the rapid synthesis of ATP from ADP and P_i.

Liposomes containing hydrogenase, vitamin K_1, fumarate reductase, and ATP synthase were found to catalyze the phosphorylation of ADP which is driven by reaction (a) (Graf *et al.*, 1985). The phosphorylation yield ($P/2e = 0.1$) was relatively high when the activity of the overall reaction was limited by the electron transport reaction. The velocity of phosphorylation was about 1% of that measured with the bacterial membrane. The $\Delta\tilde{\mu}_H$ generated across the membrane of the liposomes by reaction (a) was 0.1 V or less. When driven by an artificial $\Delta\tilde{\mu}_H$ of this magnitude the phosphorylation was similarly slow (Bokranz *et al.*, 1986).

The electron transport chain catalyzing reaction (c) has not yet been investigated. It may be speculated that the chain differs from that catalyzing reactions (a) or (b) (Fig. 1) in that hydrogenase or formate dehydrogenase is replaced by a H_2S dehydrogenase which would react with MK. In *Desulfuromonas acetoxidans* H_2S oxidation by fumarate was found to be dependent on the presence of the bacterial MK (Paulsen *et al.*, 1986). The H^+/e ratio brought about by reaction (c) is expected to be distinctly smaller than 1 (Table I). Therefore, the mechanism of $\Delta\tilde{\mu}_H$ generation by reaction (c) should differ from that of Fig. 1.

Sulfur Respiration

The composition and organization of the electron transport chain catalyzing reaction (d) is not yet known. From the redox potentials of CO_2/formate ($E_0' = -0.435$ V) and S/H_2S ($E_0' = -0.245$ V) it is obvious that MK ($E_0' = -0.075$ V) cannot be a member of the chain. Sulfur reduction by NADH which is catalyzed by the membrane of *D. acetoxidans* is independent of the presence of MK (Paulsen *et al.*, 1986). The cytoplasmic membrane of *W. succinogenes* grown on formate and S was found to catalyze DMN reduction by formate and H_2S oxidation by DMN with high specific activities. The latter activity is not present in bacteria grown with fumarate and formate. It may be caused by the enzyme functioning as a sulfur reductase in the growing bacteria. The relation of the sulfur reductase to

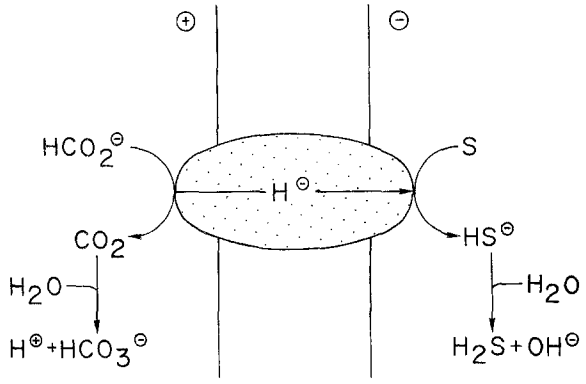

Fig. 3. Possible mechanism of $\Delta\tilde{\mu}_H$ generation ($0.5H^+/e$) in *W. succinogenes* catalyzing reaction (d).

formate dehydrogenase is currently investigated. Elucidation of the mechanism of electron transport is a prerequisite for understanding the mechanism of generation of the $\Delta\tilde{\mu}_H$.

According to the data given in Table I, the H^+/e ratio associated with S reduction by formate should be distinctly smaller than 1. A hypothetical mechanism with a H^+/e ratio of 0.5 is depicted in Fig. 3. According to this mechanism a hydride ion instead of 2 electrons (Fig. 1) is transported vectorially across the membrane. As a consequence a negative charge would disappear on the outside and would be created on the inside. Hydrolysis of the products of the electron transport reaction would give a proton on the outside and a hydroxyl ion on the inside. The mechanism would require that the sulfur site of sulfur reductase faces the inside. The sulfur reductase involved in sulfur reduction [reaction (d)] should, therefore, differ from the H_2S dehydrogenase catalyzing H_2S oxidation [reaction (c)] at least in this respect.

Desulfuromonas acetoxidans

D. acetoxidans grows anaerobically with acetate and S as sole energy substrates and forms CO_2 and H_2S as the products [reaction (e)] (Pfennig and Biebl, 1976). Acetate is metabolized via a modified citric acid cycle.

$$\text{Acetate} + 4S + 2H_2O + H^+ \longrightarrow 2CO_2 + 4H_2S \qquad \text{(e)}$$

$$\Delta G_0' = -39\,\text{kJ/mol acetate}$$

Acetyl-CoA is formed from the succinyl-CoA and acetate (Gebhardt *et al.*, 1985). ATP can be gained only from the electron transport to S of the

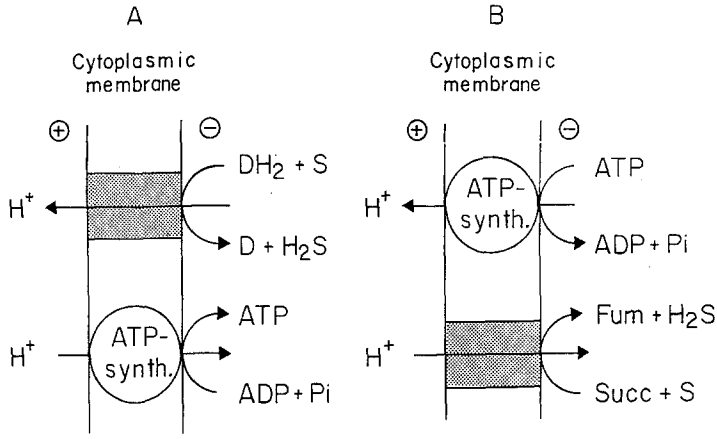

Fig. 4. Hypothetical mechanism of electron transport-coupled phosphorylation (A) and ATP-driven succinate oxidation (B) with sulfur as terminal electron acceptor in *D. acetoxidans*. DH_2, NADPH, reduced ferredoxin, or NADH.

reducing equivalents derived from isocitrate, 2-oxoglutarate, and malate. Therefore, it is likely that the ATP is formed by electron transport-coupled phosphorylation (Fig. 4A).

The oxidation of succinate (reaction f), which is a step in the citric acid cycle, is strongly endergonic.

$$\text{Succinate} + S \longrightarrow \text{Fumarate} + H_2S \qquad \text{(f)}$$

$$\Delta G_0' = +53\,\text{kJ/mol}$$

The exergonic reduction of fumarate by H_2S, which is catalyzed by the membrane fraction prepared from *D. acetoxidans*, is dependent on the bacterial MK as an electron transport component (Paulsen *et al.*, 1986). There is evidence that succinate oxidation [reaction (f)] and fumarate reduction involve the same enzyme which operates as a succinate dehydrogenase in the growing bacteria.

Reaction (f) is driven by reversed electron transport (Paulsen *et al.*, 1986). Inverted vesicles of the bacterial membrane catalyze reaction (f) provided that ATP is added. The reaction is fully inhibited by protonophores or DCCD. The activity of ATP hydrolysis, which is also catalyzed by the vesicles, is equally sensitive to DCCD as succinate oxidation driven by ATP. These results suggest that the cytoplasmic membrane of *D. acetoxidans* contains an ATP synthase which can operate as a proton translocating ATPase (Fig. 4B). The enzyme is thought to create a $\Delta\tilde{\mu}_H$ which drives reaction (f) in the experiment with the inverted vesicles. In growing bacteria

the $\Delta\tilde{\mu}_H$ is probably generated by sulfur reduction with the more negative reducing equivalents provided by the citric acid cycle. The $\Delta\tilde{\mu}_H$ is then used in phosphorylation as well as in succinate oxidation.

Reversed electron transport was earlier found to be used in the reduction of the pyridine nucleotides with more electropositive donor substrates by mitochondria (Klingenberg and Schollmeyer, 1960) or bacteria (Aleem, 1977; Knaff, 1978). While these reactions have anabolic functions, succinate oxidation by sulfur in *D. acetoxidans* represents the first example of reversed electron transport in catabolism.

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