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Phosphorylation and phosphate-ATP exchange catalyzed by the ATP synthase isolated from *Wolinella succinogenes*

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The ATP synthase, isolated from *Wolinella* (formerly *Vibrio*) *succinogenes* could be fully incorporated into liposomes without significant cleavage of the enzyme or loss of activity. These proteoliposomes, but not the isolated enzyme, catalyzed phosphate-ATP exchange and the phosphorylation of ADP which was driven by an artificially imposed $\Delta\tilde{\mu}_H$ across the liposomal membrane. Phosphorylation driven by light was catalyzed by proteoliposomes containing also bacteriorhodopsin. The three activities were similarly sensitive to protonophores or dicyclohexylcarbodiimide. This sensitivity was similar to that of the electron-transport-driven phosphorylation catalyzed by bacterial membrane vesicles. With a $\Delta\tilde{\mu}_H$ value of 280 mV to drive phosphorylation the turnover number of the enzyme was in the same order of magnitude as that measured in the electron-transport-driven phosphorylation catalyzed by the bacterial membrane. When the $\Delta\tilde{\mu}_H$ was below 150 mV, the phosphorylation activity of the incorporated enzyme was two orders of magnitude slower, and was about as fast as light-driven phosphorylation or as the exchange reaction.

Introduction

Using detergent extraction an ATP hydrolyzing enzyme was isolated from the cytoplasmic membrane of the anaerobic bacterium *Wolinella succinogenes* [1]. Structurally this enzyme resembled the ATP synthases of aerobic and phototrophic bacteria, mitochondria and chloroplasts, but its M_r value was lower and its composition simpler. In this communication we describe experiments involving incorporation of the enzyme into liposomes, and the capability of the resulting proteoliposomes to catalyze phosphorylation and phosphate-ATP exchange.

Methods

Preparation procedures

Incorporation of ATP synthase into liposomes. Soybean phospholipid (0.1 g, Sigma No. P 5638) was sonicated for 10 min at 20°C in 2.5 ml of the buffer indicated in the legends. Sonication was done using a Branson sonifier equipped with the microtip, at 40 W and 50% duty cycle. The resulting suspension was mixed with the ATP synthase isolated from *W. succinogenes* [1]. Unless otherwise indicated the protein/phospholipid ratio of the mixture was 10 mg/g. The mixture was frozen in liquid N_2 and then thawed at room temperature. This freeze-thawing was repeated twice.

Incorporation of ATP synthase and bacteriorho-

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Abbreviations: TTFB, 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazol; DCCD, *N,N'*-dicyclohexylcarbodiimide; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Mes, 4-morpholineethanesulphonic acid; FCCP, carbonylcyanide-4-trifluoromethoxyphenylhydrazone; Aminoxid WS 35, acyl(C_{11}–C_{17})amidopropyldimethylaminoxide.

dopsin. Soybean phospholipid was sonicated in 50 mM Hepes at pH 8.0 as described above. After the addition of 20 mg bacteriorhodopsin/g phospholipid, the mixture was sonicated in the same manner an additional 15 min. Then ATP synthase (20 mg protein per g phospholipid) was added, and the mixture was freeze-thawed as described above.

Analytical procedures

One unit (U) of enzyme activity (in phosphate-ATP exchange, ATP formation or hydrolysis) was equivalent to the turnover of 1 μmol phosphate per min. The enzymic activities were given as turnover numbers in most instances. The turnover numbers were calculated from the specific activities and the molar mass of the ATP synthase protein ($4.1 \cdot 10^5$ g protein/mol).

Phosphate-ATP exchange. The reaction mixture (1 ml at 37°C) contained 50 mM Hepes (pH 8)/2 mM ATP/1 mM $MgCl_2$/0.5 mM [^{32}P]phosphate (7.4 GBq/mol). The reaction was started by addition of proteoliposomes containing ATP synthase in an amount equivalent to 20 μg protein. After 5, 10, 15 and 20 min, 0.1 ml samples were taken and mixed with 1 ml of a solution containing 10 mM $(NH_4)_2MoO_4$ and 0.7 M H_2SO_4. The inorganic phosphate was extracted twice from this solution using a mixture of isobutanol and benzene (1 : 1, v/v). The extracted aqueous phase was analyzed for organic phosphate using liquid scintillation counting.

Phosphorylation driven by $\Delta\tilde{\mu}_H$ transition. The proteoliposomes containing ATP synthase were incubated for at least 5 min with 45 nmol valinomycin per g phospholipid. The phosphorylation reaction was started by injecting 50 μl of the liposomal suspension (about 20 μg ATP synthase protein) into 0.45 ml of a buffer (pH 9.0 and 22°) containing 0.2 M glycylglycin/0.8 mM ADP/0.4 mM $MgCl_2$/10 mM phosphate (test buffer). The reaction was terminated by the addition of 1 M $HClO_4$. Following neutralization with KOH, the ATP concentration was determined using the luciferase assay [2].

Light-driven phosphorylation. Proteoliposomes containing ATP synthase (80 μg protein) and bacteriorhodopsin [3] were suspended in 1 ml of a solution containing 50 mM Hepes (pH 8.0)/5 mM ADP/2.5 mM $MgCl_2$/50 mM glucose/15 U hexokinase/1 mM [^{32}P]phosphate (7.4 GBq/mol). The mixture was held in a glas water bath (37°C) that permitted illumination with a slide projector (250 W) at a distance of 10 cm. The projector was equiped with a 515 mm cut-off filter (2 mm wide OG 515, supplied by Schott, Mainz). The reaction was started by illumination. After 5, 10, 15 and 20 min, 0.15 ml samples were removed and assayed for organic phosphate as described above. A blank was kept in the dark.

Protein determination. Protein was measured photometrically using the Biuret method with KCN [4]. Bovine serum albumin was used as standard. Alternatively, protein was assayed with Amido black on Nitrocellulose sheets [5].

Results

Incorporation of the ATP synthase into liposomes

A suspension of sonicated liposomes was mixed with ATP synthase, at a protein/phospholipid

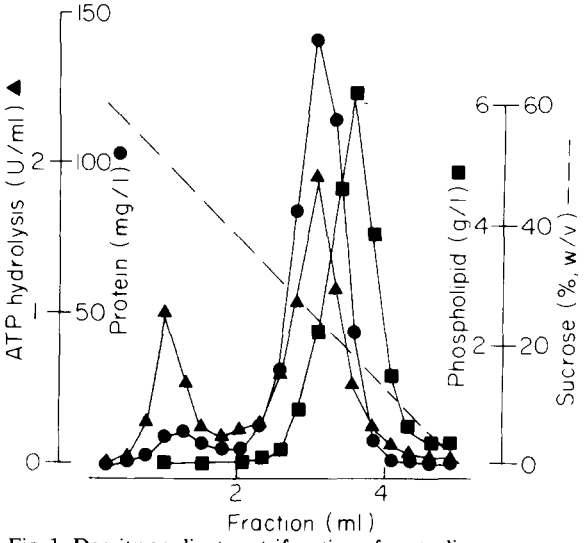

Fig. 1. Density gradient centrifugation of proteoliposomes containing ATP synthase. The prtoeoliposomes were prepared in 50 mM Hepes (pH 8.0) at a ratio of 20 mg protein/g phospholipid. The preparation (0.12 mg ATP synthase protein) was layered onto 5 ml of the same buffer (0°C) containing a linear sucrose gradient (0–60%, w/v). After centrifugation for 3.5 h at 65000 rpm in a VTi 65 rotor (Beckman Instruments), the content of the centrifuge tube was fractionated, and ATPase (photometric assay described in Ref. 1), protein and phospholipid were measured. Phospholipid was determined by counting the radioactivity of [^{14}C]dipalmitoylphosphatidylcholine, which had been added to the phospholipid used for preparing the liposomes.

ratio of about 20 mg/g, and freeze-thawed. To determine whether proteoliposomes were formed, the preparation was subjected to sucrose density gradient centrifugation (Fig. 1). After centrifugation the contents of the centrifuge tube was fractionated, and in each fraction ATP hydrolysis activity, protein and phospholipid content were determined. To be certain that the activity of enzyme molecules facing the inside of the liposomes was also recorded, the enzymic activity was measured after lysis of the liposomes with Aminoxid WS 35. Approx. 90% of the protein and 70% of the enzymic activity was found in coinciding bands, located near the phospholipid band. The remaining protein and activity was found in coinciding bands at a greater sucrose density (free enzyme). This suggested that most of the enzyme had been incorporated into the liposomes. The liposomal preparation was heterogeneous with respect to the protein/phospholipid ratio, which ranged between 25 and 100 mg/g in the main fractions of the protein band (Fig. 1).

The specific activity of the enzyme associated with the phospholipid was the same as that of the ATP synthase after isolation. In contrast, the specific activity of the free enzyme was about 5-times greater. This suggested that part of the free enzyme consisted of the ATPase, the specific activity of which is known to be 15 times that of the ATP synthase [1]. From specific enzyme activities it was estimated that about 20% of the free enzyme protein consisted of ATPase. As the free enzyme amounted to 10% of the total protein, the ATP synthase preparation contained only 2% ATPase.

When the proteoliposome preparation was freeze-thawed in the presence of labeled taurine, and then separated from the medium by gel filtration (not shown), this non-permeant solute was retained. Based upon the amount of taurine associated with the phospholipid, the internal volume was determined [6] to be 1.5 ml/g phospholipid. From this value and from the space requirement of a phospholipid molecule (0.7 nm^2), the average inner diameter of the proteoliposomes was calculated to be 35 nm. Taking into account thickness of the membrane (5 nm) the number of phospholipid molecules per proteoliposome was calculated from the inner diameter to be $1.4 \cdot 10^4$.

The accessibility of the ATP synthase to ATP in the proteoliposomes separated from the free enzyme was determined by measuring the activity of ATP hydrolysis in the presence and absence of melittin (3 mg/g phospholipid). The ratio of the activities was 0.7 suggesting that 70% of the enzyme molecules was oriented towards the liposomal outside [7]. When detergents such as Triton X-100 or Aminoxid WS 35 were used instead of melittin, lower ratios were measured. This difference in ratios is probably due to the stimulation of the enzyme by these detergents; as they were found to stimulate the isolated enzyme. The amount of Aminoxid (10 mmol per mol phospholipid) introduced into the liposomal preparation with the ATP synthase was less than 10% of that required for lysis of the proteoliposomes.

Electron microscopy

After negative staining of the liposomal preparation, unilamellar vesicles (i.e., proteoliposomes) studded with particles on the outer surface

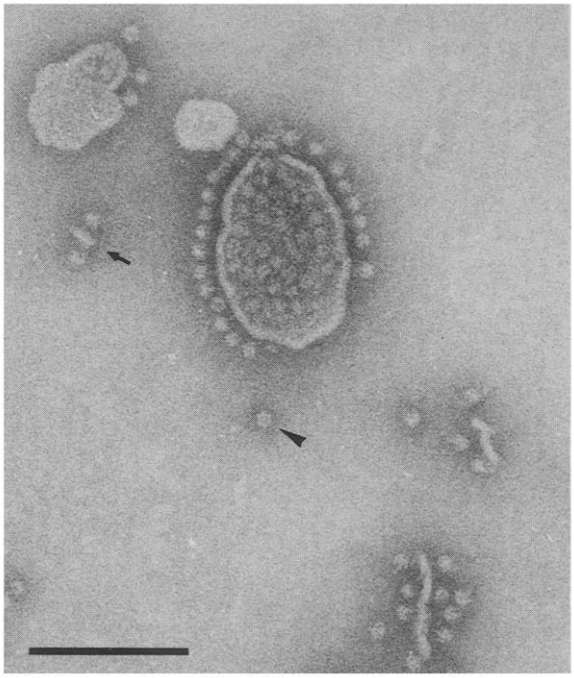

Fig. 2. Electron micrograph of the proteoliposomal preparation after negative staining. The staining was done as described previously [1]. A proteoliposome, two liposomes, free ATP synthase (→) and ATPase molecules (face view ▶) are seen. Magnification, ×207000. The bar represents 100 nm.

were seen in electronmicrographs (Fig. 2). The diameter of the proteoliposomes varied from 40 to 170 nm, with an average of 96 nm. This value is about twice that calculated from the internal volume of the preparation.

The particles had the appearance and dimensions of ATP synthase molecules [1], the base pieces of which were inserted into the liposomal membrane. While most of the particles were associated with the liposomes, some free ATP synthase and ATPase molecules were also seen. Most of these ATP synthase molecules were linked together at their base pieces.

The preparation was heterogeneous with respect to number of particles per liposome. Part of the liposomes were densely covered with enzyme molecules, while others contained only a few or no particles. This observation was in agreement with the finding that preparations were heterogeneous with respect to the protein/phospholipid ratio (Fig. 1).

Phosphate-ATP exchange

The proteoliposomes catalyzed the phosphate-ATP exchange reaction [8], while the isolated enzyme lacked this activity (Fig. 3). The pH optimum value for the activity was at about 8. With 2 mM ATP and 0.5 mM phosphate present, the

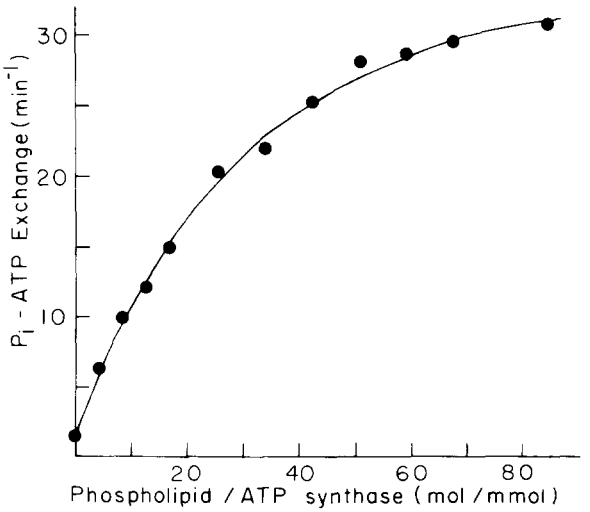

Fig. 3. Phosphate-ATP exchange activity as a function of the phospholipid/ATP synthase ratio used for the preparation of the proteoliposomes. The proteoliposomes were prepared in 50 mM Hepes (pH 8.0).

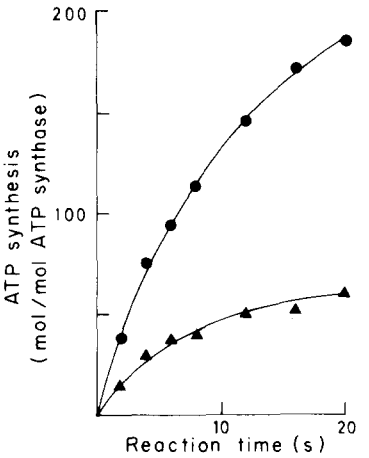

Fig. 4. Time-course of ATP synthesis driven by a $\Delta\tilde{\mu}_H$ transition. The proteoliposomes were prepared in 200 (●) or 50 mM (▲) sodium maleinate (pH 5.0) and were injected into a test buffer which also contained 0.1 M KCl.

activity remained constant for 20 min (not shown). After this period of time approx. 1/3 of the ATP had been hydrolyzed.

In the experiment shown in Fig. 2, proteoliposomes containing various amounts of ATP synthase were used. Complete incorporation of the enzyme into liposomes was corroborated using density gradient centrifugation. The turnover number of the enzyme increased as the phospholipid/protein ratio of the proteoliposomes was increased. Saturation of the activity (about 40 min^{-1}) required ratios greater than 100 mol phospholipid per mmol ATP synthase. When the ratio of phospholipid/ATP synthase was 14 mol/mmol, it was estimated (based upon the average size of the liposomes, $1.4 \cdot 10^4$ molecules of phospholipid) that each liposome contained a single ATP synthase molecule. Lower enzyme turnover numbers were measured using proteoliposomes prepared from phosphatidylethanolamine (Sigma No. P 6398), as compared to those prepared from the soybean phosphatidylcholine used in the experiment described here.

ATP synthesis driven by $\Delta\tilde{\mu}_H$ transition

Proteoliposomes prepared in a maleinate buffer at pH 5 catalyzed the formation of ATP from ADP and phosphate when injected into a buffer of pH 9 (Fig. 4). The reaction proceeded as an approximately linear function of time for 5 s after

the injection and slowed down thereafter. With proteoliposomes containing 0.2 M maleinate, the initial velocity of this phosphorylation reaction at 22°C (1200 min^{-1}) was about 40-times greater than that of the phosphate-ATP exchange reaction at 37°C (Fig. 3). The activity was less with proteoliposomes containing the buffer at a lower concentration.

In the experiment shown in Fig. 5, proteoliposomes containing 0.2 M maleinate at pH 5 were incubated with various amounts of valinomycin and then permitted to react in a buffer (pH 9) containing KCl for 5 s. Although phosphorylation was observed in the absence of valinomycin (20% of the maximum activity), the activity was increased by its presence according to a saturation curve. Saturation required more than 100 nmol valinomycin/g phospholipid, while 50% saturation was observed with 5 nmol valinomycin per g phospholipid.

Phosphorylation activity was related to the concentration of ADP or phosphate according to the Michaelis equation (data not shown). Under optimal reaction conditions (those of Fig. 5), the K_M for phosphate was 0.59 mM with 0.4 mM ADP present.

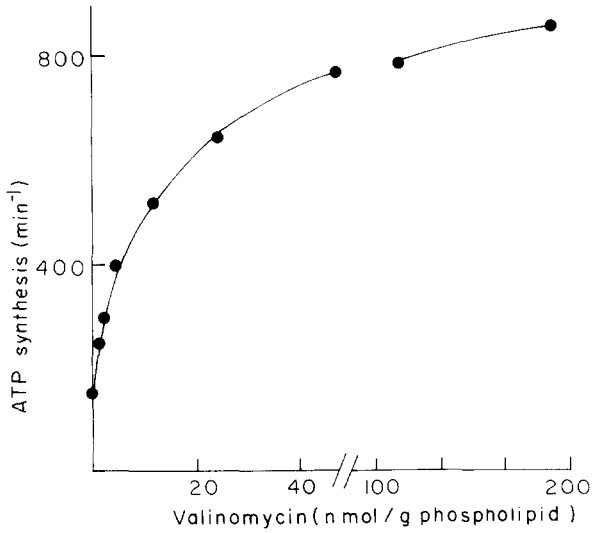

Fig. 5. The turnover number of ATP synthesis driven by $\Delta\tilde{\mu}_H$ transition as a function of the amount of valinomycin present. The proteoliposomes were prepared in 0.2 M sodium maleinate (pH 5.0) and were injected into test buffer which also contained 0.1 M KCl. The reaction time was 5 s.

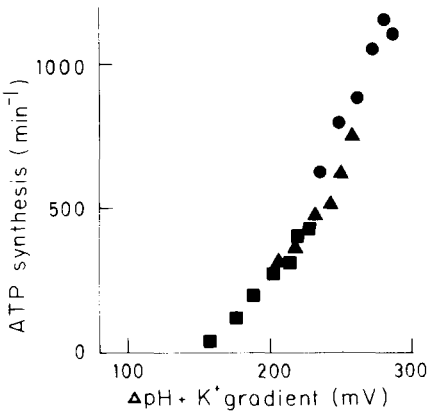

Fig. 6. The turnover number of ATP synthesis as a function of H^+ and K^+ gradient. The proteoliposomes were prepared in 0.2 M maleinate (pH 5.0) (●), 0.2 M malonate (pH 5.5) (▲) or 0.2 M Mes (pH 6.0) (■), containing 20 mM KCl. The reaction was started by injection of the preparation into a test buffer containing up to 100 mM KCl. The reaction time was 5 s. The K^+ gradient (external/internal concentration) was calculated taking into account the K^+ content of the phospholipid used.

The initial rate of ATP synthesis (expressed as the turnover number) was a function of the ΔpH and the potassium gradient (external/internal concentration) across the liposomal membrane (Fig. 6). This was shown with proteoliposomes that had been prepared with three different buffers (pH 5, 5.5 and 6) containing 20 mM K^+. The liposomal suspensions were injected into buffer solutions (pH 9) containing up to 100 mM KCl and were permitted to react for 5 s. The turnover numbers were plotted against the sum of the H^+ and K^+ gradients, expressed in terms of electrical potentials. Such plots revealed the equivalence of both gradients. The turnover numbers were as small as those of the phosphate-ATP exchange (below 100 min^{-1}) when initial potentials were below 150 mV, while the maximum turnover number (1200 min^{-1}) was observed when the initial potential was 280 mV.

Light-driven phosphorylation

When bacteriorhodopsin was incorporated into liposomes together with ATP synthase, these proteoliposomes, upon illumination, catalyzed the formation of organic phosphate from inorganic phos-

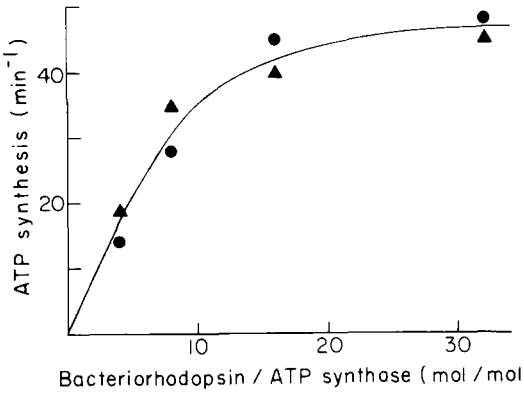

Fig. 7. Light driven ATP synthesis as a function of the bacteriorhodopsin/ATP synthase ratio. The proteoliposomes were prepared using (per g phospholipid) either 10 mg bacteriorhodopsin and 2.5–20 mg ATP synthase protein (▲) or 10 mg ATP synthase and 2.5–20 mg bacteriorhodopsin (●).

phate in the presence of ADP, hexokinase and glucose. Most of this activity (80–90%) was sensitive to protonophores. The residual activity (blank reaction) was due to the incorporation of inorganic phosphate into ADP. The blank reaction was insensitive to protonophores, did not require light and was also catalyzed by the isolated enzyme. In the experiment shown in Fig. 7, the turnover number of ATP synthase in light-driven phosphorylation was measured as a function of the molar ratio bacteriorhodopsin/ATP synthase in the liposomes. Two types of preparation were used. The first contained an average of 0.3 molecules ATP synthase per liposome and increasing amounts of bacteriorhodopsin. The second preparation contained six molecules of bacteriorhodopsin (M_r 26000) per liposome and assuming complete incorporation) and decreasing amounts of ATP synthase. The activities measured using both preparations followed nearly the same saturation curve, suggesting that the ATP synthase was rate-limiting at the higher bacteriorhodopsin-to-ATP synthase ratios. The maximum turnover number of ATP synthesis (50 min^{-1} at 37°C) was close to that of the phosphate-ATP exchange reaction (Fig. 3), and was approx. 20-times lower than that of $\Delta\tilde{\mu}_H$-driven phosphorylation at 22°C. The turnover number was constant for 20 min, indicating that the enzyme performed several hundreds of turnovers during the experiment. Using a preparation with a saturating bacteriorhodopsin/ATP synthase ratio, at various light intensities (not shown), it was found that the experiment of Fig. 7 had been done under nearly light-saturating conditions. The phosphorylation activity was related to the concentration of ADP according to the Michaelis equation (data not shown); with 1 mM phosphate present, the K_M value for ADP was found to be 15 μM.

Inhibition of the activities of liposomal ATP synthase

The phosphorylation reactions, as well as the phosphate-ATP exchange reaction, were inhibited by protonophores. Upon titration with TTFB [9], the activities were decreased hyperbolically with increasing amounts of the inhibitor (Fig. 8). The activities of the $\Delta\tilde{\mu}_H$ driven phosphorylation and of the exchange reaction followed the same titration curve. 50% inhibition was achieved with 0.3 μmol TTFB per g phospholipid, while full inhibition required more than 10 times this amount. 50% and full inhibition of light-driven phosphorylation was achieved with 0.6 μmol and with more than 10 μmol TTFB per g phospholipid, respectively. The same concentrations of FCCP yielded similar re-

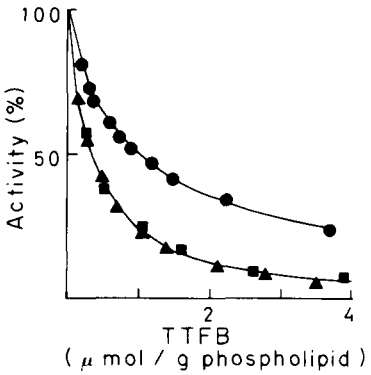

Fig. 8. Titration of the activities of phosphorylation and phosphate-ATP exchange with TTFB. The exchange reaction (▲) was measured with proteoliposomes (20 mg ATP synthase protein/g phospholipid) prepared as described in the legend of Fig. 2. $\Delta\tilde{\mu}_H$-driven phosphorylation (■) was measured using proteoliposomes prepared with 0.2 M maleinate (pH 5.0). The test buffer also contained 0.1 M KCl. The reaction time was 5 s. Light-driven phosphorylation (●) was assayed as described in the Methods section. TTFB was added in a dimethylsulfoxide solution. Full (100%) activity corresponded to the following turnover numbers: 29 min^{-1} (▲), 57 min^{-1} (●) and 625 min^{-1} (■).

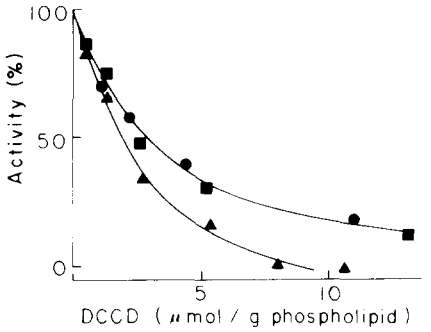

Fig. 9. Titration of phosphorylation and phosphate-ATP exchange activities with DCCD. ■, $\Delta\tilde{\mu}_H$-driven phosphorylation. ●, light-driven phosphorylation. ▲, phosphate ATP exchange. The experiments were done as described in the legend of Fig. 7. DCCD, in dimethylsulfoxide solution, was added to the proteoliposomes 10 min before the reaction was started. Full (100%) activity corresponded to the following turnover numbers: 980 min^{-1} (■), 25 min^{-1} (●) and 15 min^{-1} (▲).

sults (data not shown). In earlier experiments with bacterial membrane vesicles, electron transport-driven phosphorylation was found to be 50% and fully inhibited with 0.5 μmol and 5 μmol FCCP per g membrane protein, respectively.

The phosphorylation reactions as well as the exchange activity were inhibited by DCCD (Fig. 9). Maximum inhibition, with any given amount of DCCD, required incubation of the proteoliposomes with the inhibitor for 10 min. The inhibition curves were hyperbolic. The phosphorylation activities followed the same titration curve; 50% inhibition occurred in the presence of 3 μmol DCCD/g phospholipid. This amount was equivalent to 120 mol DCCD/mol ATP synthase. The exchange activity was slightly more sensitive to DCCD with 50% inhibition being achieved with 2 μmol DCCD/g phospholipid. As a comparison, full inhibition of the electron-transport-driven phosphorylation catalyzed by membrane vesicles was observed with 80 mol DCCD per mol ATP synthase [10]. The ATP hydrolysis activity of the bacterial membrane, or the isolated or liposomal ATP synthase were not effected by incubation with 1000 mol DCCD per mol ATP synthase.

Discussion

The electron transport from H_2 to fumarate (reaction a) is catalyzed

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

by the cytoplasmic membrane of *W. succinogenes*, and is coupled to phosphorylation of ADP [10–12]. The phosphorylation is blocked by protonophores or DCCD. This finding suggests that the free energy of reaction (a) is conserved in a $\Delta\tilde{\mu}_H$ across the membrane, which is then used by an ATP synthase to drive phosphorylation [13]. This view is supported by the finding that liposomes containing the hydrogenase, fumarate reductase and ATP synthase isolated from *W. succinogenes* catalyze phosphorylation driven by reaction (a) [14]. The turnover number of the ATP synthase in this reaction was, however, only about 1% of that measured with the bacterial membrane (Table I). To determine the reason for this discrepancy we have investigated the energy transduction capability of the *W. succinogenes* ATP synthase.

We have found that the turnover number of the reincorporated enzyme in light-driven phosphorylation and in the phosphate-ATP exchange reaction is as slow as that in the reconstituted system of electron-transport-driven phosphorylation [14]. When the phosphorylation is driven by an artificially imposed $\Delta\tilde{\mu}_H$ of sufficient magnitude (nearly 300 mV), however, the turnover number is nearly two orders of magnitude greater and amounts to 25 and 50% of that of the enzyme in growing bacteria and in membrane vesicles, respectively (Table I). This suggests that the isolated enzyme has retained its original capability of energy transduction. This view is supported by the finding that the phosphorylation catalyzed by the proteoliposomes is similar in its sensitivity to protonophores or DCCD, as the electron-transport-driven phosphorylation catalyzed by bacterial membrane vesicles.

Phosphorylation turnover number is dramatically dependent upon the magnitude of the initial $\Delta\tilde{\mu}_H$ applied. Small values were measured when the initial $\Delta\tilde{\mu}_H$ was below 150 mV, while attainment of maximum velocities required values of nearly 300 mV. Similar results were obtained earlier when an artificial $\Delta\tilde{\mu}_H$ was applied to mitochondrial particles [17], to thylakoids from spinach chloroplasts [18] or to proteoliposomes containing the ATP synthase of the thermophilic bacterium 'PS3' [19]. It should be mentioned that

TABLE I

TURNOVER NUMBERS OF THE ATP SYNTHASE OF *W. SUCCINOGENES* CATALYZING PHOSPHORYLATION AT 37°C

The turnover number for growing bacteria was determined using the specific growth rate (0.46 h^{-1}), the growth yield (1.56 g dry cells/mol ATP [15,16] and the bacterial enzyme content (25 μmol ATP synthase/g dry cells [1]). The enzyme content of the membrane vesicles used was 0.1 μmol ATP synthase per g protein [1]. The sidedness of the enzyme in the proteoliposomes (30% inside) was corrected for. A temperature increase of 10°C was assumed to double the enzymic activity.

Preparation	Driving force	Turnover number (min^{-1})
Growing bacteria	electron transport	20000
Membrane vesicles	electron transport	10000
Proteoliposomes	artificial $\Delta\tilde{\mu}_H$	5000
Proteoliposomes	bacteriorhodopsin + light	70
Proteoliposomes	electron transport	60 [14]

the $\Delta\tilde{\mu}_H$ generated by electron transport across the membrane of vesicles from *W. succinogenes,* was found to be 180 mV [20]. Based upon the results presented in Fig. 6, this potential should permit only 10% of the phosphorylation turnover number actually measured with the vesicles (Table I). The discrepancy could be explained on the basis that the initial $\Delta\tilde{\mu}_H$ given in Fig. 6 decayed rapidly to a lower value after start of the reaction.

The low turnover number of the ATP synthase in the reconstituted liposomal system of electron-transport-driven phosphorylation [14] can now be understood because of the $\Delta\tilde{\mu}_H$-dependence of this parameter. In actuality, the $\Delta\tilde{\mu}_H$ generated by the reconstituted electron transport was amximally 100 mV [14]. The low rate of light-driven phosphorylation may also be explained by a low $\Delta\tilde{\mu}_H$ generated with bacteriorhodopsin. In general, the $\Delta\tilde{\mu}_H$ generated by light across the membrane of liposomes containing bacteriorhodopsin were found to be less than 150 mV [21–23]. Correspondingly, the turnover numbers of phosphorylation of the ATP synthase were generally below 100 min^{-1} [21,24–26]. The highest rates of light-driven phosphorylation (250 min^{-1}) thus far measured, using liposomes containing bacteriorhodopsin and a mitochondrial ATP synthase [27] are about 5-times greater than those observed in our studies, but are 20-times lower than those obtained with artificially imposed $\Delta\tilde{\mu}_H$ (Table I).

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