ATP SYNTHESIS DRIVEN BY PROTONMOTIVE FORCE IMPOSED ACROSS *ESCHERICHIA COLI* CELL MEMBRANES

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1. Introduction

ATP synthesis driven by the imposition of H^+ or/and K^+ ion gradients across the membrane of the grana [1–9], mitochondria [10–12] and chromatophores [13,14] have strengthened the concept that the transmembrane protonmotive force may be used for the reversal of ATP hydrolysis, as was originally proposed by Mitchell [15]. Recently it has been reported [16–17] that ATP hydrolysis by cytoplasmic membrane particles prepared by sonication or mechanical disruption of *E. coli* cells could be coupled with inward translocation of H^+ ions. DCCD-sensitive extrusion of H^+ ions has been observed in experiments with *E. coli* cells [18]. The ATP hydrolysis-couple accumulation of lipophilic anions in sonicated *E. coli* membrane particles and enhancement of 1-anilino naphthalene-8-sulfonate fluorescence [18,19] support the view that H^+ ion translocation across *E. coli* membrane through the ATPase system is electrogenic.

Here we report an examination of the reversal of ATP hydrolysis in *E. coli* cells by protonmotive force. We have shown that artificially imposed membrane potential and transmembrane pH gradient drive ATP synthesis.

While the present paper was in preparation it was reported [20] that the imposition of a K^+ ion gradient across membranes of *S. lactis* and *E. coli* cells leads to the increase in the intracellular ATP concentration.

2. Methods

E. coli strains B (wild type) and ML 308-225 (i^-, z^-, a^+, y^+) a generous gift of Dr H. R. Kaback (Nutley, USA) were grown in the liquid minimal salt medium A with 0.25% glucose as the sole carbon source under the conditions described earlier [18]. The harvested cells were treated with Tris–EDTA and sucrose-washed cells were prepared as described by West and Mitchell [21]. The K^+-loaded cells were prepared by incubation of Tris–EDTA-treated cells in 0.1 M potassium phosphate buffer (pH 7.0) for 20 min at 37°C. Then the cells were pelleted in a refrigerated centrifuge, washed once with chilled 0.25 M sucrose and suspended in the same solution. DCCD-treated cells were prepared by incubation of Tris–EDTA-treated cells in 0.1 M potassium phosphate buffer (pH 7.0) supplemented with 1.5 mM DCCD for 20 min at 37°C. Then the cells were washed and suspended as described above. Intracellular ATP was extracted as described by Hempfling [22] and assayed with the hexokinase–glucose 6-phosphate dehydrogenase system.

3. Results and discussion

3.1. *Evidence for membrane potential generation by a K^+ gradient across valinomycin-treated* E. coli *membrane*

Recently the uptake of lipid-soluble cations and several amino acids driven by a K^+ gradient across valinomycin-treated *E. coli* membrane vesicles has been demonstrated [23,24]. The changes in the intensity of ANS^- fluorescence have been observed [19] in suspensions of intact cells under similar conditions. All these effects were postulated to be due to transient membrane potentials generated by the separation of

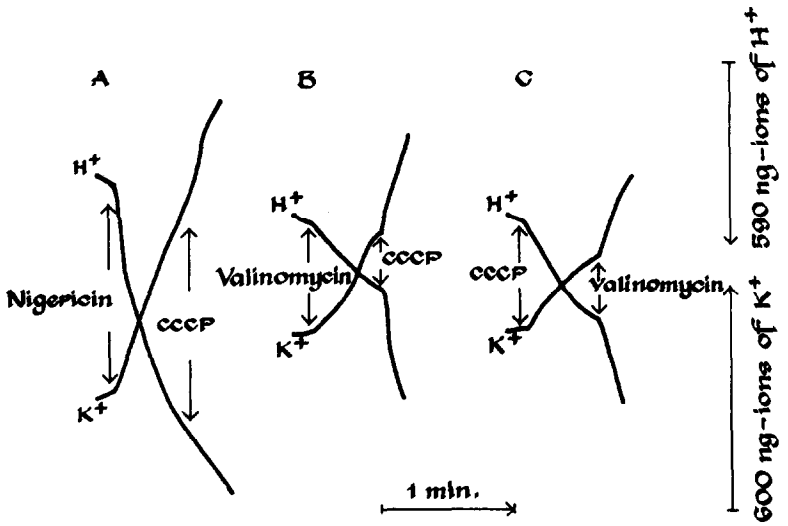

Fig.1. Effect of nigericin, valinomycin and FCCP on H^+ influx and K^+ efflux from K^+-loaded cells. *E. coli* ML 308-225 was suspended at a cell density of 1.2 mg dry wt/ml in 0.4 M sucrose with 3 mM glycyl-glycine (pH 3.9) Additions: nigericin, valinomycin (both to a final concentration of 1 μg/ml) and 1×10^{-6} M FCCP.

positive and negative charges by valinomycin-facilitated transport of K^+ along a concentration gradient. Contrarily to this it could be postulated that valinomycin-facilitated diffusion of K^+ ions from the cells and cell membrane vesicles is electroneutral. Therefore these effects could be due to a high-energy state of the *E. coli* membrane generated by the reversal of an electroneutral K^+ pump.

The results presented in fig.1 strongly support the concept of electrogenic K^+ diffusion. The K^+-loaded cells were suspended in a K^+-free medium. Treatment of cells with nigericin leads to the rapid exit of K^+ to the medium and to the uptake of H^+ ions (Expt A, fig.1). The addition of the protonophore FCCP has no effect on the rate of K^+ exit and H^+ uptake. Experiments B and C of fig.1 show that after treatment of cells with valinomycin or FCCP the maximal effects are no longer observable. Only the combination of valinomycin with uncoupler induces the maximal rate of the K^+ exit and the H^+ uptake. Therefore, the maximal rate of the K^+ diffusion along the concentration gradient is achieved under conditions of electroneutral K^+/H^+ exchange catalyzed by nigericin or by a combination of valinomycin and uncoupler. The slow rate of the K^+ exit from cells whose membrane was made permeable to K^+ in the absence of protonophore indicates that K^+ diffusion is limited by a negative electrostatic potential inside the cells. The compensation of this potential by the protonophore-induced uptake of H^+ ions facilitates K^+ diffusion along its concentration gradient.

3.2. *ATP synthesis in* E. coli *cells driven by membrane potential and pH gradient*

ATP synthesis in the cells driven by the membrane potential has been examined (table 1). Addition of valinomycin to the K^+-loaded cell suspension incubated in the presence of sodium cyanide causes an increase in the intracellular ATP concentration (Expt 1, table 1). The ATP synthesis is dependent on the amount of valinomycin added (Expt 2, table 1) and is inhibited in the presence of the uncoupler CCCP (Expt 3, table 1) or in the ATPase inhibitor-treated cells (Expt 4, table 1). The addition of nigericin (Expt 5, table 1) caused only a slight increase in the ATP yields. In experiments 6 and 7 the cells were incubated anaerobically in the absence of sodium cyanide. The induction of oxidative phosphorylation by injection of hydrogen peroxide leads to an increase in the intracellular ATP concentration (Expt 6, table 1), the process being sensitive to uncoupler and ATPase inhibitor (Expt 7, table 1). ATP yield in oxidative phosphorylation is

Table 1
ATP synthesis in K^+-loaded cells

No.	Addition (final concentration in parenthesis)	nmoles of ATP synthesized per mg of dry cell weight
1	Valinomycin (30 μg/ml)	0.69
2	Valinomycin (15 μg/ml)	0.22
3	Valinomycin (30 μg/ml) to the cell suspension supplemented with 6×10^{-6} M CCCP	0.02
4	Valinomycin (29 μg/ml) to the DCCD-treated cell suspension	0
5	Nigericin (20 μg/ml)	0.03
6	H_2O_2 (20 mM)	0.70
7	H_2O_2 (20 mM) to the cell suspension supplemented with 2.5×10^{-5} M CCCP or with 1×10^{-3} M DCCD	0

E. coli B was suspended at a cell density of 71–92 mg dry wt/ml and incubated for 3 min at 37°C in 0.25 M sucrose with 25 mM Tris–citrate (pH 8.0) and 15 mM NaCN (Expts 1–5). In Expts 6 and 7 the same cells were suspended at a density of 39 mg wt/ml in 0.25 M sucrose with 25 mM Tris–citrate (pH 6.0) and catalase (0.1 mg/ml) and incubated for 20 min at 37°C. Then the additions shown in the table were made and the ATP concentration increase in cells after 30 seconds was assayed.

comparable with ATP yield in membrane potential-driven phosphorylation. It must be pointed out that both processes are sensitive to uncouplers of oxidative phosphorylation.

ATP synthesis driven by a membrane potential should be related to the magnitude of the potassium gradient imposed. The data in fig.2 indicate that ATP is formed when K^+-loaded cells are treated with valinomycin in K^+-free medium (Expt 1, fig.2). An increase in the intracellular ATP concentration is observed during the first 30 seconds of incubation and is followed by a decay. The K^+-loaded cells suspended in 0.2 M KCl failed to synthesize ATP under the standard assay conditions indicating a requirement for a K^+ gradient across the cell membrane (Expt 2, fig.2). This conclusion is confirmed by the results of experiment 3 indicating the absence of ATP synthesis in cells which were not loaded with K^+.

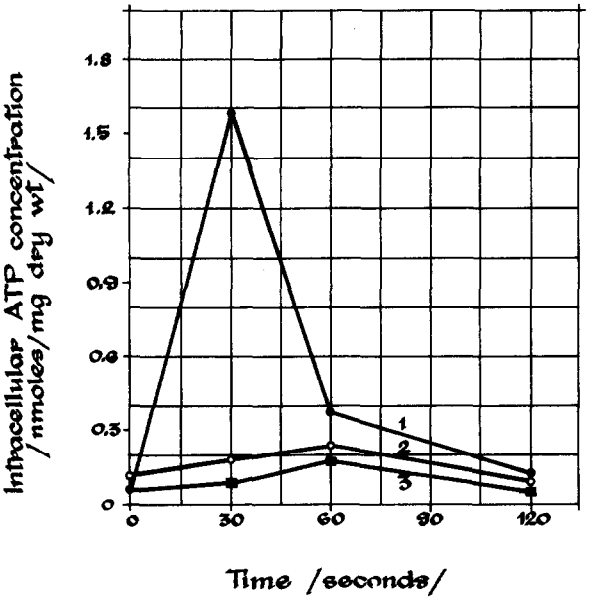

Fig.2. Dependence of ATP yield on the potassium concentration gradient across the cell membrane. K^+-loaded *E. coli* B cells (see Expts 1 and 2) were suspended at a density of 84 mg wt/ml in 0.25 M sucrose with 25 mM Tris–citrate (pH 8.0) and 15 mM NaCN (Expt 1) or in 0.2 M KCl with 25 mM Tris–citrate (pH 8.0) and 15 mM NaCN (Expt 2). Sucrose-washed cells (see Expt 3) were suspended at a density of 81 mg dry wt/ml in the sucrose medium. After incubation for 3 min at 37°C the valinomycin was added, to a final concentration of 30 μg/ml.

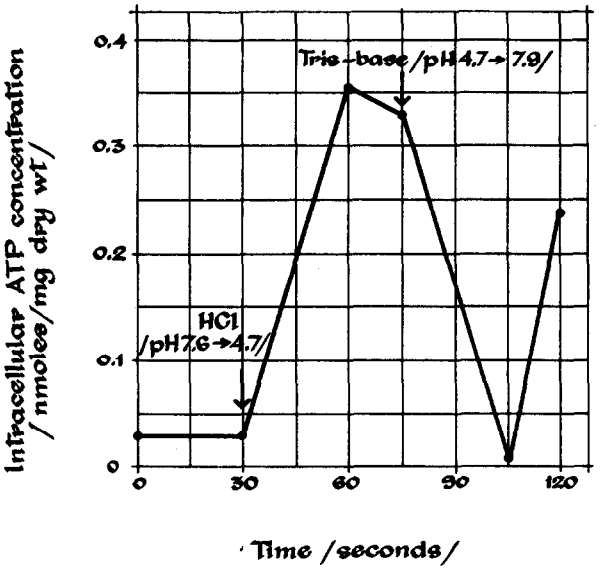

Fig.3. Dependence of intracellular ATP concentration on the transmembrane pH gradient direction. *E. coli* B was suspended at a cell density of 53 mg dry wt/ml in 0.25 M sucrose with 25 mM Tris-citrate (pH 8.0) and 15 mM NaCN and incubated for 20 min at 37°C. The medium was acidified by the addition of 1 N HCl or alkalinized by the addition of solid Tris-base. pH changes are shown in the figure.

Fig.3 shows a phosphorylation following base-acid transition in *E. coli* cell suspension. The amount of ATP in the cells is markedly reduced when the direction of the pH gradient is reversed by alkalization of the incubation medium. The imposition of the 'wrong-side' pH gradient leads to a transient decrease in intracellular ATP concentration.

In our experiments maximal ATP yields were obtained by the imposition of a membrane potential, but not a pH gradient. It could be that a membrane potential and a pH gradient besides being thermodynamic forces for ATP synthesis have different regulatory effects on the ATP synthetase activity in *E. coli.*

These experiments indicate that uncouplers abolish membrane potential generation by K^+ ion diffusion along a concentration gradient and inhibit ATP synthesis driven by the imposed membrane potential in *E. coli.*

Consideration of these facts and the well known sensitivity of oxidative phosphorylation to the uncouplers leads to the view that ATP synthesis in *E. coli* is driven by a protonmotive force.

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