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Formation of an ion transport supercomplex in *Escherichia coli*

An experimental model of direct transduction of energy

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Hydrogen gas production was observed to occur during ATP-driven H^+/K^+ exchange in anaerobically grown *E. coli*. Neither process was found in aerobically grown cells or anaerobic cells grown on nitrate medium or when the osmotic pressure was decreased or K^+ removed, or finally when DCCD, arsenate or CCCP was applied. Dithiothreitol restored the process even in the presence of CCCP but not in other cases of inhibition. A model of a multienzyme transport supercomplex is proposed. The supercomplex consists of three genetically independent mechanisms: F_0F_1 H^+-ATPase to provide energy, the K^+-transporting Trk system as energy sink and formate-hydrogen lyase as donor of reducing equivalents. Within this supercomplex direct transduction of energy is accomplished via oxidation of 2 SH to S-S.

Exchange, H^+-K^+; Hydrogen evolution; Transport supercomplex; Transmembrane electrochemical proton gradient; Thiol-disulfide interconversion; (*E. coli*)

1. INTRODUCTION

Experimental evidence was obtained recently that in anaerobically grown *E. coli*, the F_0F_1 H^+-ATPase may unite with a K^+ transporter, the so-called Trk system [1], into a single supercomplex [2,3]. Such a supercomplex functions as an ion-exchanging H^+/K^+ pump with a ratio of $1ATP:2H^+:1K^+$. This phenomenon was not observed in aerobically grown *E. coli* [4] and in mutant strains of *E. coli* with defects in F_0F_1 [5] or in the Trk system [6]. In other words, in anaerobic bacteria the F_0F_1 enzyme transfers part of the energy of ATP hydrolysis directly to the Trk system for creating a K^+ gradient of 2000:1 [4], without participation of the long-range messenger $\Delta\tilde{\mu}H^+$.

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Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DTT, dithiothreitol; FHL, formate-hydrogen lyase

The present data allow us to propose a possible mechanism of interaction between F_0F_1 and the Trk system.

2. MATERIALS AND METHODS

Experiments were carried out with the wild-type *E. coli* K-12 (λ). Anaerobic cells were grown in a medium containing peptone with glucose. To obtain anaerobic cells oxidizing formate through nitrate/nitrite respiration [7], 100 mM $NaNO_3$ was introduced into the growth medium. Aerobic cells were grown in a minimal salt medium with succinate. Bacteria were grown for 18–22 h at 37°C. Preparation of bacteria for experiments was detailed in [8]. Ion fluxes were measured using cation-selective glass electrodes as described [8]. The $\Delta\psi$ values were calculated from the distribution of tetraphenylphosphonium cations (TPP^+) with TPP^+ electrodes in cells treated with 10 mM EDTA [9,10].

To measure the oxidation reduction potentials both a platinum electrode (E_h') and an electrode manufactured from titanium-silicate glass (E_h) at the Laboratory of Glass Electrochemistry (Leningrad State University) were used. In contrast with platinum electrodes, those made from electron-conducting glass are insensitive to gaseous O_2 and H_2 and cannot serve as a catalyst for redox reactions. These distinctions between two types of electrode allowed us to use platinum electrodes to record the kinetics of H_2 evolution in anaerobic *E.*

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coli during ATP-driven H^+/K^+ exchange. To verify this approach we also measured H_2 production chemically according to the reaction

$$2KMnO_4 + 3H_2SO_4 + H_2 \longrightarrow K_2SO_4 + 2MnSO_4 + 4H_2O$$

where a solution with $KMnO_4$ is bleached in the presence of H_2.

A closed experimental chamber with electrodes was connected through a tube with a test-tube containing the solution. It was established that only anaerobically grown *E. coli* bleached the solution. This effect was detected during the period when the platinum electrode indicated a sharp reduction (broken lines in figures) in contrast to the true E_h observed with the titanium-silicate electrode. Thus, the kinetics of H_2 production recorded with the platinum electrode were found to be correct. The kinetic curves in the figures represent one of 3–5 independent experiments.

3. RESULTS

3.1. *H_2 production by formate-hydrogen lyase*

It was shown in [4] that the difference between the rates of H_2 extrusion before and after the bend in the H^+ kinetic curves (between 5 and 10 min, fig.1) is the H^+ efflux passing through the F_0F_1 [5] while the intense accumulation of K^+ during the same period occurs via the Trk system [6]. Both counter-fluxes are interrelated with a rigid stoichiometry of ATP:$2H^+1K^+$ [2,3,9].

The important feature of the findings in fig.1 is the coincidence in time of H^+/K^+ pump activity and H_2 evolution. Anaerobic growth of *E. coli* leads to synthesis of membrane-bound formate-hydrogen lyase [7] which splits formate into CO_2 and H_2. Synthesis of FHL need not be observed in anaerobic *E. coli* grown in nitrate medium or in aerobically grown *E. coli*. Fig.2 indicates that cells without FHL did not produce H_2 (E_h and E'_h curves are the same in fig.2, cf. fig.1) and showed no H^+/K^+ pump activity (see also [4]).

3.2. *Absence of H_2 production without H^+/K^+ pump operation*

Another approach demonstrates much more clearly the link between FHL and the H^+/K^+ pump. We showed previously, using appropriate mutant strains of *E. coli*, that a decrease in external osmotic pressure or application of DCCD blocks the F_0 proton channel of the F_0F_1 enzyme, whereas the removal of K^+ from the medium obviously eliminates Trk activity [5]. Fig.3 indicates that production of H_2 is also lacking under these conditions. It is important to note that neither H_2 production nor H^+/K^+ exchange was restored by DTT in the experiments illustrated in fig.3.

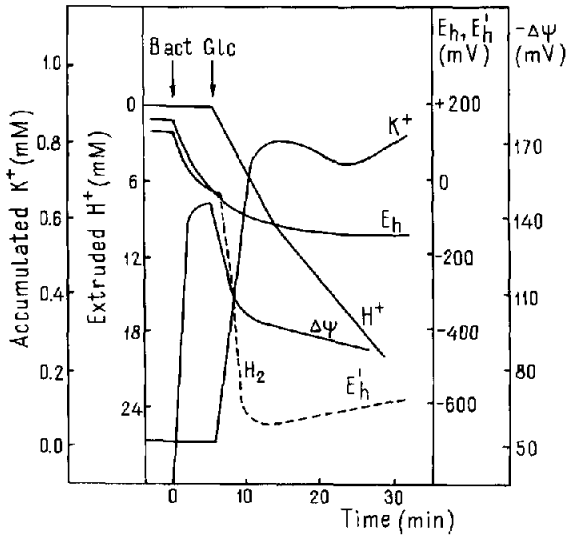

Fig.1. Simultaneous recording of H^+ and K^+ fluxes, membrane potential ($\Delta\psi$) and redox potential (E_h, E'_h) measured with a titanium-silicate and a platinum electrode, respectively, in anaerobically grown *E. coli*. The broken line shows the period when the platinum electrode (E'_h) registered H_2 production (cf. E_h and E'_h curves); after growth, bacteria were washed with distilled water and resuspended in the experimental medium containing 3 mM KCl, 1 mM NaCl, 0.4 mM $MgSO_4$, Tris-phosphate buffer (pH 7.8) and 50 mM glucose. Bacterial count: about 5×10^9/ml. During experiments the pH fell from 7.8 to 6.8. However, the decrease in pH had no effect on the data (except for the slow decrease in $\Delta\psi$) because the characteristics were the same when pH was maintained at 7.8. The transfer of bacteria from distilled water into high-osmolarity solution was equivalent to an increase in external osmolarity. This procedure is necessary for the activation of ATP-driven H^+/K^+ exchange [8].

3.3. *$\Delta\tilde{\mu}H^+$ as a regulator of SH groups in the membrane*

Despite the noticeable drop in $\Delta\psi$ during glycolysis (fig.1) the absolute value of the membrane potential remained sufficiently high. According to the theory developed by Robillard and Konings [12] such a $\Delta\psi$ value could still support normal transport activity by maintaining the necessary asymmetric distribution of SH and S-S groups inside the membrane.

Elimination of $\Delta\tilde{\mu}H^+$ by CCCP resulted in the suppression of H_2 evolution and H^+/K^+ exchange (fig.4). DTT completely restored the pump and

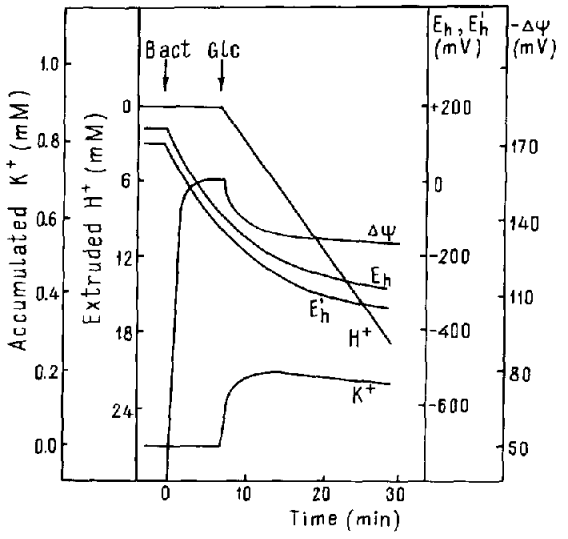

Fig.2. Data analogous to those shown in fig.1 but in aerobically grown *E. coli*. Similar data were obtained for anaerobic cells grown in the presence of 100 mM $NaNO_3$. In this case, 10 mM $NaNO_3$ was added to the experimental medium.

FHL operation. However, DTT could not restore $\Delta\psi$-driven K^+ uptake [1,4] in aerobic cells treated with CCCP (not shown).

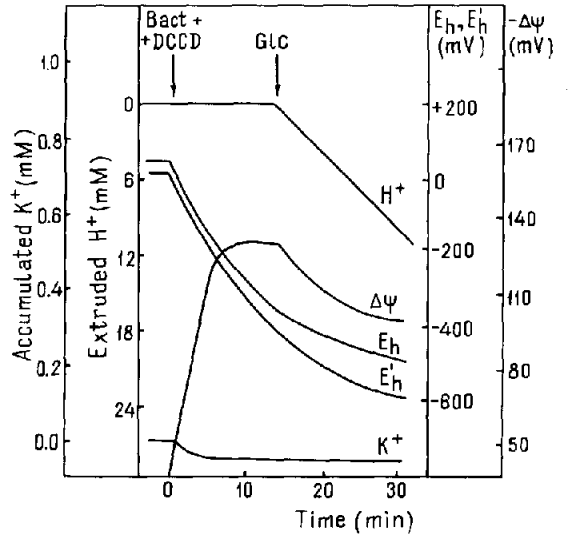

Fig.3. Data analogous to those in fig.1 but after application of 0.5 mM DCCD. Similar data were obtained under three other conditions: (i) when bacteria were washed with a sucrose solution of high osmolarity and transferred to a medium of low osmotic pressure [8]; (ii) when K^+ was removed from the medium, and (iii) in the presence of 10 mM sodium arsenate.

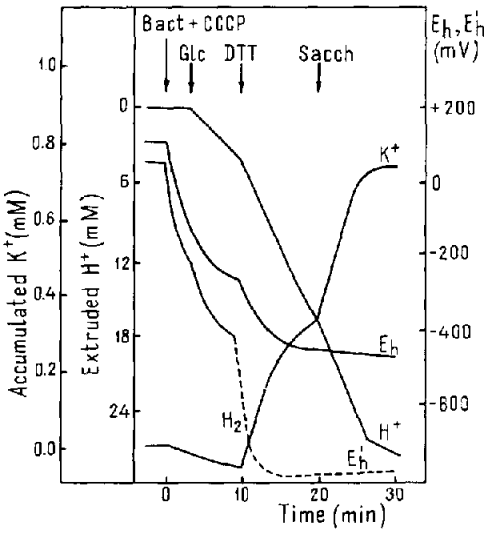

Fig.4. Inhibition of H^+/K^+ exchange and H_2 production in anaerobic *E. coli* by 10 μM CCCP and recovery of all the characteristics by 5 mM DTT. To reactivate H^+/K^+ exchange by an osmotic upshift [8] 300 mM sucrose was added. Conditions as in fig.1 except that the initial concentration of K^+ was 1 mM.

4. DISCUSSION

Based on our findings [2–6,8,9], including the present data, we can propose a model of a multienzyme transport supercomplex (fig.5). The reaction $2SH \longrightarrow S\text{-}S + H_2$ is accompanied by evolution of H_2 and is characterized by a ΔG of -35 kJ/mol, 20 kJ/mol of which is evidently utilized for counter-gradient accumulation of K^+ (2000:1 [4]). At the same time, the F_0F_1 H^+-ATPase transfers one H^+ against the $\Delta\tilde{\mu}H^+$ and the second H^+ only against ΔpH (electroneutral exchange of H^+ vs K^+ at a H^+/K^+ stoichiometry of 2:1, see [3]). Translocation of 2 H^+ requires about 15 kJ/mol in anaerobic *E. coli* at pH 7.8 and a $\Delta\psi$ of 150 mV. Since the phosphate potential in *E. coli* is about 50 kJ/mol [13], the energy required for operation of the supercomplex does not exceed that of ATP hydrolysis.

A difference in the electrochemical potential of H^+ is essential for maintaining sulfhydryl groups in the system [12]. As shown in fig.4, $\Delta\tilde{\mu}H^+$ can be substituted with DTT. This means that the artificial chemical regulator of SH groups, DTT, is equivalent to the natural electrochemical form, $\Delta\tilde{\mu}H^+$. The compulsory occurrence of $\Delta\tilde{\mu}H^+$ or application of DTT for the transfer of reducing

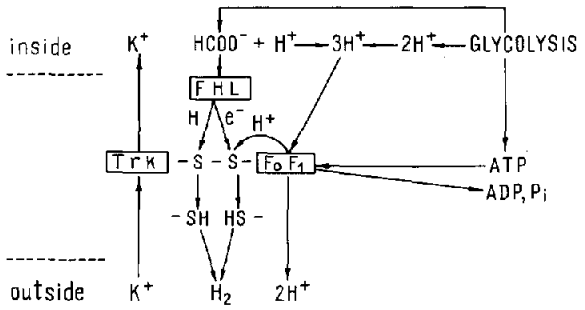

Fig.5. Model of the multienzyme transport supercomplex composed of F_0F_1 H^+-ATPase as a donor of energy, formate-hydrogen lyase (FHL) as a donor of reducing equivalents and the K^+ transport Trk system, as an acceptor of energy.

equivalents is somewhat extraordinary because the formate/CO_2 couple has a redox potential of -0.42 V while that of the 2SH/S-S couple is only -0.34 V.

Extrapolation of our findings to other transport systems leads to the conclusion that direct transduction of energy through a dithiol-disulfide interchange is impossible without the participation of $\Delta\tilde{\mu}H^+$ (see also [12]). Putting it another way, oxidative phosphorylation or photophosphorylation cannot proceed through direct transfer of energy but only with mediation of $\Delta\tilde{\mu}H^+$.

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