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Relationship between the F_0F_1-ATPase and the K^+-transport System within the Membrane of Anaerobically Grown *Escherichia coli*. *N*, *N'*-Dicyclohexylcarbodiimide-Sensitive ATPase Activity in Mutants with Defects in K^+-Transport[1]

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A considerable (2-fold) stimulation of the DCCD-sensitive ATPase activity by K^+ or Rb^+, but not by Na^+, over the range of zero to 100 mM was shown in the isolated membranes of *E. coli* grown anaerobically in the presence of glucose. This effect was observed only in parent and in the *trkG*, but not in the *trkA*, *trkE*, or *trkH* mutants. The *trkG* or the *trkH* mutant with an *unc* deletion had a residual ATPase activity not sensitive to DCCD. A stimulation of the DCCD-sensitive ATPase activity by K^+ was absent in the membranes from bacteria grown anaerobically in the presence of sodium nitrate. Growth of the *trkG*, but not of other *trk* mutants, in the medium with moderate K^+ activity did not depend on K^+ concentration. Under upshock, K^+ accumulation was essentially higher in the *trkG* mutant than in the other *trk* mutant. The K^+-stimulated DCCD-sensitive ATPase activity in the membranes isolated from anaerobically grown *E. coli* has been shown to depend absolutely on both the F_0F_1 and the *Trk* system and can be explained by a direct interaction between these transport systems within the membrane of anaerobically grown bacteria with the formation of a single supercomplex functioning as a H^+-K^+ pump. The *trkG* gene is most probably not functional in anaerobically grown bacteria.

KEY WORDS: F_0F_1; K^+-transport; ATPase activity; interaction between membrane transport systems; supercomplex; anaerobically grown bacteria.

INTRODUCTION

Many bacteria are known to extrude protons through proton pumps and to accumulate potassium ions via transport systems. Such a H^+-K^+-exchange has been shown to have different characteristics in aerobically and anaerobically grown bacteria. H^+-K^+-exchange with a variable stoichiometry in aerobically grown *E. coli* (Martirosov and Trchounian, 1986) and other bacteria (Trchounian *et al.*, 1987b), as well as in *E. coli* grown anaerobically in the presence of sodium nitrate (Bagramyan and Martirosov, 1989), occurs via separate mechanisms for H^+ and K^+ transport. DCDD-sensitive[3] H^+-K^+-exchange with fixed stoichiometry of $2H^+/K^+$ in anaerobically grown *E. coli* (Durgaryan and Martirosov, 1978; Martirosov and Trchounian, 1981a, b, c, 1983, 1986) or other bacteria (Trchounian *et al.*, 1987a, b) is carried out through the F_0F_1 (Martirosov and Trchounian, 1981c, 1983) and via the *Trk* (or *Trk*-like) system (Martirosov and

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[3] Abbreviations: F_0F_1, H^+-ATPase complex; F_1, ATPase, F_0, proteolipid, H^+-channel; *Trk*, constitutive system for K^+ uptake in *E. coli*; BCA, bicinchoninic acid. DCCD, *N*, *N'*-dicyclohexylcarbodiimide; DNP, 2,4-dinitrophenol; EDTA, ethylenediaminotetraacetic acid; MES, 2*N*-morpholinoethanesulfonic acid; P_i, inorganic phosphate; SDS, sodium dodecyl sulfate; KO, phosphate-buffered media without K^+ (Epstein and Davies, 1970); KONO, phosphate-buffered media containing neither K^+ nor NH_4^+ (Rhoads *et al.*, 1976).

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Trchounian, 1981b, 1986). Experimental evidence for intramembrane interaction of these systems with each other and their association into the same mechanism, functioning as a H^+-K^+-pump has been obtained in our laboratory (Martirosov and Trchounian, 1982; Trechounian *et al.*, 1987b, 1992; Martirosov *et al.*, 1988; see reviews by Trchounian, 1992, 1993). But the mechanism and the regulation of such an interaction between these systems are not clear. There is no relation or coordinated action between the *unc*-operon for the F_0F_1 and the *trk* genes for the K^+-transport. Moreover, new *trk* genes in different parts of the *E. coli* chromosome have been identified recently (Dosch *et al.*, 1991). The *Trk* activity previously considered to represent the activity of a single system, named *TrkA*, is proposed to be the sum of two mechanisms distinguished by products of the *trkG* or the *trkH* gene (Dosch *et al.*, 1991; Schlosser *et al.*, 1993). The role of each gene product in *Trk* activity remains unclear. Are all *trk* genes functional in bacteria grown in different conditions? Can they determine an interaction between the F_0F_1 and the *Trk* system or separate operation of the *Trk* system? Has the *Trk* system an ATPase activity?

Results presented in this paper indicate K^+-dependent DCCD-sensitive ATPase activity in the isolated membranes of anaerobically grown *E. coli*. This phenomenon can be explained by the intramembrane interaction between the F_0F_1 and the *Trk* system with formation of a single supercomplex functioning as a H^+-K^+-pump (see reviews by Trchounian, 1992, 1993). Results also allow one to assume an unusual role of the *trkG* gene in *Trk* activity.

MATERIALS AND METHODS

Bacteria

E. coli mutants provided by Prof. W. Epstein at the Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, Illinois, were used throughout (Table I).

Grown of Bacteria

Bacteria were grown in flasks or tubes filled with medium containing 2% peptone, 0.5% NaCl, 0.2% K_2HPO_4, and 0.2% glucose for 9–10 h at 37°C. To obtain anaerobic cells with nitrate/nitrite respiration (Konings and Kaback, 1973), 100 mM $NaNO_3$ was introduced into the growth medium. Growth was estimated by change of suspension turbidity measured with a model Spectronic 20 colorimeter (Bausch & Lomb, USA).

Table I. *E. Coli* Strains Used in Experiments

Strain	Genotype[a]
TK2076[b]	mal lacZ nadA trp pyrE *kdpABC5 trkD1*
TK2205[b]	lacZ nadA *kdpABC5 trkA405 trkD1*
TK2321[b]	nadA trp metE *kdpABC5 trkD1 trkG82*
TK2654[b]	lacZ nadA trp *kdpABC5 trkD1 trkH1*
TK2685[b]	*TK2076 trkG92*
FRAG92[b]	lacZ gal *kdpABC5 trkD1 trkG82* Δzda[c]
FRAG96[b]	lacZ gal *kdpABC5 trkD1 trkH1*
FRAG111[b]	lacZ gal *kdpABC5 trkD1* Δ(*trkE419*)
FRAG116[c]	*FRAG92* Δ*unc*
FRAG117[c]	*FRAG96* Δ*unc*

[a] All strains were also F^- rha thi.
[b] Dosch *et al.* (1991).
[c] The designation of Δ*zda* represents a spontaneous deletion of the tetracycline element selected by fusaric acid resistance (Dosch *et al.*, 1991).
[d] Strains were provided by Prof. W. Epstein (Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago Illinois). According to his personal communication, the *unc* deletion isolated by Prof. R. Simoni (Department of Biological Sciences, Stanford University, Stanford, Califonia) starts in or near *uncB* and extends into or past *uncD*. The deletion removes all the genes between *uncB* and *uncD*, and probably removes most of those two as well. The only genes which might be present are *uncI* and *uncC*.

Determination of K^+-Dependence of Growth

Growth rates were measured as described (Epstein and Davies, 1970). Bacteria grown in the peptone medium with glucose were washed twice with KO, which contains 46 mM Na_2HPO_4, 23 mM NaH_2PO_4, 0.4 mM $MgSO_4$, 8 mM $(NH_4)_2SO_4$, and 8 μM $FeSO_4$, as well as a small amount of K^+, usually up to 0.02 mM, as a contaminant (Epstein and Davies, 1970) and inoculated to a density of approximately 10^8 cells/ml in series of tubes containing media of different K^+ concentrations at 37°C. Turbidity was measured at intervals with the colorimeter. The growth rate was determined over the interval where the logarithm of turbidity increased linearly with time.

Isolation of Bacterial Membranes

Bacterial membranes were isolated by the method of Kaback (1971). Bacteria were harvested by centrifugation at 5000 rpm for 10 min at 4°C, washed twice with 10 mM Tris-HCl (or Tris-MES)

buffer, pH 8.0, transferred into a small volume of 30 mM Tris-HCl buffer, pH 8.0, containing 10 mM sodium-EDTA, 0.5 mM $MgCl_2$, 20% sucrose, and lysozyme, 0.5 mg/ml, and incubated for 30 min. Spheroplasts were harvested by centrifugation, resuspended in a small volume of 100 mM Tris-HCl buffer, pH 6.6, containing 20 mM $MgSO_4$, 20% sucrose, and homogenized by a Potter-Elvehjem tissue grinder (Wheaton, USA) with addition of DNase, 3–5 mg/ml. The homogenate was diluted 300–500 times with the same buffer, incubated for 15 min at 37°C; 10 mM EDTA was added, and after 15 min 15 mM $MgSO_4$. The pellet, after centrifugation at 16,000 *g* for 30 min, was resuspended in 100 mM Tris-HCl buffer, pH 6.6, containing 20 mM $MgCl_2$, 10 mM EDTA, and DNase, 0.1 mg/ml, homogenized by centrifugation at 45,000 *g* for 30 min. The last procedure was repeated. The membrane pellet was overlaid with a small volume of 50 mM Tris-HCl buffer, pH 7.5, and stored on ice until use.

Membranes from bacteria grown in the presence of nitrate were isolated from bacteria by a modified procedure (Konings and Kaback, 1973). Spheroplasts (about 2 g) was lysed by rapid dispersion in 10 ml of 10 mM Tris-MES buffer, pH 6.6, containing 2 mM $MgSO_4$ and DNase, 10 μg/ml. Dispersion was achieved by means of a hypodermic syringe fitted with an 18 gauge needle. Lysate was incubated at 37°C for 30 min on a shaker platform; after 15 min, 5 mM $MgSO_4$ and DNase, 20 μg/ml, were added. Incubation of lysate was continued for 15 min. The suspension was then centrifuged at 800 *g* for 1 h. Membranes were harvested from supernatant by centrifugation at 46,000 *g* for 1 h. Protein was determined spectrophotometrically by using BCA protein assay reagent (Pierce Chemical Co., USA).

Determination of ATPase Activity

ATPase activity was expressed as the amount of P_i liberated per minute per mg of protein. The reaction was initiated by adding 3 mM ATP-Tris to the membranes in 50 mM Tris-HCl buffer, pH 7.5, containing 1 mM $MgSO_4$ and K^+ or other ions (see Results), at 37°C and stopped after 10, 15, and 30 min with 5% SDS. Protein was about 50 to 80 μg per sample of 0.2 ml. P_i was determined spectrophotometrically by the method of Jorgensen and Petersen (1982). ATPase activity in such membranes increased linearly with time and protein (Fig. 1). The

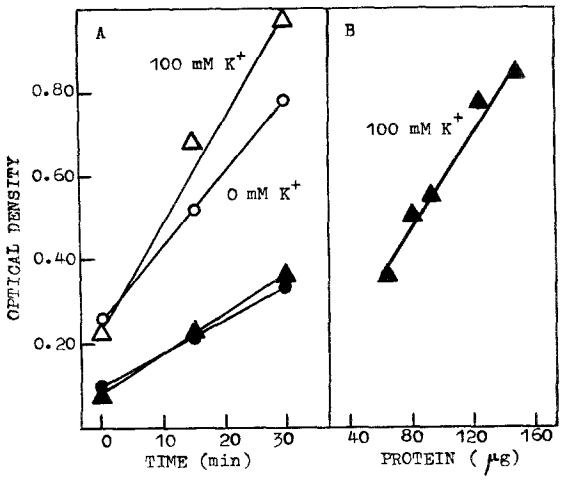

Fig. 1. Liberation of P_i by the reaction of the membranes isolated from *E. coli TK2076* with ATP is linearly dependent on time (A) or protein (B). Total (○, △) and DCCD-sensitive (●, ▲) liberations of P_i are represented in units of optical density for the K^+ concentration of 0 or 100 mM indicated. Protein was 0.65 μg in sample (A); the reaction was stopped after 30 min (B). Average results are shown; standard errors are within the designations.

DCCD-sensitive component of ATPase activity was estimated as the difference between activities in the absence and presence of DCCD, 0.1 mM; membranes were incubated for 10 min with DCCD prior to assay.

Study of K^+-Transport

K^+ uptake was estimated by determination of K^+ with an internal standard flame photometer (Instrumentation Lab., Inc., Lexington, Massachusetts, model 143). Initial transport rates were expressed as micromoles of substance taken up per minute per gram dry weight. Chloramphenicol, 50 mg/ml, was added to inhibit protein synthesis. Bacteria were harvested by using 0.45-μm pore size membrane filters (Millipore, USA), suspended with KONO (Rhoads *et al.*, 1976) and incubated for 30 min at 30°C. For several experiments bacteria were treated with 10 mM DNP in KONO. Cells were collected and transferred into KONO, containing 300 mM sucrose, at 37°C. K^+ uptake was initiated by adding 20 mM glucose and K^+. Cell samples were collected on gridded 0.45-μm pore size filters, washed briefly with 0.4 M glucose containing 1 mM Tris-HCl buffer, pH 7.0, and dried and analyzed for K^+. The dry weight of bacteria was determined after washing with 70% ethanol.

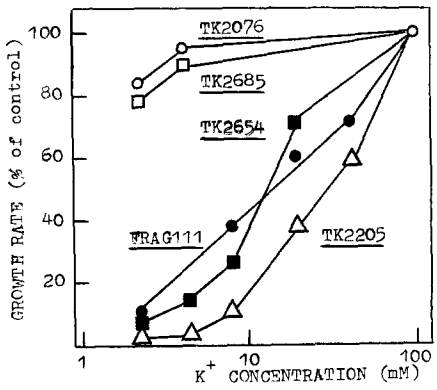

Fig. 2. Dependence of the anaerobic growth rate on the K^+ concentration in the medium for *E. coli trk* mutants. Bacteria were grown in anaerobic conditions, the doubling time for all strains at 105 mM K^+ was 111 ± 6 min, and the growth rate for this concentration of K^+ was 100%. Symbols: ○, *TK2076* (precursor); △, *TK2205* (*trkA405*); ■, *TK2654* (*trkH1*); □, *TK2685* (*trkG92*); ●, *FRAG111* (△*trkE*). Average results from three independent experiments are represented.

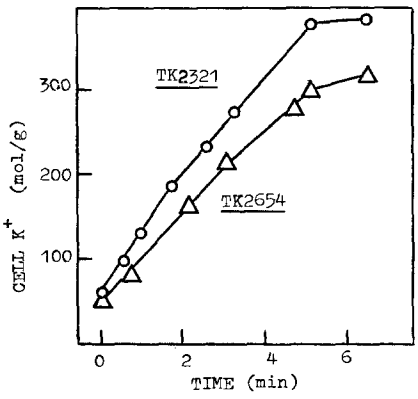

Fig. 3. Comparable kinetics of net K^+ uptake in *E. coli trk* mutants. Cells were depleted of K^+ by treatment with DNP (see Materials and Methods), and uptake was measured by adding glucose; the K^+ concentration used was 1 mM. Symbols: △, *TK2654* (*trkH1*); ○, *TK2321* (*trkG82*). Results in figure (in this and following figures) represent one of the two or three independent experiments.

RESULTS

K^+-Dependence of Growth and K^+-Accumulation in *trk* Mutants

Anaerobically grown *E. coli* mutants with defects in different *trk* genes have different phenotypes. Among them the *trkG* mutant is similar to the wild type, while *trkA*, *trkE*, and *trkH* mutants are different. Growth of the *trkG* mutant does not depend on K^+ activity in the medium with moderate K^+ activity, but growth of other mutants depends on K^+ concentration, and therefore they can grow with a high rate in medium with a high K^+ concentration (Fig. 2). Growth of *trk* mutants in aerobic conditions has been shown recently by Epstein and co-workers (Dosch *et al.*, 1991) to have other characteristics. The K_m for growth of the *trkA* or the *trkE* mutant is considerably higher than that of the mutants, but *trkG* and *trkH* mutants appear to be the same and similar to the parent and wild type (Table II). Results indicate that *trk* mutants have different K^+-dependent growth in anaerobic and aerobic conditions. There is a difference between anaerobically grown *trkG* and *trkH* mutants.

The K^+ accumulation in *trk* mutants has been studied thoroughly (Rhoads *et al.*, 1976; Rhoads and Epstein, 1977; Dosch *et al.*, 1991; Schlosser *et al.*, 1993). The wild type and both *trkG* and *trkH* mutants accumulate K^+ with a high rate, the latter being higher in the *trkG* mutant than in the *trkH* one (Table II). Such an uptake was estimated in cells which were depleted of K^+ by DNP (Rhoads *et al.*, 1976; Dosch *et al.*, 1991) and not inhibited by DCCD (Rhoads and Epstein, 1977). A similar K^+ uptake is observed for anaerobically grown mutants, which were depleted of K^+ by DNP (Fig. 3). However, K^+ accumulation is inhibited by DCCD (not shown), as

Table II. Comparison of Growth and Transport Characteristics of *trk* Mutants of *E. Coli* Grown in Anaerobic (A) and Aerobic (B) Conditions

Strain	*trk* genotype	Growth K_m (mM)		Transport kinetics (B)[b]	
		A	B[b]	K_m (mM)	V_{max} (mmol/g·min)
TK2076[c]	Wild type	0.2[d]	0.08	1–1.7[a]	0.20–0.60[a]
TK2654[c]	*H1 D1*	14.1[d]	0.11	1–1.1[a]	0.31–0.37[a]
TK2685[c]	*G92 D1*	0.5[d]	0.11	3–4[a]	0.37–0.46[a]

[a] Data were ranges obtained in measurements.
[b] Dosch *et al.* (1991).
[c] Or similar mutant.
[d] Average values represented were from two or three experiments.

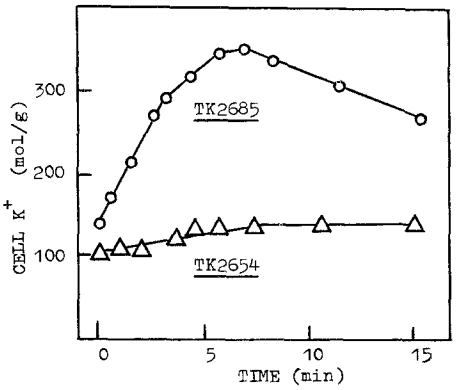

Fig. 4. Comparable kinetics of net K^+ uptake in *E. coli trk* mutants. Cells were depleted of K^+ by incubation in KONO, and uptake was measured by adding glucose; the K^+ concentration used was 2 mM. Symbols: △, *TK2654* (*trkH1*); ○ *TK2685* (*trkG92*).

shown earlier for anaerobically grown and cyanide-treated *E. coli* (Durgaryan and Martirosov, 1978; Martirosov and Trchounian, 1981a, 1986) and other bacteria (Trchounian *et al.*, 1987a, b). A significant difference in K^+ uptake by *trkG* and *trkH* mutants is seen when bacteria are depleted of K^+ by incubation of cells in KONO medium. The *trkG* mutant accumulates K^+ at a high rate, while the *trkH* mutant has only a residual K^+ uptake activity (Fig. 4). Treatment of cells with DNP leads to fairly rapid loss of K^+ regardless of the K^+ transport genotype, whereas incubation in the KONO alone is slower and depends on the transport genotype. Such a treatment with DNP is assumed to have an influence on the K^+ transport system. However, different phenotypes for *trkG* and *trkH* mutants have been confirmed. Results about different effects of a defect in the *trkG* gene on K^+ uptake in aerobically and anaerobically grown cells are in good agreement with data about the different characteristics of K^+ accumulation and H^+-K^+-exchange in bacteria grown in different conditions (Martirosov and Trchounian, 1986; Trchounian *et al.*, 1987a).

DCCD-Sensitive ATPase Activity in *trk* Mutants

Right-side-out membrane vesicles were used to study a DCCD-sensitive ATPase activity determined by the F_0F_1-ATPase which could be dependent on K^+ concentration in the medium due to the association with the *Trk* system proposed (Martirosov *et al.*, 1988; Trchounian *et al.*, 1992). It was necessary to have the determined amounts of K^+ transported from outside to inside by the *Trk* system as well as to use DCCD from outside to determine DCCD-sensitive ATPase activity. Membrane vesicles of such type have also been used for the study of the uptake of ion and other substances (Konings and Kaback, 1973; Altendorf *et al.*, 1986; Dean *et al.*, 1988, 1989), as well as of ATP-P_i-exchange (Nelson *et al.*, 1979).

Table III. ATPase Activity in Membrane Isolated from Anaerobically Grown *E. coli TK2076* at Different Concentrations of Cations

Concentration of cations (mM)		ATPase activity (nmol P_i/min · mg protein)		
		Total	DCCD-sensitive	
KCl	(4)[a]			
0		76.3 ± 7.5[b]	31.3 ± 1.7	(100%)
5		98.2 ± 9.1	49.0 ± 3.0	(157%)
100		114.0 ± 11.6	62.8 ± 3.1	(198%)
KNO_3	(3)			
0		86.3 ± 4.6	36.3 ± 3.5	(100%)
5		99.7 ± 6.1	54.9 ± 4.1	(151%)
100		104.3 ± 4.3	62.9 ± 1.7	(174%)
RbCl	(2)			
0		79.8 ± 3.8	33.4 ± 4.3	(100%)
5		92.9 ± 5.0	47.3 ± 6.2	(142%)
100		104.7 ± 5.9	55.7 ± 2.9	(167%)
NaCl	(2)			
0		84.3 ± 5.7	39.8 ± 3.0	(100%)
5		79.9 ± 0.7	40.5 ± 6.6	(102%)
100		76.6 ± 7.1	38.1 ± 0.5	(96%)

[a] The figures in brackets are the number of experiments.
[b] Average results with standard errors.

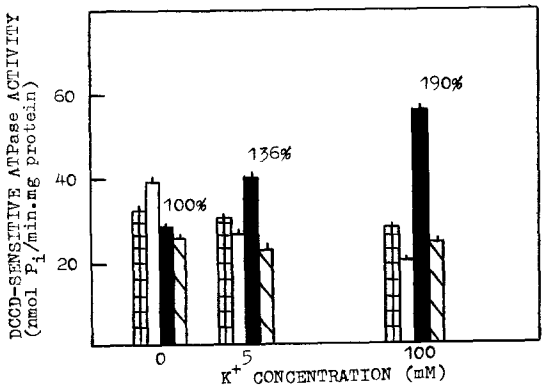

Fig. 5. Dependence of the DCCD-sensitive ATPase activity in membranes isolated from *E. coli trk* mutants on the K^+ concentration in the medium. Symbols: ⊞, *TK2205* (*trkA405*), (3); □, *TK2654* (*trkH1*) (2); ■, *TK2685* (*trkG92*) (2); ⧅, *FRAG111* (△*trkE*) (4); the figures in brackets are the number of experiments. The assignment of the values in percent are for the mutant *TK2685*; the ATPase activity in the medium without K^+ was 100%. Average results are shown with standard errors.

The DCCD-sensitive ATPase activity in the membranes isolated from anaerobically grown *E. coli* is shown to be considerably stimulated by K^+ or Rb^+, but not by Na^+ (Table III). The same effect is observed when KCl is replaced by KNO_3 (Table III). Total ATPase activity is also increased in parallel with DCCD-sensitive activity, the latter being more than 55–60%. A stimulation of the DCCD-sensitive ATPase activity by K^+ is observed in the parent (Table III) and in the *trkG* mutant, but not in other *trk* mutants (Fig. 5). K^+ inhibits the DCCD-sensitive ATPase activity more insignificantly in the *trkH* mutant than in the *trkA* or the *trkE* mutant. The last two mutants were shown to have none or only a residual *Trk* activity (Dosch *et al.*, 1991; Schlosser *et al.*, 1993). Results show that the K^+-stimulated DCCD-sensitive ATPase activity is most probably determined by the *Trk* system. This system was shown to uptake K^+ or Rb^+, but not Na^+ (Bakker *et al.*, 1984).

If the DCCD-sensitive ATPase activity stimulated by K^+ is the F_0F_1-ATPase activity, then it must be absent in *unc*-mutants with a nonfunctional F_0F_1. DCCD-sensitive ATPase activity is missed in the membranes isolated from the *trkG* or the *trkH* mutant with an *unc*-deletion (Table IV). Total ATPase activity in these mutants is much lower than in mutants without *unc* deletion. But this small ATPase activity is stimulated a little by K^+, Rb^+, or Na^+ also (Table IV).

Stimulation of DCCD-sensitive ATPase activity by K^+ is also absent in the membranes isolated from bacteria grown in the presence of nitrate (Table V).

A K^+-stimulated DCCD-sensitive ATPase activity was shown previously in protoplasts of anaerobically grown *E. coli* (Martirosov *et al.*, 1988) and anaerobic *L. salivarius* (Trchounian *et al.*, 1987a). It was not observed in the *E. coli uncA* or *trkA* mutants (Martirosov *et al.*, 1988).

All the above results have shown that the K^+-stimulated DCCD-sensitive ATPase activity is the F_0F_1-ATPase activity due to the *Trk* system.

DISCUSSION

K^+-stimulated DCCD-sensitive ATPase activity in anaerobically grown *E. coli* is the principal finding of this paper. It is necessary to note that (1) the absolute values of ATPase activity and its DCCD-sensitive component shown in many papers (Bragg, 1979; Nelson *et al.*, 1979; Leimgruber *et al.*, 1981; Fillingame *et al.*, 1991; La Roe and Vik, 1992) are very different, and our data are within the range of their values (2) the effects of monovalent cations on DCCD-sensitive ATPase activity are shown to be also different: Li^+, Na^+, K^+, or Cs^+ can inhibit ATPase activity in *E. coli* (by no more than 20%) or increase it (Bragg, 1979); (3) DCCD is a rather nonspecific inhibitor for the F_0F_1-ATPase, but this reagent is

Table IV. ATPase Activity in Membranes Isolated from Anaerobically grown *E. coli trk* Mutants with *unc*-deletion

Strain	Concentration of cations (mM)	ATPase activity (nmol P_i/min · mg protein	
		Total	DCCD-sensitive
FRAG115	0	11.5 ± 1.8	0.5 ± 0.2
(▲*unc*)	KCl: 5	11.9 ± 1.3	0.3 ± 0.1
(2)[a]	100	13.8 ± 1.5	0.3 ± 0.1
FRAG116	0	12.5 ± 1.8	1.4 ± 0.4
(△*unc trkG*)	KCl: 5	11.5 ± 2.6	0.1 ± 0.2
(2)	100	14.9 ± 2.5	0.4 ± 0.2
	0	9.4 ± 0.5	ND[b]
	RbCl: 100	13.0 ± 0.4	ND
	NaCl: 100	12.2 ± 1.7	ND
FRAG117	0	11.7 ± 1.1	ND
(△*unc trkH*)	KCl: 5	12.3 ± 2.2	ND
(2)	100	14.0 ± 2.4	ND

[a] The figures in brackets are the number of experiments.
[b] Not determined.

Table V. ATPase Activity in Membranes Isolated from *E. coli* Grown Anaerobically in the Presence of Nitrate[a]

Strain	Concentration of K^+ in medium (mM)	ATPase activity (nmol P_i/min · mg protein)		
		Total	DCCD-sensitive	
TK2685	0	47.5 ± 1.5	32.1 ± 1.6	(100%)
(*trkG*)	5	44.5 ± 1.8	29.5 ± 1.3	(92%)
(2)[b]	100	42.1 ± 1.2	23.4 ± 1.3	(73%)
FRAG116	0	8.3 ± 0.4	0.3 ± 0.1	
(△*unc*	5	8.0 ± 0.4	0.2 ± 0.1	
trkG)	100	8.0 ± 0.2	0.2 ± 0.1	
(2)				

[a] 10 mM sodium nitrate and 10 mM sodium formate were added into the reaction medium (see Materials and Methods).
[b] The figures in brackets are the number of experiments.

shown to have no effect on ATPase activity in protoplasts of anaerobically grown *E. coli* under the *uncB* mutation with defect in the large subunit of the F_0 (Martirosov *et al.*, 1988). Our data show that the DCCD-sensitive ATPase activity in membranes isolated from anaerobically grown *E. coli* is the F_0F_1-ATPase activity and is stimulated by the K^+ due to the *Trk* system, but not the one due to monovalent cations or K^+ only. The results indicate a relationship between the F_0F_1-ATPase and the *Trk* system. These two systems are assumed (Martirosov and Trchounian, 1982; Trchounian *et al.*, 1987b, 1992; Martirosov *et al.*, 1988; Bagramyan and Martirosov, 1989; see reviews by Trchounian, 1992, 1993) to interact with each other directly within the membrane and to form a single supercomplex functioning as a H^+-K^+-pump (Fig. 6). For such a

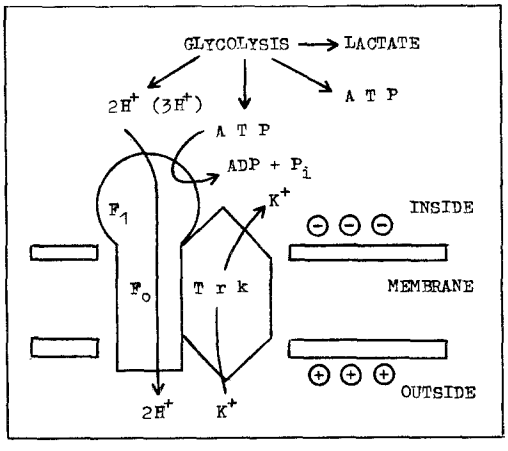

Fig. 6. The proposed model for a direct interaction of the F_0F_1-ATPase with the *Trk* system within the membrane of anaerobically grown *E. coli* with the formation of a single supercomplex, functioning as a H^+-K^+-pump.

phenomenon the following can be predicted: (1) DCCD-sensitive ATPase activity should be absent in mutants with a nonfunctional F_0F_1-ATPase and in the medium depleted of K^+, (2) K^+-stimulated ATPase activity should be completely inhibited by DCCD and missed in mutants with a nonfunctional *Trk* system. However, it is necessary to note that there is contaminated K^+ (up to 0.02 mM) in a medium without K^+ (Epstein and Davies, 1970).

DCCD has been shown to inhibit the ATPase activity in isolated membranes of *E. coli* by 60–65% (Fillingame *et al.*, 1991; La Roe and Vik, 1992). The same inhibition is also known for the ATPase activity of another bacteria (Leimgruber *et al.*, 1981). Coupled to the F_1F_0-ATPase is at least 80–90% inhibition by DCCD under conditions where DCCD is present at effective concentrations and for a sufficient period of time. Too high a concentration of DCCD should only increase inhibition. However, DCCD does not completely inhibit ATPase activity in purified preparations of the F_0F_1-ATPase (Leimgruber *et al.*, 1981), which can be explained by separate F_1-ATPase, insensitive to DCCD, or by dependence of the effect of inhibition on DCCD concentration. In mutants with a nonfunctional *Trk* system, interaction between the F_0F_1 and the *Trk* system can be destroyed; therefore, the F_0F_1 can function separately. This situation appears to be similar to the effects of different *unc* mutations on the F_0F_1-ATPase activity, which do not always set in stop of the operation (Gibson and Cox, 1977; Martirosov and Trchounian, 1981c, 1983; Gibson, 1983).

The effects of different mutations on the function of individual transport proteins within the supercomplex have been studied. However, our findings suggest a direct interaction between the F_0F_1 and

the *Trk* system with the formation of a supercomplex within the membrane (Fig. 6). Such a direct interaction between these systems is observed for bacteria grown anaerobically in the presence of glucose. Thus, a stimulation of DCCD-sensitive ATPase activity is absent in bacteria grown anaerobically in the presence of nitrate. For the latter bacteria a direct interaction between the F_0F_1 and the *Trk* system has not been proposed: an absence of the H^+-K^+-exchange with a fixed stoichiometry of $2H^+/K^+$ was shown (Bagramyan and Martirosov, 1989).

An association of the F_0F_1 with the *Trk* system into the same mechanism has to lead to economy of ATP and decrease the dissipation of converted energy. The F_0F_1-ATPase not only generates a protonmotive force but also creates a high K^+ gradient between the cell and the medium. A model for such a direct interaction between these transport proteins via dithiol–disulfide interchange has been proposed (Bagramyan and Martirosov, 1989).

Direct interaction is assumed to be a feature of membrane proteins. Intramembrane interaction between different membrane systems in bacteria is discussed for the F_0F_1-ATPase and a respiratory chain (Guffanti *et al.*, 1984; Guffanti and Krulwich, 1988, 1992), as well as for the membrane component of the phosphoenol pyruvate-dependent phosphotransferase system for glucose transport and the lactose transport (Kalachev *et al.*, 1981). The problem is to find the nature of the link between transport systems within the membrane and to understand the mechanism and the regulation of interaction between proteins within the supercomplex.

The role of the *trkG* gene for the *Trk* activity in anaerobically grown *E. coli* must be studied. This gene is not an intrinsic *E. coli* gene. It may have entered the cells with the prophage *rac* (Dosch *et al.*, 1991; Schlosser *et al.*, 1993). This gene is also assumed to be non functional or is related to another mechanism for K^+ uptake in anaerobically grown bacteria. A product of this gene may regulate an interaction of the *Trk* system with the proton pump and determine a different form of *Trk* in aerobically grown cells. Other *trk* genes are proposed to have a direct relation to the *Trk* activity.

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