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THE MAINTENANCE OF THE ENERGIZED MEMBRANE STATE AND ITS RELATION TO ACTIVE TRANSPORT IN *ESCHERICHIA COLI*

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SUMMARY

1. An ATPase mutant of *Escherichia coli* and two partial revertants of that mutant were examined for the ability to generate a high energy membrane state with D-lactate or ATP, as measured by the quenching of the fluorescent dye quinacrine.

2. All three strains showed reductions in the aerobically-driven quenching of fluorescence compared to the wild type, but the reduction could be reversed by the addition of either *N,N'*-dicyclohexylcarbodiimide or the crude soluble ATPase of the wild type.

3. The mutant exhibited a decreased ability to accumulate sugars and amino acids and showed an increased permeability to protons.

4. One partial revertant showed a slight increase in active transport and a slight decrease in proton permeability.

5. The other partial revertant showed a large increase in transport ability and a large decrease in proton permeability.

6. A model is proposed in which the conformation of the Mg^{2+}-ATPase is important in the utilization of energy derived from the electron transport chain and this function is independent of the catalytic activity of the Mg^{2+}-ATPase.

INTRODUCTION

Many bacteria can utilize for active transport energy derived either from the hydrolysis of ATP via the Mg^{2+}-ATPase or from the oxidation of reduced substrates via the electron transport chain [1–11]. It would appear that the former process requires a functional Mg^{2+}-ATPase [3, 6, 8, 9], but the role of that protein in the coupling of aerobic metabolism to active transport is not clear [1, 5, 7–10]. Several mutants of *Escherichia coli* containing Mg^{2+}-ATPase proteins with reduced activity have been shown to have normal respiration-driven active transport [3, 6, 11]. Yet other ATPase mutants have reduced ability to couple transport to oxidation, and within that group

Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; TMMG buffer, 0.05 M Tris · HCl H 8, 10 mM $MgCl_2$, 1 mM β-mercaptoethanol and 10 % glycerol (v/v).

of mutants there is a wide range of coupling abilities [1, 5, 7–10].

This paper describes the coupling of aerobic metabolism to active transport in Mg^{2+}-ATPase mutant and two partial revertants of that mutant. A correlation was found between the ability of such strains to accumulate sugars and amino acids, and the ability of those strains to maintain the energized membrane state as measured by the energy-dependent quenching of quinacrine fluorescence and the proton permeability of the membranes.

METHODS

Bacteria and media

Cultures were grown in a basal salts medium [12] supplemented with various carbon sources as noted. Solid media consisted of nutrient agar, neomycin agar (basal salts medium containing 11 mM glucose and neomycin sulfate supplemented with 2 % agar), and succinate agar (basal salts medium containing 85 mM sodium succinate supplemented with 2 % agar).

E. coli strain 7 is a wild-type K-12 derivative [13]. Strain NR76 was derived from strain 7 by selection for spontaneous resistance to 50 μg/ml of neomycin sulfate on neomycin plates by the procedure described previously [5]. Spontaneous partial revertants were selected from strain NR76 by growing single-colony isolates of NR76 in nutrient broth, harvesting and washing with sterile 0.9 % NaCl, and spreading onto succinate plates at a concentration of 10^9 cells per plate. After 5 days at 37 °C large and small colonies were streaked onto nutrient agar plates to obtain single colony isolates. Two such isolates, a slow-growing one (NR76A) and a rapidly-growing one (NR76B) were retained for further study.

Preparation of membranes

The inner membrane vesicles used for transport assays were prepared by the method of Kaback [14]. Cultures were grown to stationary phase in basal salts medium supplemented with 55 mM glycerol. The cells were harvested by centrifugation and washed once with a buffer consisting of 0.05 M Tris · HCl pH 8, 10 mM $MgCl_2$, 1 mM β-mercaptoethanol, and 10 % glycerol (v/v) (TMMG buffer). The cells were resuspended in 5 vol./g wet cells in TMMG buffer and lysed by a single passage through a French Pressure Cell (American Instrument Co.) at 22 000 lb/inch^2 ± 2000 lb/inch^2. The suspension was centrifuged at 27 000 × *g* for 10 min, and the pellet was washed once with a small volume of TMMG buffer. The supernatant solutions were combined and centrifuged at 105 000 × *g* for 1 h. The membranes were washed twice with TMMG buffer and resuspended in the same buffer to 3–5 mg of protein per ml. All of the above steps were performed at 4 °C. The membranes could be stored at 4 °C for up to a week without significant loss of Mg^{2+}-ATPase activity.

The Mg^{2+}-ATPase was solubilized by the following procedure. Membranes prepared as above were centrifuged for 1 h at 105 000 × *g* and resuspended in a buffer consisting of 1 mM Tris · HCl pH 8, containing 0.5 mM EDTA, 1 mM β-mercaptoethanol, and 10 % glycerol (v/v) (stripping buffer) at a concentration of 3–5 mg protein per ml. The suspension was centrifuged for 2 h at 105 000 × *g*, and the supernatant solution containing the soluble Mg^{2+}-ATPase was carefully removed. This procedure was performed at 23 °C. The pellet (stripped membranes) was resuspended in TMMG buffer at 4 °C to 3–5 mg of protein per ml.

Fluorescence assays

Fluorescence measurements were performed at 23 °C in a total volume of 3 ml of a mixture consisting of 50 mM Tris · HCl pH 8, containing 2.5 mM $MgCl_2$ and 1500 units of catalase. To this mixture was added 0.6—1 mg of membrane protein. Just prior to the initiation of the assay, hydrogen peroxide was added to 1.8 mM in order to saturate the system with oxygen, followed by quinacrine dihydrochloride to 0.2 μM. Fluorescence was measured using an Amicon-Bowman Spectrofluorometer with an excitation wavelenght of 420 nm and an emission wavelength of 500 nm. The relative fluorescence was recorded on a Heathkit IR-18M recorder with the zero point set with buffer alone and a full scale deflection approximately 10 % higher than the reading with membranes plus quinacrine.

Transport assays

The transport of [^{3}H] proline, [^{14}C] thiomethyl-β-D-galactoside, α-[^{14}C] methylgalactoside or *o*-nitrophenyl-β-D-galactoside in whole cells or membrane vesicles was performed as described previously [5]. The β-galactoside permease was induced by the inclusion of 0.5 mM isopropyl-β-D-thiogalactoside to the growth medium.

Measurement of proton movements in whole cells

The measurement of external pH was performed as described previously [10]. The recorder output was converted to x, y coordinates using a digitizer (Edwin Industries Corp., Silver Spring, Maryland). These values were analyzed with a Univac 1108 computer using a program incorporating the expressions derived by Mitchell and Moyle [15].

Protein determinations

Protein was determined according to a modification of the method of Lowry et al. [16].

Chemicals

L-[G-^{3}H] proline (2.4 Ci/mmol) was purchased from New England Nuclear Corp, [U-^{14}C] thiomethyl-β-D-galactoside (24.6 Ci/mol) from Schwartz/Mann, α-[U-^{14}C] methylglucoside (12.3 Ci/mol) from Calbiochem, catalase from Worthington Biochemical Corp, and carbonic anhydrase and quinacrine dihydrochloride from Sigma Chemical Co. All other compounds were analytical grade.

RESULTS AND DISCUSSION

Properties of the Mg^{2+}-ATPase of strains NR76, NR76A, and NR76B

Strain NR76 was found to have less than 3 % of the Mg^{2+}-ATPase activity of its parent, strain 7. The partial revertant NR76A consistently exhibited more activity than NR76, between 3 and 4 % that of strain 7. The other partial revertant NR76B was found to have 7–10 % of the membrane-bound Mg^{2+}-ATPase activity of the original wild-type, strain 7. Other properties of these strains and of the Mg^{2+}-ATPase of these strains will be presented in a subsequent publication (Adler, L. and Rosen, B. P., unpublished).

Energy-dependent quenching of quinacrine fluorescence

Various fluorescent dyes have been used as probes of the energized state of the membrane. Cyanine dyes are thought to measure directly the membrane potential [17], while dyes such as quinacrine are postulated to measure the H^+ concentration at the surface of the membrane [18]. In both cases energization of the membrane causes a quenching of fluorescence [17–19]. 9-Amino-6-chloro-2-methoxyacridine fluorescence is also quenched by the formation of the high-energy membrane state, and this compound has been used to determine the efficiency of energy coupling in mutants of *E. coli* defective in the Mg^{2+}-ATPase [20].

When membranes of the wild-type strain 7 are incubated in the presence of the fluorescent dye quinacrine, the addition of a respiratory substrate such as D-lactate results in a quenching of the fluorescence (Fig. 1A). This change is unaffected by inhibitors of the ATPase such as *N,N'*-dicyclohexylcarbodiimide (DCCD) (not shown) but is completely reversed by electron transport chain inhibitors such as KCN. As shown in Fig. 1A also, the addition of ATP in the presence (or absence) of KCN also results in a fluorescence quenching. The quenching by ATP is both slower and less pronounced than that caused by D-lactate, but it is completely reversed by the addition of DCCD. Both the respiratory-driven and ATP-driven quenching are reversed by the addition of an uncoupler (not shown). Thus, it appears that the fluorescence dye is responding to energization of the membrane, whether established through oxidation of D-lactate via the electron transport chain or by hydrolysis of ATP via the Mg^{2+}-ATPase. Membranes from which the Mg^{2+}-ATPase has been removed (stripped

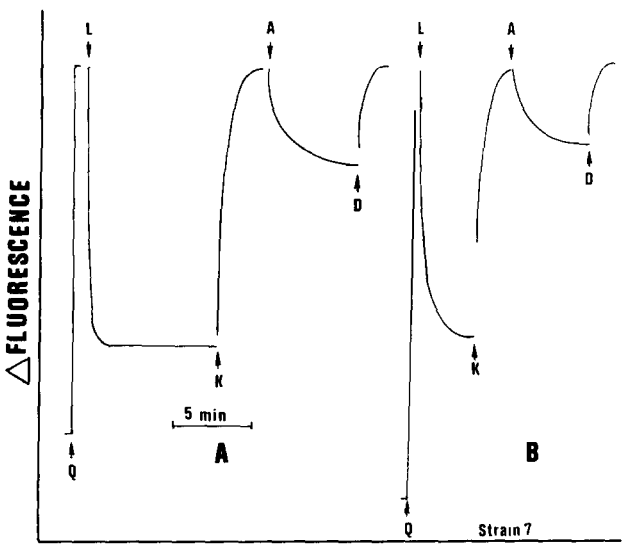

Fig. 1. Quenching of quinacrine fluorescence by control and reconstituted membranes of strain 7. Fluorescence measurements were performed as described under Methods. Reconstitution experiments were performed by adding 0.2 ml stripped membranes and a crude soluble fraction derived from 0.3 ml of control membranes to the cuvette. After 5 min at 23 °C fluorescence measurements were performed. Additions: L, 5 mM D-lactate; Q, 0.2 μM quinacrine; K, 10 mM KCN; A, 1.67 mM ATP; D, 0.1 mM DCCD. A, control membranes from strain 7. B, stripped membranes from strain 7 reconstituted with crude soluble fraction from strain 7.

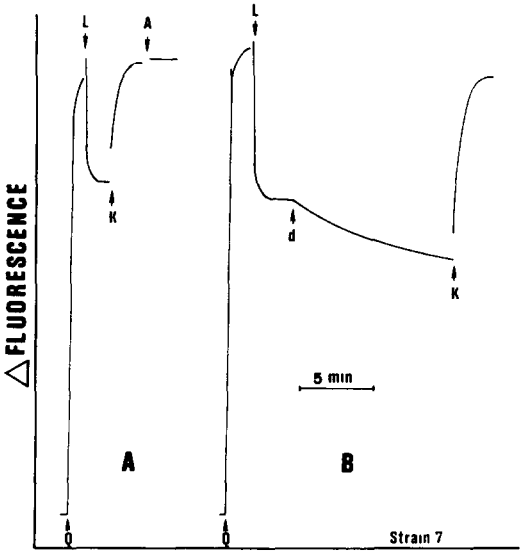

Fig. 2. Quenching of quinacrine fluorescence by stripped membranes of strain 7. Fluorescent measurements were performed as described under Methods. Additions: d, 30 μM DCCD; other additions were as given in the legend to Fig. 1. A and B, stripped membranes from strain 7.

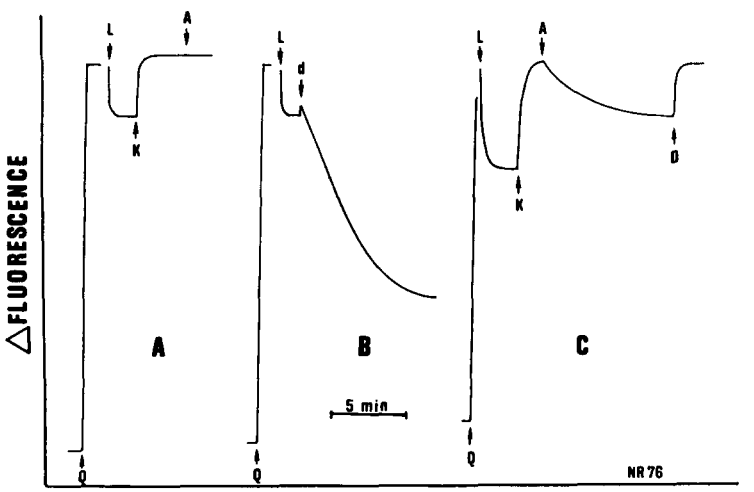

Fig. 3. Quenching of quinacrine fluorescence by membrane of strain NR76. Fluorescence measurements were performed as described under Methods. Reconstitution of control membranes of NR76 with the soluble fraction from strain 7 was performed as was the reconstitution described in the legend to Fig. 1. Additions were as given in the legend to Fig. 1. A and B, control membranes from strain NR76. C, control membranes from strain NR76 reconstituted with crude soluble fraction from strain 7.

membranes) still exhibit the respiratory-driven fluorescence quenching, but the extent of quenching is greatly reduced (Fig. 2A); the ATP-driven quenching is absent in stripped membranes (Fig. 2A). Addition of 30 μM DCCD to the stripped membranes causes an addition slow quenching (Fig. 2B). Stripped membranes to which the ATPase has been added (reconstituted membranes) respond to both D-lactate and ATP in a manner almost identical to that of control (unstripped) membranes (Fig. 1B). Although it is possible that some other component of the crude soluble fraction is important for the reconstitution, it has been shown that both the crude and purified Mg^{2+}-ATPase will reconstitute other respiratory-driven and ATP-driven processes [20, 21].

Control membranes from the Mg^{2+}-ATPase mutant NR76 exhibit only a small quenching in response to D-lactate and do not respond at all to ATP (Fig. 3A). Addition of 30 μM DCCD increases the extent of the respiratory-driven quenching to a level near to that of the wild-type (Fig. 3B). Addition of crude soluble fraction from the wild-type both increases the respiratory-driven quenching and restores the ability of control membranes from the mutant to quench the quinacrine fluorescence with ATP. Strain NR76A, the derivative of strain NR76 containing slightly higher Mg^{2+}-ATPase activity, showed properties essentially the same as NR76. Thus, it is not necessary to first remove the mutant Mg^{2+}-ATPase from the membrane of these strains in order to reconstitute with the wild-type Mg^{2+}-ATPase. It is possible that there are Mg^{2+}-ATPase binding sites on the membranes of these mutants which are unfilled, either in vivo or as a result of the preparation of the membranes. There may also be an exchange of one Mg^{2+}-ATPase for another or of the subunits of the Mg^{2+}-ATPase. A comparison of Figs 3B and 3C indicates that there is not full reconstitution. Addi-

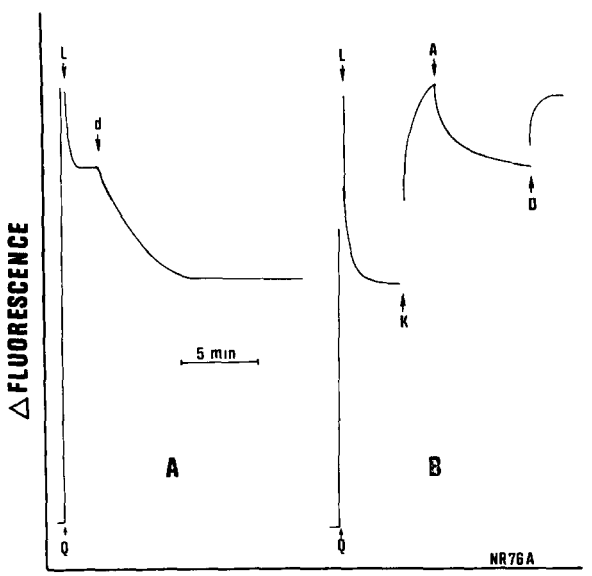

Fig. 4. Quenching of quinacrine fluorescence by stripped and reconstituted membranes from strain NR76A. Details are given in the legends to Figs 1 and 2. A, stripped membranes from strain NR76A. B, stripped membranes from strain NR76A reconstituted with crude soluble fraction from strain 7.

tion of 30 μM DCCD causes a considerably greater respiratory-driven quenching than does rebinding of the ATPase. If the membranes of either NR76 or NR76A are first stripped, the addition of the wild-type ATPase causes the same extent of fluorescence quenching (Fig. 4B) as does the addition of 30 μM DCCD (Fig. 4A). This shows that there is an impediment to the binding of the wild-type Mg^{2+}-ATPase in control membranes, and that this impediment is removed by the stripping procedure.

The data in Fig. 5 show the ability of control membranes of strain NR76B, the other derivative of strain NR76, to catalyze the quenching of quinacrine fluorescence. The Mg^{2+}-ATPase in strain NR76B has activity intermediate between that of strains NR76 and 7. Membranes of NR76B respond to D-lactate (Fig. 5A). The extent of quenching caused by D-lactate is further increased by the addition of 30 μM DCCD (Fig. 5B). Thus, control membranes of NR76B yield a respiratory-driven quenching less than the wild-type but considerably greater than either NR76 or NR76A. Unlike NR76 and NR76A, but like strain 7, membranes from NR76B can causes the quenching of quinacrine fluorescence through the hydrolysis of ATP by the Mg^{2+}-ATPase (Fig. 5A). Reconstituted stripped membranes of NR76B show properties similar to those of strain 7 membranes. No significant changes were seen in control membranes of NR76B upon the addition of crude soluble Mg^{2+}-ATPase from the wild-type.

Results of such experiments can be interpreted only qualitatively. If, as has been suggested, the quenching of quinacrine fluorescence is a measure of the proton concentration at the surface of the membrane [18], then it appears that the wild-type, strain 7, can establish such a state either by the electron transport chain or by the Mg^{2+}-ATPase. The mutant NR76, which has a defective Mg^{2+}-ATPase, cannot utilize ATP for the generation of the high-energy membrane state. Moreover, the extent of that energized state generated through the oxidation of D-lactate is reduced in NR76, even though D-lactate-dependent oxygen consumption in NR76 was found to be nor-

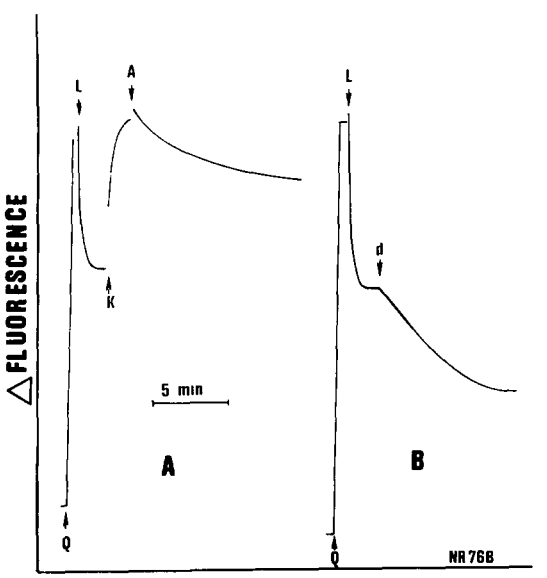

Fig. 5. Quenching of quinacrine fluorescence by control membranes from strain NR76B. Conditions are as presented in the legends to Figs 1 and 2. A and B, control membranes from strain NR76B.

mal in experiments not shown here. Although the assay is too qualitative to distinguish differences between NR76 and its derivative NR76A, the spontaneous partial revertant NR76B has clearly regained the ability to maintain an energized membrane state at a high level through the aerobic metabolism for that function. The fact that the stripped membranes from NR76, NR76A, or NR76B exhibit properties indentical to that of the wild type after addition of the crude soluble Mg^{2+}-ATPase of strain 7 suggests that the removal of an altered Mg^{2+}-ATPase and the binding of a normal one is related to the energization of the membrane.

It appears, therefore, that all four strains examined can, to some extent, generate an energized membrane state. The question arises whether strains NR76 and NR-76A are defective in the ability to generate or the ability to maintain such a state. As is shown below, the latter possibility appears the more likely.

Active transport in strains 7, NR76, NR76A, and NR76B

A second measure of the efficiency of energy-coupling is the ability to accumulate substrates via active transport system. While all Mg^{2+}-ATPase mutants investigated have been shown to be defective in the coupling of ATP derived from glycolysis to active transport [1, 3, 5, 9], not all of these have been found to be deficient in the ability to couple respiratory-derived energy to transport [1, 3, 5–11]. It has been suggested that the Mg^{2+}-ATPase serves a structural role in the maintenance of the high energy state of the membrane [5, 10, 21], and the isolation of mutants with defects in that protein which are unable to couple aerobic metabolism to transport supports that concept. Stronger evidence for such a proposal would be the restoration of active transport concomitant with a reversion of the lesion resulting in the Mg^{2+}-ATPase mutation. The two partial revertants of strain NR76 have Mg^{2+}-ATPase activities which are greater than the mutant NR76 but less than the wild-type, suggesting an alteration in the struc-

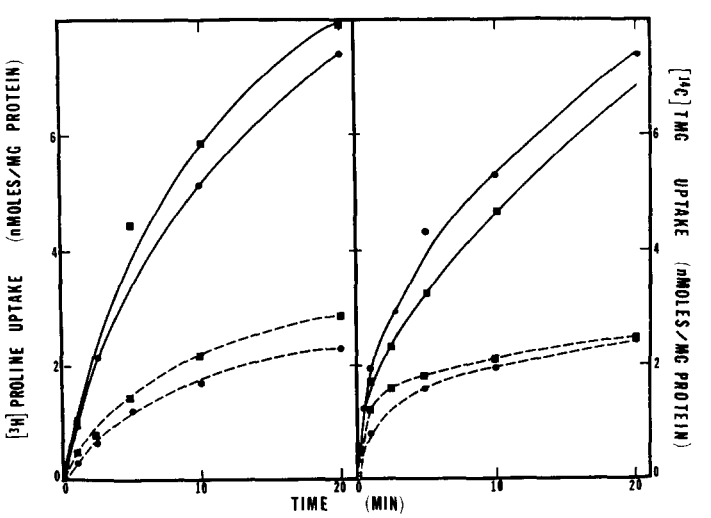

Fig. 6. Transport of proline and thiomethyl-β-D-galactoside (TMG) in whole cells. Transport assays were performed as described under Methods. Left, uptake of [^{3}H]proline at 0.36 μM, final concentration. Right, uptake of [^{14}C]thiomethyl-β-D-galactoside (TMG) at 10 μM, final concentration. (■—■), strain 7; (●—●), strain NR76B; (■- -■), strain NR76A; (●- -●), strain NR76.

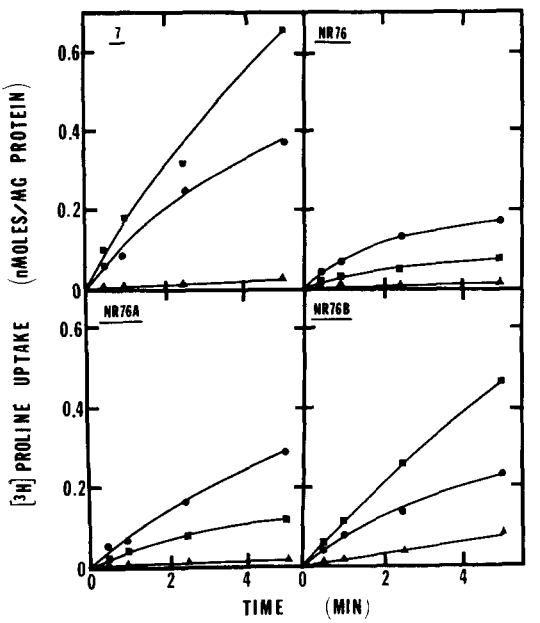

Fig. 7. Proline transport in membrane vesicles. Transport assays were performed as described under Methods, using 0.36 μM [^{3}H]proline, final concentration. (■—■), +20 mM D-lactate; (●—●), +20 mM D-lactate and 30 μM DCCD; (▲—▲), no energy source.

ture of the Mg^{2+}-ATPase protein. As shown in Fig. 6A for proline and 6B for thiomethyl-β-D-galactoside, the ability of strains NR76A and NR76B to accumulate substrates is greater than strain NR76. But the restoration of transport ability does not parallel the gain in Mg^{2+}-ATPase activity in that strain NR76B exhibits only 7–10 % of the wild-type Mg^{2+}-ATPase activity and yet transports proline and thiomethyl-β-D-galactoside as well as strain 7. The same effect is seen in membrane vesicles (Fig. 7). The ability of such vesicles to couple the oxidation of D-lactate to proline transport is not proportional to the Mg^{2+}-ATPase activity of these strains. Yet, there is a relationship, since a strain lacking that protein, strain NR70, shows no proline transport in vesicles [5], while strain NR76, which contains a nonfunctional Mg^{2+}-ATPase can couple respiration to transport, but poorly. Partial reversion of the Mg^{2+}-ATPase, as in strains NR76A and NR76B, leads to better energy coupling. These observations lend support to the concept of a structural role of the Mg^{2+}-ATPase protein. DCCD has been shown to stimulate transport of substrates in strain NR70 [5, 10], and compounds such as DCCD have been postulated to bind to the same site on the membrane as that to which the Mg^{2+}-ATPase binds, thus filling the same structural role as the Mg^{2+}-ATPase in energy-coupling [5, 10, 21]. As seen in Fig. 7 for membrane vesicles the addition of DCCD stimulates proline transport in strains NR76 and NR76A, but inhibit transport in strain 7. Strain NR76B, while still deficient in Mg^{2+}-ATPase activity, is no longer deficient in the coupling of D-lactate oxidation to transport, and DCCD does not stimulate transport in vesicles of that strain. Transport of proline and thiomethyl-β-D-galactoside in whole cells is affected by DCCD in a similar manner: DCCD stimulates transport in NR76 and NR76A but inhibits transport in strains

NR76B and strain 7. Additional experiments not shown here demonstrate that neither the in vivo hydrolysis of *o*-nitrophenyl-β-D-galactoside nor the uptake of α-methyl-glucoside are defective in strains NR76, NR76A, and NR76B. Thus, the defects found for these strains very probably do not reside in the β-galactoside permease nor do they reflect a generalized alteration in membrane permeability, but, rather, are localized in the Mg^{2+}-ATPase.

Proton translocation associated with galactoside fluxes

Mitchell [22] has suggested that the primary driving force for active transport is the electrochemical gradient of protons derived from either the oxidation of a substrate of the electron transport chain or the hydrolysis of ATP by the Mg^{2+}-ATPase. Any changes in the membrane which would decrease the ability of that membrane to maintain the proton gradient would thus decrease the ability of the membrane to accumulate substrates via active transport systems. The Mg^{2+}-ATPase mutants NR70 and DL54 have been shown to have defects in active transport and simultaneously are unable to maintain a transmembrane pH gradient [10, 23]. If the role of the Mg^{2+}-ATPase in the coupling of oxidative energy to active transport is related to establishment of such a pH gradient, then alterations in the Mg^{2+}-ATPase which lead to a decrease in transport ability should be reflected in a decrease in the ability to establish the pH gradient, and, conversely, alterations in a defective Mg^{2+}-ATPase, leading to a restoration of transport ability should likewise be a reflection of an increased proton gradient. The symport model for active transport proposes that the coupling of the electrochemical gradient to active transport is through a carrier protein which transports both its substrate and a proton, one going against its chemical gradient at the expense of the electrochemical gradient of the other [22]. The uptake of β-galactosides in *E. coli* has been shown to be associated with the concomitant uptake of protons [10, 24]. Under conditions where the formation of a proton gradient is prevented, e.g. anaero-

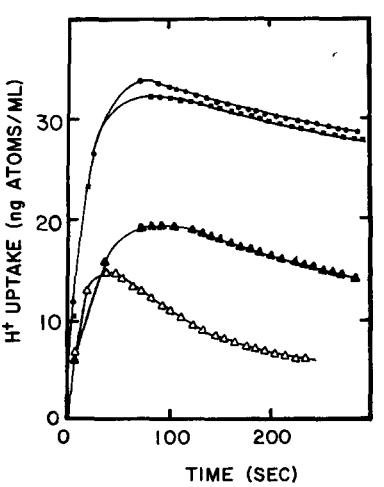

Fig. 8. Proton translocation in whole cells. pH measurements were performed as described under Methods. The reaction was initiated by the addition of 5 mM thiomethyl-β-D-galactoside at zero time. The points are corrected for the slope of the baseline (● — ●), strain 7; (■ — ■), strain NR76B; (▲ — ▲), strain NR76A; (△ — △), strain NR76.

TABLE I

HALF-TIMES FOR GALACTOSIDE-ASSOCIATED PROTON EFFLUX

Proton efflux was measured following the addition of 5 mM thiomethyl-β-D-galactoside to anaerobic suspensions of cells, as described under Methods. The recorder tracings were digitized, and the mean half-times for the exponential fall in medium pH were calculated from four experiments.

Strain	$t_{\frac{1}{2}} \pm$S.D. (s)	%
7	869±98	100±11
NR76B	720±231	83±27
NR76A	460±107	53±12
NR76	219±89	25±10

biosis, addition of a β-galactoside to the external medium has been associated with a transient uptake of protons, suggesting that as the β-galactoside goes down its chemical gradient, it brings a proton up its chemical gradient. (Electrogenic uptake of protons in such experiments is prevented by addition of the permeant anion, thiocyanate.) The effect is transient because protons leak out of the cells via a first-order process.

Fig.8 shows the response of anaerobic cultures of strain 7, NR76B, NR76A, and NR76 to the addition of thiomethyl-β-D-galactoside. In each case the medium becomes rapidly alkaline, followed by a subsequent acidification. The $t_{1/2}$ values for the release of protons subsequent to the alkalinization were calculated from four such experiments (Table I). The results show that the membrane of strain NR76 has a greatly increased permeability to protons compared to the wild-type. Strain NR76A likewise has a greater permeability than strain 7, but less than NR76. The membrane of NR76B is only slightly more permeable to protons than is the wild-type. Thus, there is a correlation between the ability to accumulate substrates and the permeability of the membrane to protons.

GENERAL CONCLUSIONS

The ability to couple aerobic metabolism to such energy-requiring processes as the phosphorylation of ADP via the Mg^{2+}-ATPase, the transhydrogenation of NADP by NADH, and active transport has been related to the generation and maintenance of a proton-motive force [22]. Neither the ability to generate the force nor the ability to maintain it is in itself sufficient for energy coupling. Thus, oxidation of a compound by the electron transport chain may cause the extrusion of protons from the cell, i.e., generation of a proton-motive force. But, if the membrane has a greatly reduced resistance to a proton "current", a short circuit would ensue, preventing the utilization of the energy derived from the oxidized compound. The results of this study are consistent with the hypothesis that the Mg^{2+}-ATPase fulfils a structural role in the maintenance of the high energy state of the membrane. The Mg^{2+}-ATPase mutant NR76 is capable of oxidizing substrates via the electron transport chain and thereby creates an energized membrane state, as measured by the quenching of quinacrine fluorescence. The magnitude of this effect is much less than the wild-type organism, although how much less cannot be determined from the fluorescence measurements.

The mutant can couple this energy to the transport of amino acids and sugars, but the coupling of the energy derived from electron transport is less than that of the wild-type. As would be predicted by the hypothesis, the permeability of the membrane of the mutant to protons is greatly increased. But is this change a direct result of the alteration in the Mg^{2+}-ATPase protein? The fact that one partial revertant of this mutant, strain NR76A, has a slightly decreased permeability to protons and a slightly increased ability to accumulate substrates, while a second partial revertant NR76B, has a greatly decreased proton permeability and a greatly increased ability to accumulate proline and thiomethyl-β-D-galactoside suggest that that is the case. There does not appear to be a correlation between the ATP-hydrolyzing activity of the Mg^{2+}-ATPase and the ability to maintain an aerobically-generated energization of the membrane. Reversion of the mutant NR76 to less than 10% of the wild-type activity, as found for NR76B, results in a nearly complete recovery of transport ability. The fact that the quenching of fluorescence by D-lactate catalyzed by NR76B membranes is not as great as that of strain 7 may be a reflection of the preparation of the membranes or of the qualitative nature of the assay itself.

A model for the role of the Mg^{2+}-ATPase in aerobically-driven transport is shown in Fig. 9. The electron transport chain can generate an electrochemical gradient

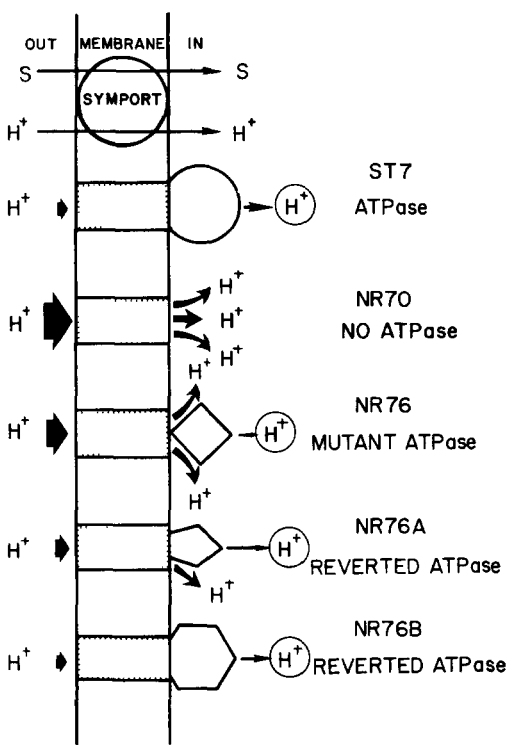

Fig. 9. Model for the structural role of the Mg^{2+}-ATPase in the maintenance of the energized membrane state. A proton gradient is established through the oxidation of compounds by the electron transport chain. Protons can reenter the cell either through symports or through the ATP synthetase system. The size of the arrows suggests the magnitude of each reaction. Enclosed protons are those utilizable for the synthesis of ATP.

of protons by the vectorial dehydrogenation of reduced substrates. Various reactions, including transport via symports and ATP synthesis via the Mg^{2+}-ATPase compete for protons. Whichever reaction occurs with the greatest velocity uncouples the other (s) if there is not sufficient energy to supply both. The ATP-synthetase complex would consist of a proton-translocating element and a synthesizing element. If the synthesizing element were able to regulate the rate at which the proton-translocating element worked, then the lack of the former, as found in the Mg^{2+}-ATPase mutant NR70, would result in such a rapid translocation of protons by the latter element that other processes utilizing the proton gradient would be uncoupled, as is found in NR70. Another mutant such as NR76, which contains an inactive Mg^{2+}-ATPase, may have a conformation of that protein which partially impedes the flow of protons through the proton pump, resulting in a large but incomplete uncoupling of active transport. Successive changes in this mutated Mg^{2+}-ATPase may result in greater regulation of the proton fluxes, and successively greater ability to accumulate substrates as found in strains NR76A and NR76B. The findings that some Mg^{2+}-ATPase mutants have unimpaired ability to accumulate small molecules is entirely consistent with this model, since a change in catalytic activity need not result in an altered interaction of the inactive protein with the proton pump. Several predictions can be made from this hypothesis. First, it should be possible to reconstitute respiratory-driven active transport in a Mg^{2+}-ATPase mutant in vitro by the binding of the wild-type Mg^{2+}-ATPase to the appropriate place on the membrane. Second, if the uncoupling seen in these Mg^{2+}-ATPase mutants is a result of a competition for protons, then an active transport system with a rate greater than that of the proton-translocating element of the ATP synthetase system should not be uncoupled, or, at most, only partially affected. Indeed, the reverse should be true. Transport by that system should uncouple oxidative phosphorylation, as has been found for the Ca^{2+} transport system of mitochondria [22, 25].

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