ACTIVE TRANSPORT IN MUTANTS OF *ESCHERICHIA COLI* WITH ALTERATIONS IN THE MEMBRANE ATPase COMPLEX

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1. Introduction

A number of mutants of *Escherichia coli* have been isolated with a defect in oxidative phosphorylation [1–7]. The mutants isolated thus far, all map in the same region of the chromosome (73.5 min) and all are affected in the membrane Mg^{2+}– Ca^{2+} ATPase complex. One class of mutants has been shown to lack ATPase catalytic activity [1,3,5,7], while a second class of mutants retains the ATPase activity [2,4,6], but in a form which is resistant to the energy transfer inhibitor DCCD** [2]. Studies of active transport in mutants of the first class have provided evidence that phosphate-bond energy is not required for the aerobic transport of certain solutes in *E. coli* [5,8,9]. This report presents a comparative study of active transport of proline and TMG in mutants representing both classes. The transport of both solutes under aerobic conditions was similar to that in the parental strain; however, whereas transport in the mutants was inhibited completely by KCN, transport in the parent was inhibited only some 50%. In addition, no transport was observed in mutant cells under anaerobic conditions. These results support the conclusion [10] that energy for transport can be derived from a high energy state or intermediate generated via respiration or ATP hydrolysis, and that transport in the anaerobic state requires a functional ATPase capable of coupling ATP hydrolysis to active transport.

2. Materials and methods

The strains used in this report are all Lac^+-transductants [11], of the parent A428 [1] and the mutants N_{I44} [1] and B_{V4} [2].

All strains were grown on Davis minimal medium [12] supplemented with proline and histidine (50 μg/ml each) and vitamin B_1 (1 μg/ml). For transport assays with TMG the cells were grown on lactose, and for amino acid transport either lactose or glucose were used as carbon sources. Cells were harvested and washed twice in unsupplemented minimal medium and suspended to give a final turbidity of about 100 Klett units as measured on a Klett–Summerson colorimeter.

Ten millilitre aliquots of the cells were preincubated in the presence of 0.5% glucose in a shaking water bath at 25°C. Inhibitors, when used, were included during the preincubation period. The assay was started by the addition of [U-^{14}C]TMG (1 Ci/mole) to a final concentration of 10^{-4} M or [U-^{3}H]proline (2 Ci/mole) at a final concentration of 10^{-4} M. At various times after the addition of the radioactive material aliquots of 0.5 ml were withdrawn, filtered on nitrocellulose filters (0.45 μ, Sartorius) and washed 3 times with 10 ml cold NaCl (0.9%). The filters were counted in Bray's solution in a liquid scintillation counter.

For anaerobic transport assays, cells prepared as described above were transferred to a closed water-

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** Abbreviations: DCCD, *N, N'*-dicyclohexylcarbodiimide; TMG, methyl-1-thio-β-D galacto pyranoside; MCCP, carbonyl cyanide, m-chlorophenylhydrazone; ACMA, 9-amino-6-chloro-2-methoxyacridine.

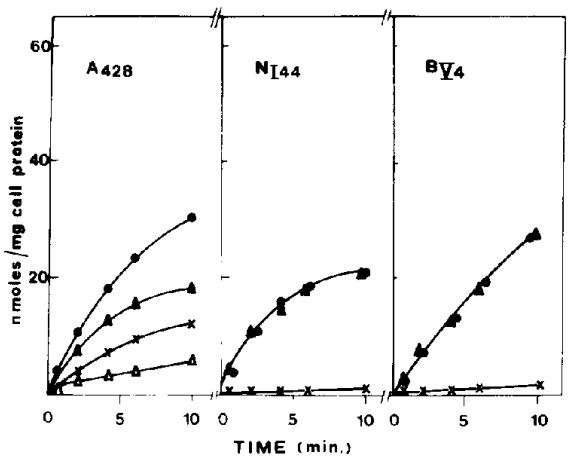

Fig. 1. Aerobic Uptake of [^{14}C]TMG by intact cells of the parent A428 and the mutants N_{I44} and B_{V4}. The experiment was conducted as described in Materials and methods. The energy source was glucose (0.5%). Additions during the pre-incubation period: None (•—•—•); DCCD, 2 mM (▲—▲—▲), KCN, 2 mM (X—X—X); KCN, 2 mM + DCCD, 2 mM (△—△—△).

jacketed vessel and bubbled with nitrogen gas for 20 min. The radioactive stock solution was submitted to the same treatment. Following addition of the radioactive solute 0.5 ml samples were withdrawn into syringes and quickly filtered, washed, and counted as described above. At the end of the 10 min period air was introduced into the vessel by simultaneously opening the vessel, stopping the bubbling, and stirring the cell suspension vigorously with a magnetic stirrer. The transport assay was then allowed to proceed for an additional 10 min under aerobic conditions.

All assays were carried out at 25°C. Inhibitors were added at the following final concentrations: KCN, 2 mM; DCCD, 2 mM; and M-CCP, 5 μM.

[U-^{14}C]TMG was obtained from New England, Nuclear and [U-^{3}H]proline from Amersham, Searle. M-CCP was purchased from Calbiochem and DCCD was a gift of Dr. Leon Goldstein. All other materials were of the highest purity commercially available.

3. Results and discussion

The results in fig. 1 illustrate the transport of the lactose analogue TMG in the parent A428 as compared with the two mutants N_{I44} (lacking ATPase catalytic activity) and B_{V4} (containing DCCD resistant ATPase). The transport in both mutants was similar to that in the parent. The addition of KCN brought about a 50% inhibition of the transport of TMG in cells of the parent, while the transport of the solute was completely inhibited by KCN in the two mutants. The energy transfer inhibitor DCCD brought an inhibition of about 40% in the parent A428, but no inhibition of transport in the two mutants could be observed. The addition of both cyanide and DCCD further inhibited the transport of TMG in A428 by some 80% indicating that the cyanide resistant transport in the parent is sensitive to DCCD. These experiments were performed using glucose as an energy source, although similar results were obtained in non-starved cells using an endogenous substrate. The complete inhibition of transport by KCN in N_{I44} indicates that the ATP formed by glycolysis cannot serve as an energy source for transport in this mutant, presumably due to the absence of ATPase. These results are in agreement with other experiments utilizing different mutants lacking ATPase activity [5, 8, 9]. It is of interest to note, that the transport in mutant B_{V4} is also completely inhibited by KCN. Although ATPase is present in this mutant [2], it cannot function to couple the hydrolysis of ATP to the generation of a high energy state, or intermediate, in agreement with cell-free studies of energy-linked transhydrogenase and energy-dependent quenching of fluorescence of the dye ACMA [14, 15].

Similar results were obtained when the transport of proline was investigated in the three strains A428, N_{I44}, and B_{V4} (fig. 2).

The transport in the parent was partially inhibited both by DCCD and by KCN, and further inhibited by a combination of the two.

The results in fig. 3 illustrate the transport of proline in the three strains under anaerobic conditions. The transport of proline in A428 in the absence of oxygen was almost identical to the transport in the presence of cyanide (fig. 2). In sharp contrast, however, no transport was observed in either of the mutants under anaerobic conditions.

The anaerobic transport of proline in A428 was inhibited some 50% by DCCD resulting in a transport activity which was similar to the aerobic transport in the presence of DCCD and KCN (fig. 2). The addition of oxygen caused an increased rate and extent of

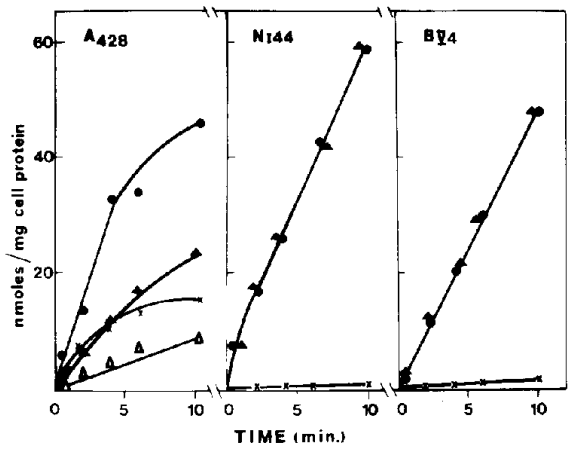

Fig. 2. Aerobic Uptake of [^{3}H]proline by intact cells of the parent A428 and the mutants N_{I44} and B_{V4}. The experiment was conducted as described in Materials and methods. The energy source was glucose (0.5%). Additions during the pre-incubation period: None (●—●—●), DCCD, 2 mM (▲—▲—▲), KCN, 2 mM (X—X—X), KCN, 2 mM + DCCD, 2 mM (△—△—△).

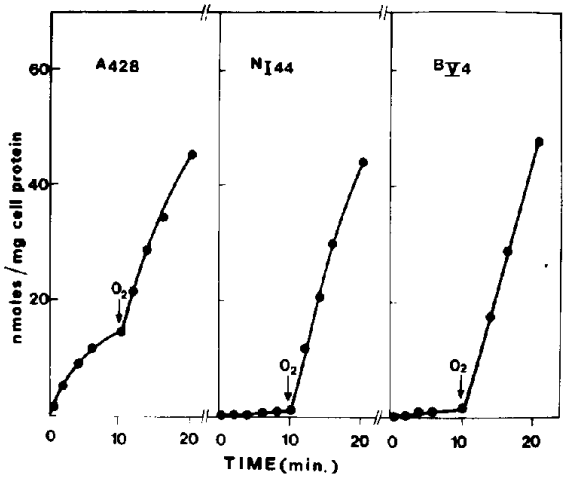

Fig. 3. The effect of anaerobiosis on the uptake of proline by intact cells of the parent A428 and the mutants N_{I44} and B_{V4}. The experiment was conducted as described in Materials and methods. The energy source was glucose (0.5%).

proline transport which was similar in the three strains. Similar results were obtained with TMG. The transport of both solutes in all strains and under all conditions was completely inhibited by the uncoupling agent m-CCP.

These results demonstrate that transport of proline and TMG in the two mutants is strictly respiration dependent, and that a functional ATPase complex is required for anaerobic transport. The inhibition of transport by DCCD in the parental strain A428 may indicate a role for functional ATPase in aerobic transport. Thus, N_{I44} and B_{V4}, both lacking a functional ATPase complex are unaffected by the inhibitor.

A number of reports have demonstrated that mutants lacking ATPase activity transport normally under aerobic conditions [5,8,9]. On the other hand preliminary studies using another class of mutants affected in the coupling process, but retaining ATPase [14] indicate that these new mutants are impaired in aerobic transport. These new mutants may resemble 'etc' mutants described by Hong and Kaback [16].

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