GALACTOSIDE TRANSPORT DISSOCIATED FROM PROTON MOVEMENT IN MUTANTS OF ESCHERICHIA COLI

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SUMMARY: Two mutants of Escherichia coli have been described in which the transport of β-galactosides is partly uncoupled from the metabolic reactions which drive active transport. It is shown that the effective inflow of H^+, caused by the addition of β-galactoside, is much less in these mutants than in the parental strains, and it is concluded that β-galactoside transport is partly uncoupled from H^+ transport.

INTRODUCTION

Two mutants of Escherichia coli have recently been isolated in this laboratory in which the β-galactoside carriers have a somewhat increased capacity to catalyse the facilitated diffusion of β-galactosides, but at the same time have a severely decreased ability to accumulate non-metabolizable β-galactosides against a concentration gradient; one mutant (ML 308-22) was in an ML strain (1), while the other (X71-54) was in a K_{12} strain (2,3). It was concluded that the defect in both cases was in the mechanism by which exergonic metabolic reactions are coupled to the active transport of β-galactosides and consequently these mutants have been referred to as having an energy-uncoupled β-galactoside transport system.

The two mutants appear to have very similar defects though they arose spontaneously in different strains, for in all respects in which they have been tested they show the same altered properties. Thus, both mutants show considerably faster efflux of ^{14}C-TMG[2], in both mutants the defect in accumulation is most striking at about pH 5.8 and becomes progressively

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[2] Abbreviations: TMG, thiomethyl-β-D-galactopyranoside; ONPG, o-nitrophenyl-β-D-galactopyranoside; TDG, D-galactopyranosyl-β-D-thiogalactopyranoside.

less marked at higher pH, and the rate of ONPG hydrolysis by intact cells is, in each case, 150-180% of the parental rate. There is a small quantitative difference in that the defect in accumulation of TMG is more severe in ML 308-22 than in X71-54. The accumulation of TMG by ML 308-22 at pH 5.8 is 8% of that of its parent (ML 308), while at the same pH, that of X71-54, is 15% of its parent (X71) (see refs. 1-4).

It was concluded from deletion mapping of X71-54 that the genetic lesion is near the transacetylase end of the y gene and that the altered phenotype is therefore due to a modified β-galactoside carrier, and not to some other defect in energy metabolism or membrane permeability (4). Because of the similar physiology of the two mutants, it seems likely that in ML 308-22 the defect is also in the y gene.

These mutants, in which the β-galactoside transport is energy-uncoupled, provide a valuable opportunity to test hypotheses concerning the mechanism of the coupling between metabolism and β-galactoside transport. One such hypothesis is the suggestion of Mitchell (5) that the β-galactoside carrier of E. coli is a β-galactoside-proton symport, i.e. that the carrier obligatorily couples the translocation of β-galactoside to the translocation of H^+. The metabolically driven efflux of H^+ was expected to establish a gradient of the electrochemical potential of H^+ which would be the driving force for β-galactoside accumulation. Recent observations on the alkalification of the extracellular medium (6), the acidification of the intracellular medium (7) and the movement of mobile counter-ions during β-galactoside inflow (8) lend strong support to the chemiosmotic coupling hypothesis, for it is clear that during β-galactoside transport by normal strains of E. coli, there is a strict coupling between the inflow of β-galactoside molecules and the effective[3] inflow of H^+ ions.

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[3] Because the inflow of H^+ cannot be distinguished from the outflow of OH^-, the term "effective inflow of H^+" is used to refer to either process.

On this hypothesis, an altered carrier, which could catalyse the net translocation of β-galactoside without the concomitant net translocation of H^+, would be able to effect rapid equilibration of β-galactoside between the inner and outer media, but would be unable to cause accumulation or retention of β-galactoside against a concentration gradient. The two energy-uncoupled mutants ML 308-22 and X71-54 were tested in experiments similar to those of West (6), and shown to have a defect in the effective H^+ inflow caused by β-galactoside inflow.

METHODS

E. coli strains used in this study included: ML 308 ($i^-z^+y^+a^+$), ML 308-22 ($i^-z^+y^{(un)}a^+$), X71 ($i^-z^+y^+a^-$, $proC^-$, try^-, B_1^-) and X71-54 ($i^-z^+y^{(un)}a^-$, $proC^-$, try^-, B_1^-. The gene which codes for the energy-uncoupled transport protein is designated as $y^{(un)}$.

Bacteria were grown aerobically at 37° to late log-phase (0.33-0.46 mg cell dry wt./ml) in mineral medium M63 (9) supplemented with NaCl (50 mM). X71 and X71-54 were grown in medium 63 supplemented with L-proline (100 μg/ml), L-tryptophan (10 μg/ml) and thiamine (0.5 μg/ml). The carbon source was either 0.2% succinic acid (w/v) brought to pH 7 with KOH, or 1% Tryptone casein hydrolysate (Difco, Box 1058A, Detroit, Michigan). Cells were harvested by centrifugation at 4° for 10 min at 12,000 x g, washed once in 120 mM NaCl containing 2 mM mercaptoethanol and resuspended in 120 mM NaCl—2 mM mercaptoethanol, at a cell density of about 40 mg cell dry wt./ml. This stock suspension was kept at 0° until used.

The measurements of extracellular pH were made in a narrow glass vessel with a plastic lid, which contained, in 2.0 ml: 180 μmoles NaCl, 60 μmoles NaSCN and 6.5-8.0 mg cell dry wt. This suspension was mixed with a magnetic stirrer and bubbled with O_2-free nitrogen so that, though the plastic lid was somewhat permeable to O_2, the suspension and air space were kept anaerobic. The extracellular pH was monitored with a combined glass

and reference electrode and a sensitive pH meter, connected to a strip-chart recorder.

Standard solutions of 5.0 mM HCl and 5.0 mM NaOH, made up in 120 mM NaCl, were evacuated and flushed with N_2. A solution of 1 M TMG was freed of O_2 by bubbling N_2 through it for 10 min. These solutions were dispensed anaerobically from small syringes (Hamilton, Box 307, Whittier, California).

Before the addition of anaerobic TMG, the suspension was incubated for 20-30 min to allow the cells to become anaerobic and come into equilibrium with the ionic composition of the medium.

RESULTS

Fig. 1 shows the strip-chart recording of extracellular pH when anae-

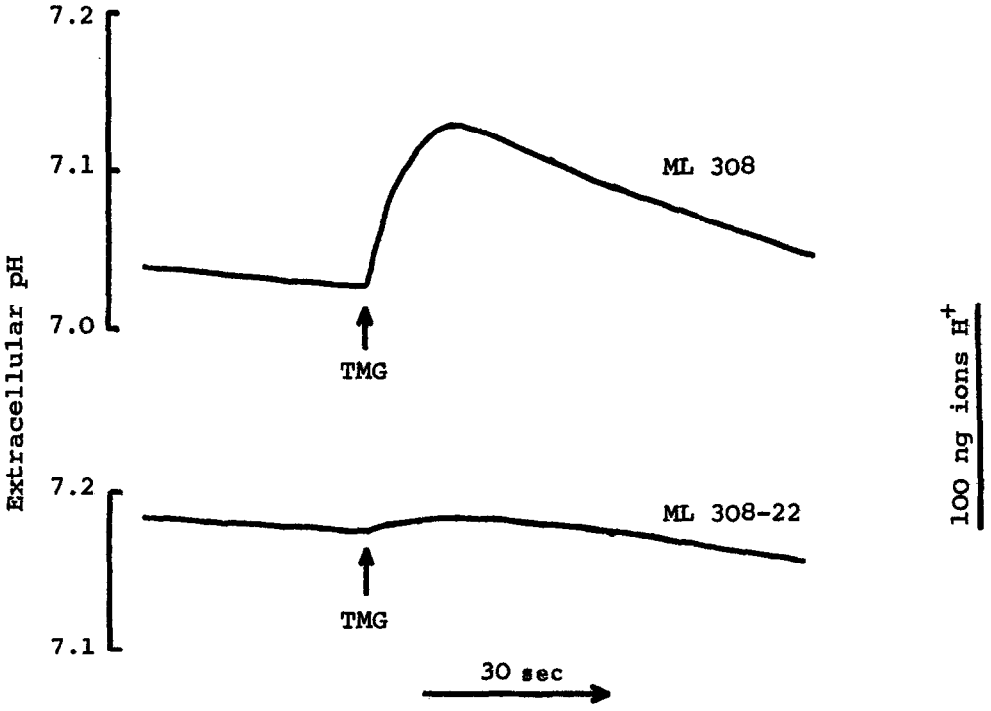

Fig.1. Extracellular pH changes on adding TMG to anaerobic suspensions of ML 308 and ML 308-22. To the 2.0 ml of suspension, containing 6.6 mg of ML 308 or 7.1 mg of ML 308-22 (cell dry wt.), was added 10 µl of anaerobic 1 M TMG. The extracellular buffering power was the same for each trace.

robic TMG solution was added to anaerobic suspensions of (a) the normal strain, ML 308, and (b) the energy-uncoupled strain, ML 308-22. The rapid alkalification of the normal suspension was comparable with that reported previously (6,8), while in the case of the energy-uncoupled mutant, both the initial rate and the final extent of the effective H^+ inflow were very much reduced. From the standard acid and alkali titrations of the extracellular buffering power, the initial rate of alkalification can be converted to a rate of effective H^+ inflow. In the experiment of Fig.1, the initial rates were 42 and 1.8 ng ions H^+/mg cell dry wt. per min for ML 308 and ML 308-22 respectively. Similar rates were obtained with these strains whether the cells were grown with Tryptone or with succinate as carbon source.

The result of a similar experiment, using the other energy-uncoupled mutant (X71-54) and its normal, energy-coupled parent (X71), is shown in Fig.2. Corrections have here been made for the baseline drift and the

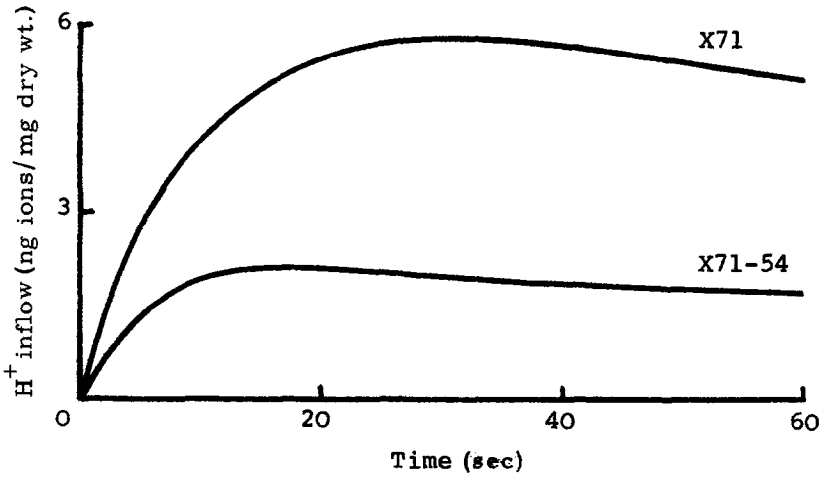

Fig.2. Effective translocation of H^+ into X71 and X71-54 on adding TMG. Extracellular pH was 7.1-7.2. Extracellular buffering power was 62 and 69 ng ions H^+ per 0.1 pH unit for X71 and X71-54 respectively, while cell dry wt. was 6.9 and 6.4 mg for X71 and X71-54 respectively. At zero time 10 µl of anaerobic TMG was added.

small differences in extracellular buffering power and cell dry wt. between the two traces. Once again, the initial rate of effective H^+ inflow was lower in the case of the energy-uncoupled mutant than in the parent (18.9 and 39.6 ng ions H^+/mg cell dry wt. per min respectively), though with these two strains the difference was smaller, as is the defect in TMG accumulation.

The addition of anaerobic TDG solution to anaerobic cell suspensions also caused the effective inflow of H^+, but the initial rate and the maximal extent were both small (Fig.3). This is presumably because the

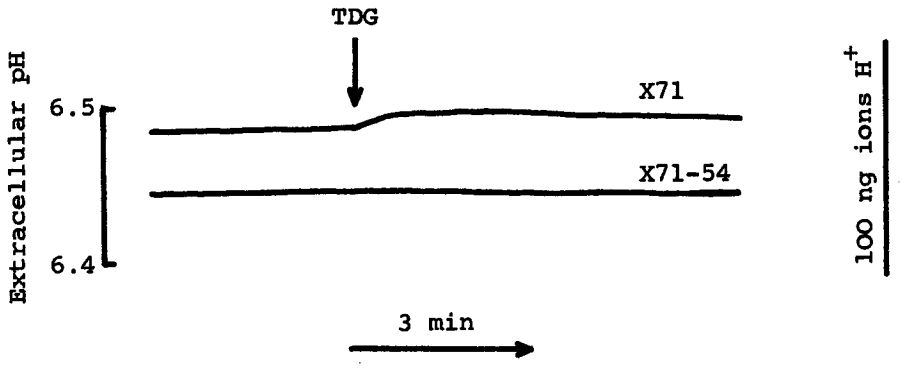

Fig.3. Extracellular pH changes on adding TDG to anaerobic suspensions of X71 and X71-54. To the 2.0 ml of suspension, containing 8.2 mg of X71 or 8.0 mg of X71-54 (cell dry wt.), was added 10 µl of anaerobic 1 M TDG.

inflow of this substrate is so slow (15% of the rate for TMG) (10) that the outward leakage of the displaced H^+ soon equals the rate of H^+ inflow. With the energy-uncoupled mutant, X71-54, the effective inflow of H^+, caused by TDG addition, was too small to be detected.

It was important to confirm that the plasma membranes of the bacteria were intact, and to test the possibility that the small effective net movements of H^+ with the energy-uncoupled strains were merely due to a greater permeability to H^+. This was done routinely by giving the anaerobic suspensions a small injection of aerobic medium (50 µl). The brief pulse

of respiration caused a rapid acidification of the extracellular medium, followed by a gradual return of the pH trace to the original baseline. Using this test procedure it was found that cells harvested during mid log-phase growth were more liable to lose their ability to establish a pH difference across the plasma membrane than were cells from late log-phase. In the experiments described in Figs.1-3, the membranes remained intact and there was no loss of the ability to establish a pH difference across the membrane. Nor was the gradual leakage of acid equivalents through the membrane faster in the two energy-uncoupled mutants than in the energy-coupled parents.

DISCUSSION AND CONCLUSIONS

It is shown in Figs. 1 and 2 that the effective inflow of H^+ induced by β-galactoside inflow into each of the energy-uncoupled mutants was considerably slower than that into the respective parental cells. It is possible that the entry of β-galactoside was correspondingly slower, but the previous work with these cells (1-4) under aerobic conditions indicates that the opposite is the case. Thus, (a) the rate of *in vivo* hydrolysis of ONPG by ML 308-22 and X71-54 was 150-180% of the rate in the corresponding parental strains, (b) the accumulation of TMG in azide-treated cells, caused by counterflow, was significantly greater in each of the energy-uncoupled mutants than in the parental strains and (c) the initial rate of ^{14}C-TMG uptake by X71-54, measured at 4°, was faster than that of its parent, X71.

We therefore, conclude that in each of the two energy-uncoupled mutants, ML 308-22 and X71-54, there is a defect in the coupling of effective H^+ inflow to β-galactoside inflow such that the stoichiometry of H^+/β-galactoside, which in the normal, energy-coupled, strain ML 308-225 had recently been shown to be 1.0 (11), is decreased severalfold. This defect is more severe in ML 308-22 than in X71-54.

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