LACTOSE TRANSPORT COUPLED TO PROTON MOVEMENTS IN ESCHERICHIA COLI

I. C. West

Department of Biological Chemistry, University of Manchester, Manchester 13, England.

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Summary. This paper gives evidence of a flow of protons into Escherichia coli when lactose is transported inwards by the lactose carrier.

Lactose and other β-galactosides are accumulated by Escherichia coli as free sugars to concentrations between 100 (1) and 2000 (2) times the extracellular concentration. These accumulated galactosides are rapidly released by adding 2mM dinitrophenol (3) or 30mM azide (1) to the suspension. Hypotheses have been put forward which explain the accumulation of galactosides as due to the interaction of a high-energy compound (e.g. ATP) with the carrier (4), or the carrier-sugar complex (5), either on the inside of the membrane so as to lower the affinity of the carrier for the sugar (5), or on the outside to increase the affinity (4). According to these hypotheses the action of uncouplers in releasing accumulated galactosides would be due to a decrease in the cellular levels of the high-energy compound. Pavlasova and Harold (6) have recently shown that uncoupling agents abolish thiomethyl-galactoside accumulation while causing little or no decrease in the level of ATP, which is therefore unlikely to be the high-energy compound immediately responsible for lactose accumulation. Arguing from the hypothesis that the primary action of these uncoupling agents is to conduct protons across the membrane, Mitchell suggested (7,8) that it is the difference in the electrochemical potential of protons between the inside and the outside of the cell which maintains the

high internal concentration of the sugar. The energy required to transport a lactose molecule into the cell against a concentration gradient would be obtained from the passage of a proton in the same direction down its electrochemical potential gradient. The hypothesis, described in Mitchell's terminology, is that the lactose porter is a proton symporter.

If the energy for lactose accumulation is derived either directly or indirectly from the electrochemical potential gradient of protons, there must be a movement of protons down that gradient. That is thermodynamically necessary. In cells metabolizing normally, the postulated flow of protons into the cell would be balanced by an equal flow of protons out of the cell. This is caused, it is assumed, by the flow of electrons down the respiratory chain (7), and by the functioning of a membrane ATPase (9). On adding lactose to a suspension of cells in weak buffer, there might be an initial slight fall in the proton concentration of the medium, but no continued rise in pH. However, if respiration and the glycolytic production of ATP are inhibited it should be possible to cause a net flow of lactose and proton into the cell. Having inhibited the normal pumping of protons out of the cell, another factor which might limit the net inflow of protons (and therefore lactose also, according to the hypothesis) is the building up of a positive charge inside the bacterium. This charge could be neutralized either by an inflow of negative ions or an outflow of positive ions. Mitchell and Moyle (10) have shown that the thiocyanate anion readily penetrates the mitochondrial membrane, and suggested that it might serve in the present experiments to accelerate proton inflow. The following experiments demonstrate a movement of protons on adding lactose.

Three constitutive strains of *E. coli* were used; ML 308 (having galactosidase and permease), ML 308-225 (lacking galactosidase) and ML 35 (lacking permease). Cells from exponential growth (10-18 mg.

dry wt.) were washed once and suspended in 5.0 ml. of a medium containing NaCl (150 mM), Tris buffer (1 mM) and iodoacetate (1 mM) adjusted to pH 7.2. This suspension was bubbled with oxygen-free nitrogen in a narrow glass vessel; the stream of bubbles provided adequate mixing. Hydrogen ion activity was measured with a combined glass and reference electrode connected to an electrometer and strip-chart recorder. HCl (0.1 M) was used to calibrate the millivolt scale.

Lactose, added to a suspension of ML 308 to give 1 mM (50 µl of 0.1 M), caused a proton uptake of about 5 nmole/min./mg. cells. If NaCNS was first added to a concentration of 50 mM the rate of proton uptake on adding lactose was increased several fold (Fig. 1). NaCNS also doubled the rate of lactose penetration into anaerobic cells poisoned with 1 mM iodoacetate. This was determined by following the reduction in optical density caused by osmotic swelling, a technique

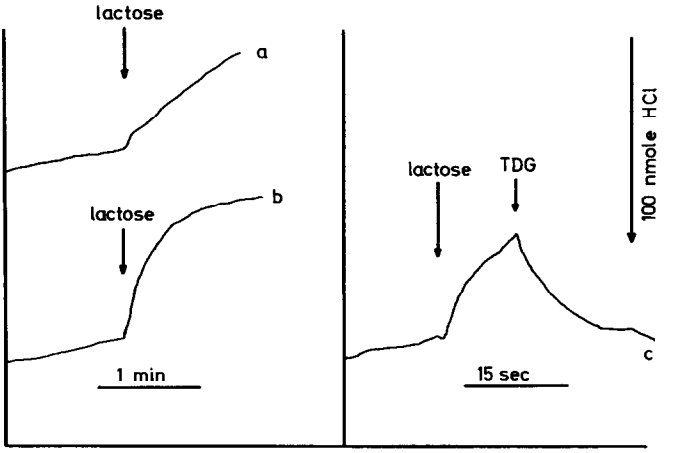

Fig. 1 Hydrogen ion activity. Strain ML 308; (a) without NaCNS (b) and (c) with 50 mM NaCNS.

described by Mitchell and Moyle (11). Low concentrations of uncoupler (4×10^{-6} M carbonylcyanide-m-chlorophenylhydrazone (CCCP)) also increased the rate of lactose penetration under these conditions by 20% (S.D. 8.7%, 8 experiments). Presumably an outflow of protons discharges the

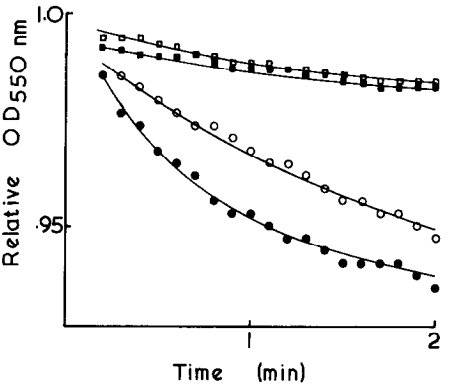

Fig. 2 Osmotic swelling in lactose solution. Cells of strain ML 308 washed and suspended for 30 min. in a solution containing NaCl (150 mM), iodoacetate (1 mM) and Tris buffer (1 mM) at pH 6.5. At time zero 0.1 ml of cell suspension was added to a cuvette containing 2.5 ml of lactose (386 mM) which had been bubbled with nitrogen for 5.0 min. The lactose solution contained in addition 40 mM NaCl (open symbols), 40 mM NaCNS (closed symbols) or 30 mM formaldehyde. (square symbols)

potential difference across the membrane. (Fig. 2)

Thiodigalactoside (TDG), a competitive inhibitor of carrier mediated lactose uptake, abruptly stopped the proton uptake, and caused a release of the accumulated protons, when added to a concentration of 1 mM (Fig. 1c). Cells treated with formaldehyde (30 mM) which also inhibits lactose transport, showed no proton uptake on adding lactose (Fig. 3a). (The pH trace fell continuously; adding lactose, or an equal volume of water, caused a slight extra acidification, which may be due to dissolved oxygen.) Likewise, cells treated for 10 min. with CCCP at 5×10^{-5} M gave no response to lactose. A strain lacking the permease gene (ML 35) was examined; again there was no proton uptake (Fig. 3b).

The ML 308 strain, having galactosidase, will take up quite large amounts of lactose, even when anaerobic and poisoned with iodoacetate, because rapid hydrolysis prevents efflux of the sugar. On the other hand, ML 308-225, lacking galactosidase, will only take up lactose under these conditions until the internal concentration is the same as

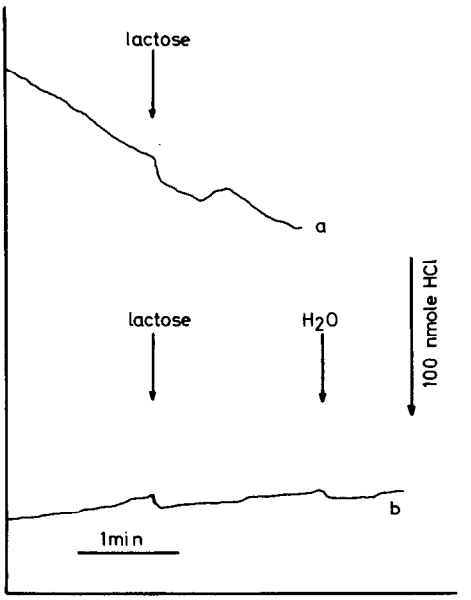

Fig. 3 Hydrogen ion activity. (a) strain ML 308-225 treated with 30 mM formaldehyde, (b) strain ML 35.

the external. (This has been confirmed with radioactive lactose.) Fig. 4a shows the pH trace when lactose was added to a suspension of ML 308-225 to give a concentration of 5 mM (25 µl of M lactose); the proton uptake reached a plateau after 3 min.. This plateau level was proportional to the amount of lactose added. The dry wt. of cells is known, the volume of cell water is 2.7 µl/mg. dry wt., measured as the water inaccessible to blue dextran and in agreement with Winkler and Wilson (1), so one can calculate how much lactose is taken up at equilibrium; the results are shown in the table. The observed proton uptake was about 75% of the calculated lactose uptake. If NaCNS was first added the initial rate of proton uptake was again much faster, but after 40 sec. protons began to leak out again (Fig. 4b). The permeability of both the anion (CNS^-) and the protonated acid (HCNS) may make thiocyanate a weak uncoupler.

In these experiments energy production was prevented and lactose

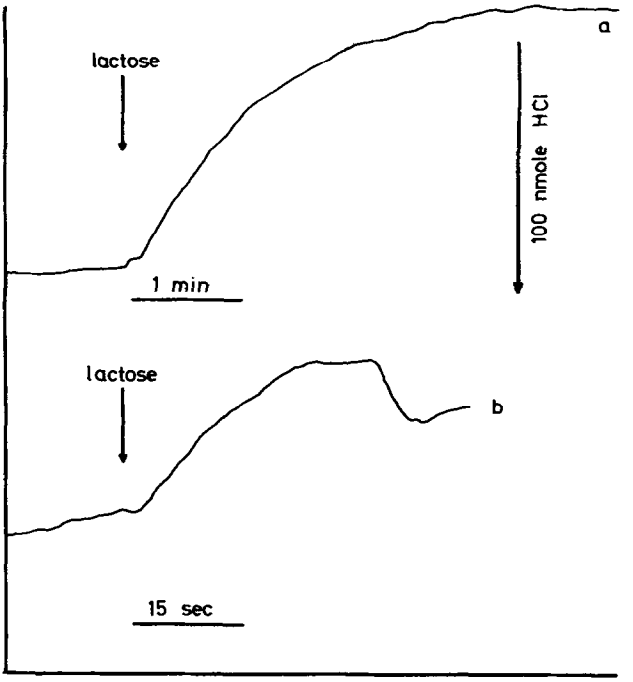

Fig. 4 Hydrogen ion activity. Strain ML 308-225; (a) without NaCNS, (b) with 50 mM NaCNS.

only moved down a concentration gradient. There was nevertheless a proton flow, which means that the two fluxes must be coupled mechanically; lactose does not traverse the membrane unless accompanied by a proton. This is indeed what one would expect if the mechanism suggested by

Table

Stoichiometry between lactose and proton uptake

External lactose concn. (mM)	Dry wt.of cell (mg.)	Calc. internal lactose (nmole)	Proton uptake (nmole)	Proton per lactose
5	14.3	193	100	0.52
2	11.7	63.2	51.2	0.81
2	18.5	100	70.7	0.71
1	11.7	31.6	46.3	1.46
1	16.7	45.1	27.7	0.61
0.5	18.5	25.0	19.1	0.76

Mitchell were to apply —— the efflux of galactosides being prevented by the low electrochemical potential of protons inside the cell.

These experiments show that, as predicted by the proton symport theory, there is a flow of protons into E. coli when lactose is taken up by the lactose carrier (or else the equivalent release of hydroxyl ions). The flow of protons is roughly proportional to that of lactose. Furthermore, the flow of lactose seems to be electrogenic and to be impeded if charge neutralization is inhibited.

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1. H.H.Winkler and T.H.Wilson, J.Biol.Chem.,241 (1966) 2200.
2. A.Kepes and G.N.Cohen, in: The bacteria, Vol.IV, eds. I.C. Gunsalus and R.Stanier (Academic Press, New York, 1962) p.179.
3. A.Kepes, Biochim.Biophys.Acta, 40 (1960) 70.
4. G.A.Scarborough, M.K.Rumley and E.P.Kennedy, Proc.Natl.Acad.Sci. 60 (1968) 951.
5. D.Schachter and A.J.Mindlin, J.Biol.Chem., 244 (1969) 1808.
6. E.Pavlasova and F.M.Harold, J.Bacteriol., 98 (1969) 198.
7. P.Mitchell, Biochem.Soc.Symp., 22 (1963) 142.
8. P.Mitchell, Symp.Soc.Gen.Microbiol., 20 (1970) 121.
9. F.M.Harold, J.R.Baarda and E.Pavlasova, J.Bacteriol., 101 (1970) 152.
10. P.Mitchell and J.Moyle, European J.Biochem., 9 (1969) 149.
11. P.Mitchell and J.Moyle, Symp.Soc.Gen.Microbiol., 6 (1956) 150.