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Galactoside Accumulation by *Escherichia coli*, Driven by a pH Gradient

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Acidification of the external medium results in thiomethylgalactoside accumulation in an energy-depleted adenosine triphosphatase-negative mutant of *Escherichia coli*.

An increasing body of evidence (4, 5, 8, 11-14) supports Mitchell's hypothesis (7) that lactose enters *Escherichia coli* by cotransport with protons. According to this view active transport of β-galactosides is driven by an inwardly directed electrochemical gradient for protons (protonmotive force). Consistent with this view is the demonstration that the addition of lactose or thiomethylgalactoside (TMG) to energy-depleted cells results in net proton entry (11-14). Furthermore, proton entry induced by a membrane potential (inside negative) results in β-galactoside accumulation in membrane vesicles of *E. coli* (4, 5). This communication demonstrates that proton entry induced by an artificially produced chemical gradient for protons (outside acid) can also provide the driving force for sugar accumulation in *E. coli*.

DL-54 (10), an adenosine triphosphatase (ATPase)-negative mutant (kindly provided by Simoni) was grown overnight in minimal medium (3) containing 0.4% glycerol. In the morning the cells were diluted in fresh medium to an optical density of 50 Klett units (filter no. 42) and allowed to double once according to the method of Wood (16). Cells were then depleted of energy reserves by incubation for 1 h in minimal medium containing 5 mM 2,4-dinitrophenol according to the method of Berger (2). Starved cells were washed, resuspended in 200 mM potassium phosphate, pH 8, 2 mM sodium iodoacetate, and 2 mM NaCN, and then incubated for 1 h. Cyanide was added to block respiration and iodoacetate to inhibit glycolysis; the latter process might produce a protonmotive force in this mutant via slight residual activity of the ATPase. [^{14}C]TMG (final concentration 50 μM) was added to the assay tube and aliquots were initially removed to determine the extent of TMG uptake at pH 8. Under these conditions the TMG uptake by starved cells was very low (Fig. 1), the intracellular concentration being one-half the medium concentration. In other similar experiments the intracellular sugar concentration was sometimes above and sometimes below that in the external medium. This presumably depended upon the magnitude and direction of the protonmotive force in energy-depleted cells. When HCl was added to the assay tube decreasing the external pH from 8 to 5.7, a transient accumulation of the radioactive substrate was observed. The peak concentration of approximately 260 μM was five times that found in the medium and 10 times that found in cells at pH 8 before the pH gradient was imposed. Control cells preincubated at pH 6 and then assayed for TMG uptake at pH 6 showed no accumulation. Proton entry with sugar during the acid pulse experiment should result in a membrane potential (inside positive) which would severely limit further transport. In these experiments, however, chloride ions probably enter the cell and prevent the development of such a potential difference. This view is supported by the observation that transport is much reduced when H_2SO_4 is added instead of HCl. The transient accumulation of TMG resulting from HCl addition was blocked by the presence of the proton conductor carbonylcyanidefluoromethoxyphenylhydrazone (10 μM), suggesting that a protonmotive force is involved in the transport event. Acid-induced TMG accumulation was also observed with the energy-depleted ATPase-positive cells (ML-308).

The ATPase-negative mutant DL-54 was utilized in these experiments to exclude the possibility that proton entry via the ATPase generated ATP which was the immediate source of energy for transport. It has been shown that in ATPase-positive cells a sufficiently large protonmotive force is able to drive adenosine 5'-triphosphate synthesis from adenosine 5'-diphosphate and inorganic phosphate (6). A peculiar feature of this particular ATPase-negative mutant is its abnormally high permeability to protons. The ATPase inhibitor *N,N*-dicyclohexylcarbodiimide (DCCD) has been shown to

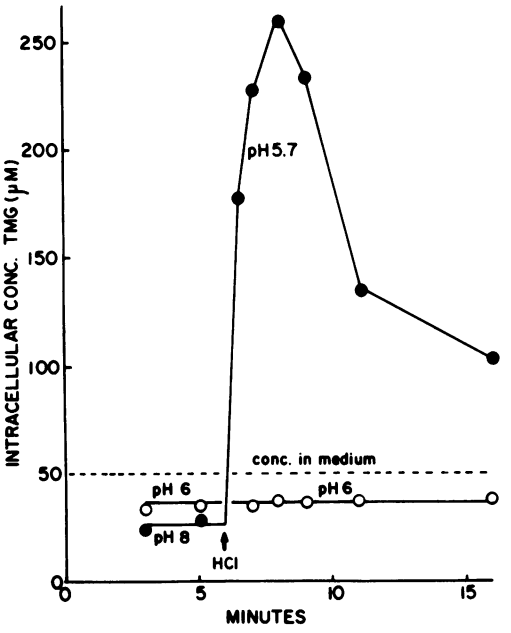

FIG. 1. *Stimulation of TMG accumulation by a pH gradient. Starved cells were divided into two portions. One group was washed twice with potassium phosphate (200 mM, pH 8) containing iodoacetate (2 mM) and cyanide (2 mM). The other group was washed twice with the same solution adjusted to pH 6. Both groups were incubated in the presence of DCCD (1 mM) for 30 min. Each group of cells was washed again in buffer adjusted to the appropriate pH. Cells in pH 8 buffer (optical density = 2,350; filter no. 42) were diluted 10-fold into the same solution. After 1 h the experiment was started by adding [^{14}C]TMG at a final concentration of 50 μM (3.9 μCi/ml). At the 3- and 5-min intervals samples (0.2 ml) were filtered and quickly washed with potassium phosphate (200 mM, pH 8). At 6 min 0.19 ml of 2 N HCl was added to 3 ml of cell suspension and at various intervals additional samples (0.2 ml) were filtered and washed with pH 6 buffer. Control cells at pH 6 (optical density = 2,870; filter no. 42) were diluted 10-fold into the same solution. After 1 h the experiment was started by adding [^{14}C]TMG. At various intervals samples were withdrawn, and the cells were filtered, washed, and counted. All manipulations were carried out at 25 C. Intracellular concentrations of TMG were calculated assuming that 1 ml of a cell suspension of optical density = 100 contained 0.6 μl of intracellular water (15).*

block the high proton permeability found in this mutant (1). Rosen (9) had previously shown that DCCD not only reduces the high proton permeability but also repairs the sugar transport defect seen in a similar ATPase-negative strain. The use of DCCD in the present studies was to reduce the proton permeability so that large protonmotive potential differences could be maintained.

These experiments indicate that an artificially imposed pH gradient (acid outside) can provide the energy for sugar accumulation in energy-depleted cells of *E. coli*. These results are consistent with the chemiosmotic hypothesis of Mitchell (7).

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