CO-TRANSPORT OF Na^+ AND METHYL-β-D-THIOGALACTOPYRANOSIDE MEDIATED BY THE MELIBIOSE TRANSPORT SYSTEM OF ESCHERICHIA COLI

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SUMMARY: Na^+-dependent transport of methyl-β-D-thiogalactopyranoside (TMG) mediated by the melibiose transport system was investigated in Escherichia coli mutants lacking the lactose transport system. When an inwardly-directed electrochemical potential difference of Na^+ was imposed across the membrane of energy depleted cells, transient uptake of TMG was observed. Addition of TMG to cell suspensions under anaerobic conditions caused a transient acidification of the medium. This acidification was observed only in the presence of Na^+ or Li^+. These results support the idea that TMG is taken up by a mechanism of Na^+-TMG co-transport via the melibiose transport system in Escherichia coli.

The mechanism for the cellular accumulation of sugars and amino acids in several animal cells has been shown to involve the obligatory coupling between the movements of Na^+ and the substrate (1). In microorganisms, on the other hand, H^+ rather than Na^+ is coupled to several substrate accumulation processes (2). There are a few microbial systems, however, in which Na^+ plays an important role. In 1971 Stock and Roseman (3) reported experiments with Salmonella typhimurium which suggested Na^+-melibiose co-transport. MacDonald and Lanyi (4) reported Na^+-gradient driven transport of amino acids in Halobacterium halobium.

Escherichia coli possesses two pathways for the entry of melibiose, one via the lactose transport system and another via a melibiose transport system (TMG permease II) (5). Accumulation of substrates through the lactose membrane carrier has been shown to be due to H^+-sugar co-transport (6,7). It was of interest to determine whether the melibiose transport system utilized H^+ or Na^+ for co-transport.

In this paper evidence is presented for Na^+-TMG co-transport in melibiose-induced cells of Escherichia coli strain lacking the lactose transport system.

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MATERIALS AND METHODS

E. coli strain 7-6 is a lactose transport negative (y^-) derivative of strain 7 (8) obtained with the method of Müller-Hill et al. (9) utilizing o-nitrophenyl-β-thiogalactopyranoside (TONPG). The TONPG procedure was also employed to select for strain NR70-1, a y^- derivative of strain NR70 (an ATPase negative derivative of strain 7) (10). Strains 7-6 and NR70-1 were grown at 37° in medium 63 (11) supplemented with 10 mM melibiose, 0.2% casamino acids and 0.5 µg/ml of thiamine. Cells were harvested in middle to late exponential phase of growth.

For transport energized by endogenous energy, cells of strain 7-6 were washed twice with 100 mM potassium phosphate buffer, pH 7.0, and resuspended in same buffer. Transport experiments were conducted with ^{14}C-TMG which is a substrate for the melibiose transport system (5). ^{14}C-TMG (2.5 µCi/µmole) was added to 1.0 ml of assay mixture containing 100 mM potassium phosphate, pH 7.0 and cells. The final concentration of the TMG was 0.10 mM. When required either 10 mM NaCl or 10 mM LiCl was added. Samples (0.2 ml) were taken at intervals, filtered on Millipore filters, and washed with 5 ml of 100 mM potassium phosphate, pH 7.0.

In another series of experiments cells with either high or low intracellular Na^+ content were energy depleted. One portion of cells("low-Na^+")was grown in medium 63 in the absence of Na^+ in order to reduce the intracellular Na^+. These cells were exposed to Na^+-free solutions during the subsequent steps of energy depletion and washing. A second portion of cells ("high-Na^+") were grown in medium 63 containing 100 mM Na^+ so that the intracellular Na concentration would be high. During the energy depletion and washing steps this Na^+-containing buffer was utilized. All cells were energy depleted by exposure to 5 mM dinitrophenol (DNP) for 1 hour at 37° and washed 3 times to remove DNP. Both the low-Na^+ and high-Na^+ cells were divided into two samples; one was washed with buffer at pH 6.0 and the other at pH 8.0. At the end of the final wash carbonylcyanide-p-trifluoromethoxyphenylhydrazone (CCFP) and KCN were added to each of the four samples to give a final concentration of 5 µM and 2 mM respectively. 10 minutes after addition of CCFP and KCN cells were diluted 1:100 into a solution containing 100 mM NaH_2PO_4 (adjusted to pH 8.0 with KOH), 2 mM KCN, 5 µM CCFP and 0.10 mM ^{14}C-TMG.

For the pH measurements, cells of strain 7-6 were washed twice with 120 mM KCl, and resuspended in small volume of 120 mM KCl. pH changes were measured as described by West (6) under anaerobic conditions. Iodoacetate which is inhibitory for TMG transport was omitted from the reaction vessel. Intracellular water space was determined as described elsewhere (12).

RESULTS

While most E. coli K_{12} strains will not produce an active melibiose transport system at 37°, the lactose transport negative strains 7-6 and NR70-1 grow well on melibiose at 37°. As shown in Figure 1, transport of TMG in melibiose-induced cells of strain 7-6, was greatly stimulated by NaCl or LiCl. K^+, NH_4^+, and choline$^+$ were ineffective. No detectable uptake of TMG was observed with uninduced cells in the presence or absence of NaCl (data not shown).

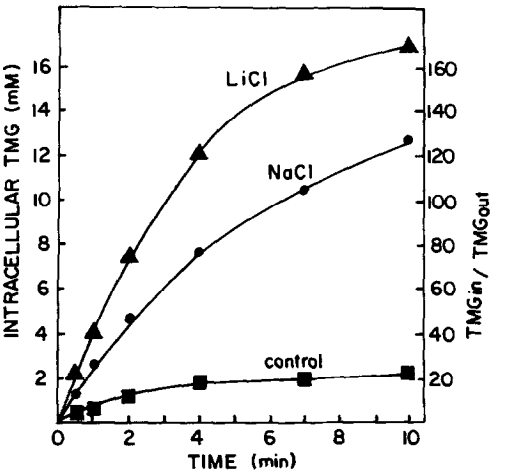

Fig. 1. Stimulation of TMG transport by Na^+ or Li^+ in strain 7-6. ^{14}C-TMG uptake was measured at room temperature. Cells were preincubated in the absence (■) or in the presence of 10 mM NaCl (●) or 10 mM LiCl (▲) for 10 min. Reactions were initiated by the addition of ^{14}C-TMG.

TMG transport driven by an artificially imposed electrochemical potential difference of Na^+.

Two general mechanisms for Na^+ stimulation of transport were considered: 1) Na^+ is a "co-factor" in the activation of a carrier which involves H^+-TMG co-transport and 2) Na^+ is directly involved in a Na^+-TMG co-transport. If TMG transport through the melibiose transport system were coupled with H^+, we should observe uptake of TMG by imposing a ΔpH across the cell membrane of energy-depleted cells as described in the case of the lactose transport system (13). All such attempts were unsuccessful. In order to distinguish between the two hypotheses given above, a series of experiments were carried out in which the driving forces on H^+ and Na^+ were in the opposite directions. In the first experiments cells were equilibrated at pH 6.0 and then exposed to the proton ionophore CCFP. Cells were then diluted 100-fold into a medium at pH 8.0. The outward movement of protons via CCFP would be expected to generate a membrane potential, inside negative. This would provide an electrical driving force for the inward movement of Na^+. In the first case conditions were arranged to

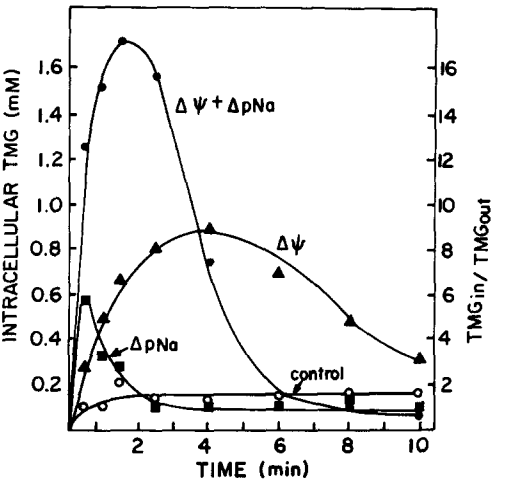

Fig. 2. TMG transport induced by artificial driving forces in energy-depleted strain NR70-1. A membrane potential, inside negative ,(ΔΨ) was imposed as a diffusion potential of H^+ (interior acid). An inwardly directed chemical gradient of sodium (ΔpNa) was imposed by the addition of sodium to the external medium. For details, see Materials and Methods. Symbols are: control (no driving force) (o); ΔΨ (▲); ΔpNa (■); ΔΨ and ΔpNa (●). pNa = $-\log_{10}$(sodium concentration).

provide both electrical (ΔΨ) and chemical (ΔpNa) forces for the inward movement of Na^+. In addition to the pH gradient described above, an inwardly directed chemical gradient for Na^+ was produced by exposing cells with low internal Na^+ (less than 0.5 mM) to an external Na concentration of 100 mM. Under these conditions a TMG accumulation of 17 fold was observed (Fig. 2). When the major driving force was electrical (the chemical gradient for Na^+ reduced to a low level) a nine-fold TMG accumulation was observed. When only a chemical gradient of Na^+ was imposed across the membrane (no pH gradient) a 5-fold accumulation was observed. When no pH gradient or chemical gradient was present no sugar accumulation was observed. Sugar accumulation driven by the artificial electrochemical gradient of Na^+ was a transient one, since the driving force was also only transient in nature. The inward movement of Na^+ in the first three cases would reduce both the membrane potential and the chemical gradient for Na^+.

pH changes induced by TMG entry.

In a second group of experiments proton movement was monitored by measuring the external pH of a suspension of anaerobic cells. Cells were grown in suc-

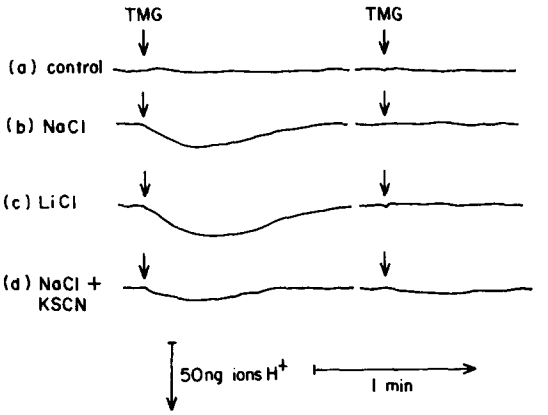

Fig. 3. Extracellular pH changes on adding TMG to cell suspension of strain 7-6 under anaerobic conditions. To the 2.0 ml of suspension, containing 12 mg (cell dry weight) of cells, and 100 µg of carbonic anhydrase, was added 20 µl of 0.5 M TMG with rapid stirring. Two minutes after the first addition, the second addition of TMG was carried out. (a) Cell suspension in 120 mM KCl; (b) 110 mM KCl + 10 mM NaCl; (c) 110 mM KCl + 10 mM LiCl; (d) 80 mM KCl + 10 mM NaCl + 30 mM KSCN. A downward deflection indicates a decrease of pH. The records were corrected for base line drift.

cinate plus melibiose, washed and resuspended in an unbuffered solution anaerobically. After a 30 minute incubation with continuous bubbling of nitrogen a small volume of an anaerobic TMG solution was added. If the entry mechanism were H^+-TMG co-transport addition of TMG should result in a rise of pH in the external medium as observed with the lactose transport system (6). If, on the other hand, Na^+-TMG co-transport takes place, a transient acidification of the medium would be predicted, since Na^+ influx associated with TMG will establish a membrane potential, interior positive, and H^+ will efflux from the cell. As shown in Figure 3, addition of an anaerobic TMG solution to an anaerobic suspension of cells caused an acidification of the medium in the presence of 10 mM NaCl (Fig. 3b) or LiCl (Fig. 3c). Again, Li^+ was more effective than Na^+. The addition of KSCN reduced the TMG-induced acidification in the presence of NaCl (Fig. 3d). This is consistent with the view that the entry of the lipid-soluble SCN^- reduced the membrane potential (inside positive) resulting from Na^+ entry with TMG and thus inhibited proton exit. In the absence of these cations (control),

no pH change elicited by an addition of TMG. The second injections of TMG failed to cause any pH change. This control excludes the possibility of inadvertantly introducing either acid or oxygen in the TMG solution.

DISCUSSION

Evidence is presented that the Na^+ stimulation of TMG uptake by the melibiose system is due to Na^+-TMG co-transport. Proton-TMG co-transport has been excluded by two types of experiments in which TMG accumulation occurs in the face of net proton movement out of the cell. In the first experiment (Fig. 2) protons moved from inside the cell (pH 6) to outside (pH 8) via the proton ionophore CCFP. This resulted in TMG accumulation in the presence of external Na^+. In the second experiment (Fig. 3) TMG addition to anaerobic cells resulted in acidification of the external medium. The requirement for Na^+ or Li^+ for this process is consistent with Na^+-TMG co-transport.

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REFERENCES

1. Crane, R.K. (1965) Fed. Proc. 24,1000-1006.
2. Harold, F.M. (1972) Bacteriol. Rev. 36,172-230.
3. Stock, J., and Roseman, S. (1971) Biochem. Biophys. Res. Commun. 44,132-138.
4. MacDonald, R.E., and Lanyi, J.K. (1975) Biochemistry 14,2882-2889.
5. Prestidge, L.S., and Pardee, A.B. (1965) Biochim. Biophys. Acta 100,591-593.
6. West, I.C. (1970) Biochem. Biophys. Res. Commun. 41,655-661.
7. West, I.C., and Mitchell, P. (1972) Bioenergetics 3,445-462.
8. Hayashi, S., Koch, J.P., and Lin, E.C.C. (1964) J. Biol. Chem. 239,3098-3105.
9. Müller-Hill, B., Crapo, L. and Gilbert, W. (1968) Proc. Nat. Acad. Sci. U.S.A. 59,1259-1264.
10. Rosen, B.P. (1973) J. Bacteriol. 116,1124-1129.
11. Cohen, G.N. and Rickenberg, H.V. (1956) Ann. Inst. Pasteur 91,693-720.
12. Flagg, J.L., and Wilson, T.H. (1977) J. Membrane Biol. in press.
13. Flagg, J.L., and Wilson, T.H. (1976) J. Bacteriol. 125,1235-1236.