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PROTON EFFLUX ASSOCIATED WITH MELIBIOSE PERMEASE ACTIVITY IN SALMONELLA TYPHIMURIUM

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SUMMARY

Transport of thiomethyl-β-D-galactoside (TMG) via the melibiose permease system (TMG permease II) in Salmonella typhimurium is known to be a sodium-dependent co-transport system. We have shown that this co-transport of sodium and TMG is associated with extrusion of protons from the cells. The rate and extent of proton extrusion during TMG uptake were measured in wild-type cells and mutants containing internal and extended deletions in the pts locus. No differences between these various strains were noted.

INTRODUCTION

Inducer exclusion is the regulatory process by which substrates of the phosphoenolpyruvate: glycose phosphotransferase system (PTS) can selectively inhibit the concomitant transport of certain non-PTS substrates such as glycerol, lactose, maltose, and melibiose. This phenomenon has been studied intensively for some time particularly in Salmonella typhimurium (6,7,8). Although attempts have been made to delineate the mechanism involved, little is clear at this time except that the product of the crr gene, believed to be Factor III of the PTS,is known to play a key role. Cordaro (2) recently hypothesized that the inhibiting substrate may act as an uncoupling agent causing a collapse or dissipation of the membrane potential required to energize transport of the non-PTS substrates. For technical reasons we have not yet been able to test this hypothesis in the exact manner prescribed by Cordaro; nevertheless, data are presented suggesting that this hypothesis may be untenable. The melibiose and glycerol permease systems of Salmonella typhimurium were analyzed for this purpose.

Stock and Roseman (10) showed that this permease is a sodium-

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dependent, co-transport system. We have shown that this co-transport occurs with a concomitant extrusion of protons from the cells.

MATERIALS AND METHODS

Organisms and Media

All cultures were grown with vigorous shaking at 37°C in a mineral salt medium containing (per liter): K_2HPO_4, 7g; KH_2PO_2, 2g; $MgSO_4 \cdot 7H_2O$, 0.1g; $(NH_4)_2SO_4$, 1g; final pH 7.2. Melibiose at a final concentration of 0.01 M was added separately as the carbon and energy source, and tryptophan (20 µg/ml) was added since some of the strains used were trp auxotrophs. The parental strains used were S. typhimurium LT-2 and strain SB3507 (trp). The latter was the parental strain for the following PTS mutants: strain SB3750 (ptsIΔ,trp); SB3687 (ptsIcrrΔ,trp) and SB3686 (ptsHIcrrΔ,trp). Strain SB3750 requires cyclic AMP (5 mM) in the culture medium to grow on melibiose. The isolation and characterization of these internal and extended deletion mutants is described elsewhere (3,5).

Measurement of Proton Movement

Cells grown to mid-log were harvested, washed twice at 4°C in 150 mM KCl in 2 mM glycylglycine buffer (pH 6.5), resuspended in the same buffer at a dry weight concentration of 10-20 mg/ml and then used immediately. All subsequent experimental manipulations were carried out at 25°C in a water-jacketed, glass vessel containing ports for additions of substrates, etc. The cells and all other additions made to the vessel were bubbled with water-saturated, oxygen-free nitrogen. After addition of cells to a concentration of 1 mg dry weight/ml, the volume of the reaction mixture was adjusted to 25 ml. Also included in this reaction mixture were: 1 mM potassium iodoacetate to inhibit glycolysis and 20 µg/ml carbonic anhydrase to catalyze equilibrium of the CO_2/carbonic acid system (9). NaCl was added, when used, at a final concentration of 50 mM. This reaction system is essentially as described by Collins et al. (1).

Changes in the pH of the reaction mixture were determined using a Beckman Combination Electrode (#41263) connected to a Model 320 Fisher expanded scale pH meter. The pH was recorded continuously on a Dohrmann Instrument Co., dual-channel recorder (Model RDC 850) set at 1 mV to give a full-scale deflection (19 cm) of 0.13 pH units. Prior to addition of the substrate, the cells were allowed to equilibrate for 25-30 min during which time the pH of the reaction mixture was periodically adjusted to 6.5 by addition of small amounts of HCl or KOH.

Measurement of Potassium Ion Movement

For this measurement the procedure described by Collins et al. (1) was used. The conditions described above were used except that choline salts were used in place of the corresponding potassium compounds, and KCl, $MgCl_2$ and Valinomycin were added at concentrations of 0.01 mM, 10 mM and 20 µg/ml respectively. Potassium ions were detected using a Beckman cation electrode (#39137) connected to a second pH meter and a common reference electrode used for both meters. The outputs from both meters were recorded simultaneously on the dual-channel recorder; the potassium channel was set at 1 mV, full scale deflection.

Chemicals

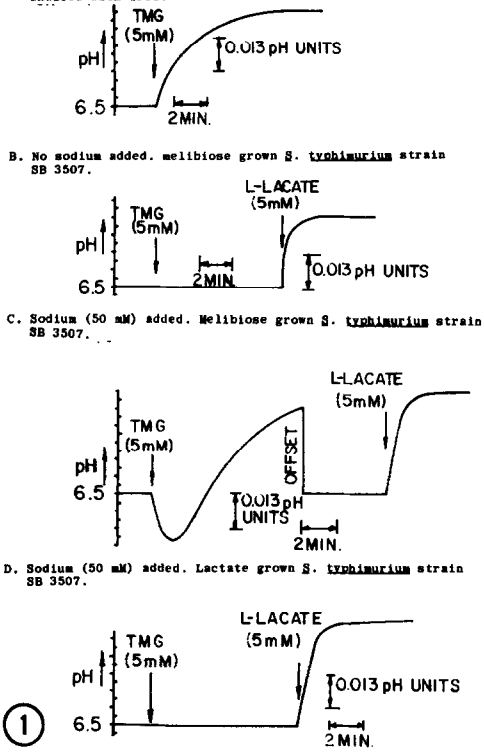
Cyclic AMP was purchased from the U. S. Biochemical Corporation (Cleveland, Ohio). Valinomycin, L-lactate, TMG, melibiose, tryptophan, iodoacetate and carbonic anhydrase were purchased from the Sigma Chemical Company (St. Louis, Mo.). All other chemicals were reagent grade and generally available.

RESULTS

The data presented in Figure 1 (graph A) show that E. coli ML30 cells induced for lac operon activities by growth in glycerol medium containing 1 mM IPTG transported TMG with proton symport as previously reported (1), i.e., the pH of the extracellular medium increased with TMG uptake. Melibiose grown S. typhimurium strain SB3507 cells which contain the TMG permease II system (10) could not transport ^{14}C-TMG (data not shown) nor did any change in proton flow occur when TMG was added to this same reaction mixture which lacked sodium (graph B). These latter cells, however, could take up L-lactate with a proton symport. When 50 mM NaCl was added to this reaction (graph C), TMG uptake occurred (shown by ^{14}C-TMG uptake studies, data not shown) and was associated with an efflux of protons from the cells. This uptake and concomitant proton efflux did not occur when the cells had been pre-grown in lactate and therefore lacked the TMG permease II system (graph D); the cells were able to transport lactate normally. These data complement the studies of Stock and Roseman (10) who first reported this system to be a sodium-dependent co-transport system. We can now conclude that this process occurs with concomitant efflux of protons.

The data presented in Figure 2 were obtained as described above using melibiose grown strains of S. typhimurium. In these experiments strains having internal and extended deletions in the pts locus were analyzed. As can be seen, essentially identical proton changes accompanied the transport of TMG and L-lactate in all strains tested. This was true whether or not cAMP was added to the growth medium.

The data presented in Figure 3 showed that no proton or potassium ion changes occurred when glycerol was added to the reaction mixtures containing S. typhimurium strain SB3507 (parental) cells which had been pre-grown overnight on 0.02 M glycerol as the sole carbon and energy source.



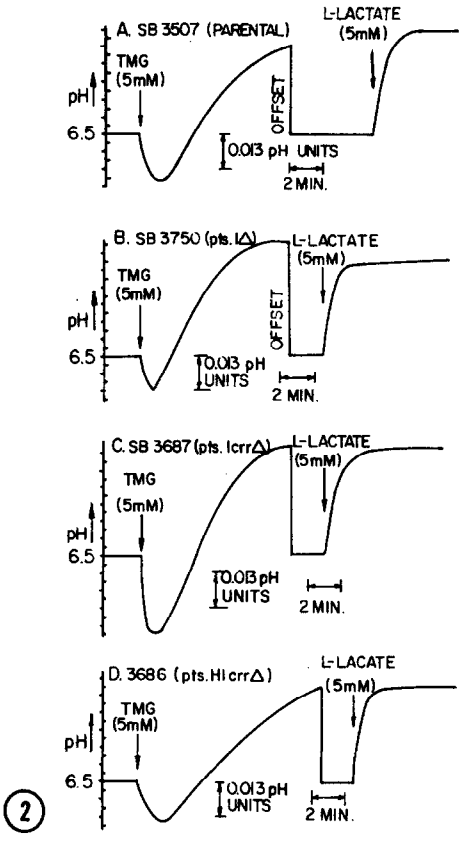


Figure 1. Proton movement associated with the sodium-dependent TMG permease II system in S. typhimurium. The reaction mixture described in Materials and Methods was used in each of these experiments. No sodium ions were added to the reaction mixtures for the experiments shown in graphs A and B. Sodium ions were added to the reaction mixtures for the experiments shown in graphs C and D. The cells and substrates used in each experiment are indicated in each graph.

Figure 2. Proton movement associated with TMG and lactate transport in parental and pts deletion mutants of S. typhimurium. pH changes associated with TMG and lactate transport in melibiose grown cells were measured in the sodium supplemented reaction mixtures as described in Materials and Methods. Strain SB 3750 required 5 mM cAMP to grow on melibiose, whereas the other strains did not. Graph A: strain SB 3507 (parental); graph B: strain SB 3750 (ptsIΔ); graph C: strain SB 3687 (ptsIcrrΔ); graph D: strain SB 3686 (ptsHIcrrΔ).

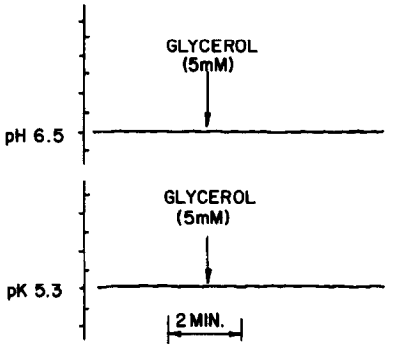


Figure 3. Proton and K^+ movements associated with glycerol transport in S. typhimurium strain SB 3507.

DISCUSSION

These studies established that the melibiose permease system (TMG permease II) of S. typhimurium which requires co-transport of sodium (10) is associated with efflux of protons.

These studies also suggested that the mechanism proposed by Cordaro (2) to explain the inducer exclusion phenomenon may be untenable. He postulated that PTS substrates could inhibit uptake of appropriate non-PTS substrates by dissipating the membrane potential required to energize transport of the latter substrates, and furthermore, that this possibility could be tested using appropriate mutant strains. This could be done, for example, by pre-inducing wild-type, a leaky ptsI mutant, and a ptsIcrr deletion for the non-PTS transport system of interest. Measurement of pH changes during transport of the non-PTS substrate in the presence and absence of the PTS substrate would then show whether or not the latter substrate dissipated the membrane potential; crr mutants were predicted to be resistant to such a dissipation.

Because iodoacetate must be present in the reaction mixture to stabilize the endogenously produced proton efflux by the cells, and since iodoacetate by itself can largely overcome the inducer exclusion effect (manuscript in preparation), the experiment suggested by Cordaro could not

be conducted as such. We were able to demonstrate, however, that proton efflux associated with TMG uptake in S. typhimurium was not altered from the wild-type state by the absence of enzyme I (strain SB3750), enzyme I and Factor III (strain SB3687), or HPr, enzyme I and Factor III (strain SB3686). Inasmuch as strains possessing or completely devoid of the key component for inducer exclusion, Factor III, did not show variation in the proton movements associated with TMG uptake, it seems to us unlikely that the inducer exclusion mechanism is triggered by appropriate changes in the organism's membrane potential. Even more convincing here is the fact that glycerol transport is very susceptible to the inducer exclusion effect, and yet it is well known (4,11) and again confirmed in this study (Figure 3) that glycerol transport is not associated with either a change in proton or potassium movements in the cells.

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