A SODIUM-DEPENDENT SUGAR CO-TRANSPORT SYSTEM IN BACTERIA*

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Received May 17, 1971

Summary

A characteristic difference between active transport systems in bacterial and animal cells is that the latter usually require sodium ions and operate by the process of co-transport. The melibiose permease system (TMG permease II) of Salmonella typhimurium has now been identified as a sodium-dependent co-transport system. Co-transport, and the analogous mechanism, counter-transport, may be the underlying mechanisms for the active transport of many solutes by bacterial cells, although they may be difficult to detect.

The active transport of sugars and amino acids by many animal cells generally requires sodium ions, and is thought to occur by the process of co-transport (for reviews see (1, 2)). That is, a solute-specific membrane protein transports the solute with sodium ion, the former being translocated up and the latter down its respective concentration gradient. The net result, the active transport of solute, is accomplished by removing sodium from the cell through a separate sodium pump.

Active transport systems have been extensively studied in bacterial cells, but co-transport has not previously been demonstrated. Evidence is presented here showing that the melibiose permease system in Salmonella typhimurium (designated TMG permease II (3)) is a sodium-dependent co-transport system.

MATERIALS AND METHODS

All transport experiments were conducted with S. typhimurium strain LT-2 grown to the middle of the exponential phase at 37° in Medium 63 (4) without

*This work was supported by Grant AM09851 from the National Institutes of Health, Grant P-544 from the American Cancer Society, and a grant from the Cystic Fibrosis Research Foundation. Contribution No. 630 from the McCollum-Pratt Institute.

‡Supported by NIH Predoctoral Training Grant GM-57 to the Department of Biology.

iron. (The melibiose transport system is not temperature-sensitive in this organism, as it is in a strain of Escherichia coli (3, 5).) The carbon sources used for growth were as follows: 0.5% melibiose for induced, and either 0.2% glycerol or 0.1% DL-lactate for uninduced cells. Cells were harvested by centrifugation at 4°, washed twice with either Medium 63 or 0.1 M Tris-HCl buffer, pH 7.3, and stored at 0° until used. Stored or fresh cells gave similar results.

Uptake studies were conducted with ^{14}C-methyl-labeled thiomethyl β-D-galactopyranoside (TMG), a nonmetabolizable substrate transported by the melibiose system, or with $^{22}Na^{+}$. The specific activity of the TMG was 8.5 x 10^5 cpm/μmole with one exception (Fig. 4), and that of $^{22}Na^{+}$ was 2.6 x 10^5 cpm/μmole. All experiments were conducted under constant aeration with stirring at 24° after preincubation of the cells in the specified media for the indicated times at 24°. Two milliliters of the suspension were then mixed by means of a stop-flow device with 2 ml of solution containing the identical components supplemented with the labeled substrate and other additions as specified (designated Substrate Solution). Mixing was complete within 1 sec. Samples (0.1 ml) were removed from the 4.0-ml suspension at various times by means of an automatic pipet coupled to a micro-switch and high speed chart. Each sample was immediately diluted into 10 ml of the same medium without the isotopic substrate, the time of dilution was recorded on the chart, and the suspension was filtered through glass fiber filters (Reeve Angel, No.984H, 2.4 cm). The entire procedure from dilution of sample to completion of filtration took less than 2 sec. Control experiments showed that the uptake of TMG was essentially stopped by the dilution step, that the filter quantitatively retained the bacteria, that uptake was proportional to cell number, and most important, the rate of efflux of TMG from pre-loaded cells when in the dilution medium was insignificant compared with the rate of uptake in the 4-ml suspension. This last control validated the accuracy of the uptake data. By contrast, the efflux of Na^{+} was very rapid (see below). The filters were dried and counted by liquid scintillation methods. All values are corrected for controls, which were samples treated in a manner identical with the above, but without cells. The blank values averaged around 300 cpm in the TMG experiments (with TMG at 2.5 x 10^{-4} M), and between 800 and 2000 cpm, depending on the diluent, in the $^{22}Na^{+}$ (1.0 mM) experiments.

Uptake is expressed as millimicromoles of labeled substrate per mg, dry weight, of cells. Rates are expressed as uptake per min, determined from

initial slopes of curves obtained as shown in Fig. 1.[1]

RESULTS

The principle constituent of the transport medium was 0.1 M Tris-HCl, pH 7.3. This buffer did not affect uptake of TMG when compared to Medium 63 (Fig. 1). Fig. 1 also shows the stimulatory effect of NaCl on the uptake of TMG. The constituents of the modified Medium 63 (potassium phosphate, $MgSO_4$, and $(NH_4)_2SO_4$), as well as potassium lactate, did not affect the initial transport rate of TMG, when tested alone or in combination in the Tris-HCl. All further experiments were conducted with Tris-HCl.

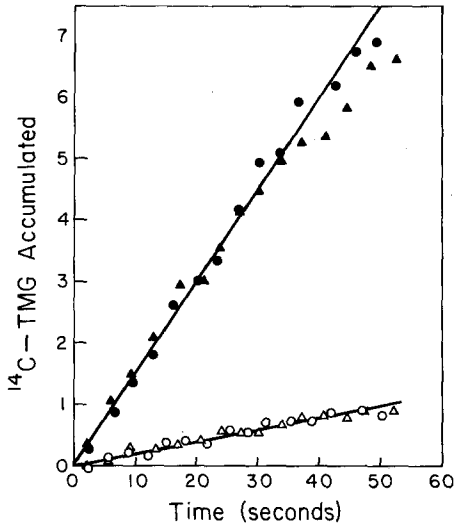

Fig. 1. Effects of Na^+ and Tris-HCl on TMG uptake. Cells were grown in inducing medium as described in the text; half of the culture was harvested and washed in Medium 63 containing 0.1% potassium lactate, the remainder was washed with a solution containing 0.10 M Tris-HCl, pH 7.3, 0.1% potassium lactate (pH 7.3), and 10 mM $MgSO_4$. Before use, aliquots of the cells were suspended in the following media and preincubated for 15 min at 24°: [Δ], Tris-HCl; [▲], Tris-HCl containing 50 mM NaCl; [○], Medium 63; [●], Medium 63 containing 50 mM NaCl. Two milliliters of each suspension were rapidly mixed with 2 ml of Substrate Solution of identical composition, containing 2.5×10^{-4} M ^{14}C-TMG. Samples were removed and assayed as described in the text. The calculated value for equilibration of ^{14}C-TMG between cell water and external medium (by a diffusion process) is 0.5 on the ordinate.

[1] Depending on the TMG and NaCl concentrations employed, the rates in some experiments declined sharply during the first min (compare Figs. 1 and 4). Where possible, initial rates were determined from the values for the first 10 to 30 sec. In some cases (e.g. Fig. 4), initial rates were estimated mathematically from the progress curves.

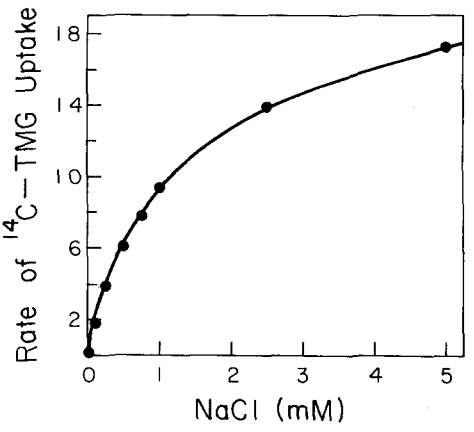

Fig. 2. Effect of Na^+ concentration on TMG uptake. Induced cells were harvested and washed with Tris-HCl as described in the text and Fig. 1. The Substrate Solutions contained 5.0 x 10^{-4} M ^{14}C-TMG in Tris-HCl. Both the cell suspensions and Substrate Solutions were supplemented with the indicated concentrations of NaCl, and both were preincubated for 5 min before mixing. The calculated value for equilibration of isotope is 1.0.

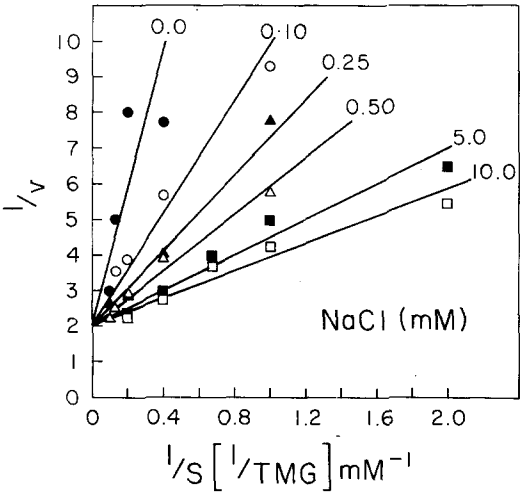

Fig. 3. Effect of Na^+ and TMG concentrations on the rate of uptake of TMG. Induced cells were harvested, washed twice with Tris-HCl and suspended in the same medium. Substrate Solutions contained twice the indicated concentrations of ^{14}C-TMG and NaCl. After preincubation for 5 min at 24°, 2-ml aliquots of cell suspensions and Substrate Solutions were mixed and assayed as described in the text to determine initial rates of uptake.[1]

The effect of NaCl concentration on the initial rate of TMG uptake (at 2.5 x 10^{-4} M) is shown in Fig. 2; stimulation by 5 mM NaCl was over 100-fold. The same stimulation was observed without preincubation of the cells with Na^+. Kinetic analysis of uptake rates (Fig. 3) shows the dependence of the apparent

K_m of TMG uptake upon Na^+ concentration. The stimulatory effect of the Na^+ causes a decrease in the K_m without affecting the V_{max} of entry.

The effect observed with Na^+ is specific (with the exception of Li^+). For example, the following uptake rates of TMG (at 2.5×10^{-4} M) were obtained using the indicated salts (all chlorides at 10 μequiv/ml): none, 1.38; Na^+, 8.34; Li^+, 18.7; K^+, 1.33; Cs^+, 1.05; NH_4^+, 0.93; Mg^{++}, 1.30; Mn^{++}, 1.11; Ca^{++}, 0.93; choline$^+$, 1.85. The stimulatory effect of NaCl was due to Na^+, and not Cl^-.[2]

The experiments described above demonstrate one parameter of a co-transport system, a sodium-dependent sugar uptake. Another parameter, sugar-dependent sodium uptake was more difficult to demonstrate because of the complication of sodium efflux by a separate sodium pump. Nevertheless, this dependence was observed (Fig. 4). Sodium was rapidly taken up by induced cells in the presence of TMG. The efflux of Na^+ was so rapid that the internal Na^+ concentration only

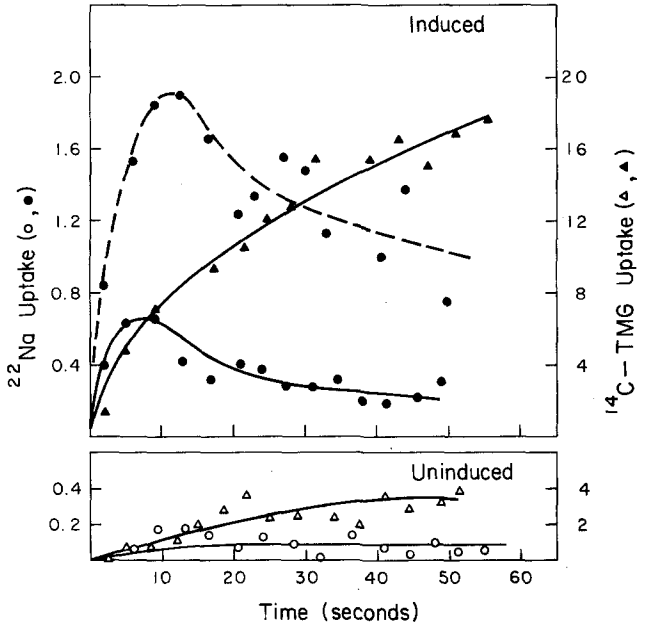

Fig. 4. Effect of TMG on Na^+ uptake. Cells were grown in inducing or non-inducing (glycerol) medium, harvested, washed with Tris-HCl, and suspended in the same medium. Substrate Solutions contained either of the following in Tris-HCl: 2 mM ^{22}NaCl, 20 mM TMG; 2 mM NaCl, 20 mM ^{14}C-TMG (specific activity, 90,000 cpm/μmole). $^{22}Na^+$ uptake was measured with: [●], induced cells; [○], uninduced cells. ^{14}C-TMG uptake was measured with: [▲], induced cells; [△], uninduced cells. Two experiments [●] are shown for $^{22}Na^+$ uptake by induced cells; in the upper (dashed) curve, the 0.1-ml samples were diluted in Tris-HCl containing 1 mM NaCl, while in the lower curve, the diluent contained 10 mM NaCl.[2] The calculated values for equilibration of isotopes between internal and external water are 2 mμmoles of $^{22}Na^+$/mg, dry weight, and 20 mμmoles for ^{14}C-TMG. $^{22}Na^+$ uptake by induced cells in the absence of TMG was negligible (comparable to the values shown for uninduced cells in the presence of TMG).

briefly approached the external concentration. Accurate stoichiometry has not yet been obtained, but one of the experiments shown in Fig. 4 suggests (from initial rates) that the ratio of Na^+:TMG uptake is 1. As the net influx of Na^+ decreased, the rate of uptake of TMG also decreased. The Na^+-dependent TMG uptake was specific in the sense that the transport of other solutes, including methyl α-glucoside, by these cells was not stimulated by Na^+.

DISCUSSION

Previous work showed that many sugars are transported by bacterial cells by the process of group translocation, that is, they are phosphorylated by a phosphotransferase system during transport (6). A major problem, however, has been to explain how amino acids and other sugars are actively transported. The results presented above provide evidence that one such sugar, melibiose, is transported by Na^+-dependent co-transport. Is the co-transport system more widespread in bacterial species?

Halophilic bacteria require Na^+ for growth, while growth of some other bacteria is markedly stimulated by this ion (7). Amino acid transport in a marine pseudomonad was dependent on Na^+, but the effect was interpreted to be independent of co-transport (8). However, these and other results should be re-examined in light of the rapid efflux of excess Na^+ via the sodium pump described above, the possibility that co-transport systems have a very low K_m for sodium, and the difficulty in removing contaminating sodium from the transport media.[2]

Another possibility raised by the present work is that solutes may be transported by counter-flow, i.e. by the flow of another solute in the opposite direction. These possibilities are being explored.

ACKNOWLEDGMENT

We are most grateful to Drs. Robert Simoni, Milton Saier, and Pamela Talalay for their many helpful suggestions.

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[2] Despite special acid washing of glassware, including the stop-flow apparatus, sodium contamination was a persistent and troublesome problem. Approximate rate measurements were obtained using plastic vessels, and the uptake of TMG (2.5×10^{-4} M) by cells in Na^+-free medium was barely detectable. A major difficulty in the $^{22}Na^+$ experiments was adsorption (or exchange) of the isotope to the glassware, particularly to the filters used for separating the cells from the medium. This difficulty necessitated the use of 1 or 10 mM NaCl in the diluent (Fig. 4). While the latter should lower the $^{22}Na^+$ in the cells, it is important to note that a 10-fold difference in NaCl content of the diluent gave less than a 3-fold difference in the content of cellular $^{22}Na^+$ at the peak value.

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