Biochimica et Biophysica Acta 904 (1987) 191–200
Elsevier

BBA 73732

Cation specificity for sugar substrates of the melibiose carrier in *Escherichia coli*

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(Received 14 May 1986)

Key words Melibiose carrier, α-Galactoside, β-Galactoside, Lactose, Proton cotransport; Sodium cotransport, Sugar transport, (*E coli*)

A study has been made of the sugar substrate specificities and the cation specificities of the melibiose transport system of *Escherichia coli*. The following β-galactosides were found to be transported: lactose, L-arabinose-β-D-galactoside, D-fructose-β-D-galactoside, *o*- and *p*-nitrophenyl-β-D-galactosides. These β-galactosides were cotransported with Na^+ but not with H^+. The α-galactosides raffinose, melibiose and *p*-nitrophenyl-α-galactoside were transported with either H^+ or Na^+. Of the monosaccharides tested D-galactose could use either Na^+ or H^+ for cotransport whereas D-fucose, L-arabinose and D-galactosamine could use only Na^+. The sugar specificity requirements for H^+ cotransport are therefore more exacting than those for Na^+ cotransport.

Introduction

Substrate-cation cotransport systems are found in all living cells [1,2]. The proton is used as the energy-coupling cation in most examples of cotransport in bacteria, yeast, lower and higher plants. Animal cells, on the other hand, commonly possess carriers that depend exclusively on Na^+. A few examples are known in which the transport protein recognizes both H^+ and Na^+ [3,4]. A representative of the latter type is the melibiose carrier of *Escherichia coli* [4]

This transport system for α-galactosides in *E coli* was first described by Prestidge and Pardee in 1965 [5]. They showed that it was induced by melibiose and galactinol (*meso*-inositol-α-D-galactoside) but not by lactose. Unlike the lactose carrier protein, it was temperature-sensitive (active at 30 °C but inactive at 37–42 °C).

In 1971 Stock and Roseman [6] found that thiomethyl-β-D-galactoside transport by the melibiose carrier of *Salmonella typhimurium* was dependent on Na^+ for cotransport, and the phenomenon was confirmed with membrane vesicles of this organism [7]. It was later demonstrated that the melibiose carrier of *E. coli* can use either Na^+ or H^+ for cotransport with melibiose [4] and there appears to be competition between these two cations for the cotransport function [8]. This competition has also been shown to occur in experiments on the binding of another substrate to this carrier. Na^+ stimulates the binding of *p*-nitrophenyl-α-galactoside to the transport protein [9–11] and this stimulation is competitively inhibited by H^+ [11].

In this paper the sugar-cation specificities of the melibiose carrier have been further investigated by measurement of cation fluxes and sugar

Abbreviations TMG, thiomethyl-β-D-galactoside, β-ONPG, *o*-nitrophenyl-β-D-galactoside, α-PNPG, *p*-nitrophenyl-α-D-galactoside, Mops, 4-morpholinepropanesulfonic acid, Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine

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uptake. It was found that the uptake of α-galactosides is associated with the cotransport of H^+ or Na^+ while the uptake of β-galactosides occurs with Na^+ but not with H^+. An additional observation of interest was that whereas several of the monosaccharides tested can be cotransported with Na^+, only D-galactose can cause uptake of H^+ as well as Na^+.

A preliminary communication of part of this data has been presented elsewhere [8].

Materials and Methods

Bacteria

The strains and plasmids used are shown in Table I. The *melA* gene codes for α-galactosidase, the *melB* gene for melibiose transport [17]. The *lacZ* gene codes for β-galactosidase, the *lacY* gene for lactose transport. DW1 was isolated by the following method: A P_1 lysate from a pool of cells containing TN10 at random positions was added to RA11 and plated on melibiose minimal plates containing tetracycline. One of the resultant recombinants (over 90% linkage of melibiose to TN10) was plated on Bochner plates [18] and tetracycline-sensitive clones isolated. Among these *melΔ(AB)* was obtained (DW1). The P_1 lysate was a gift from Dr. Donna Seto-Young.

Bacterial growth

Cells were routinely grown in minimal medium 63 [19] with the addition of 1% bactotryptone and 0.5 μg/ml thiamin. Plasmid-containing cells were grown in the presence of 10 μg/ml tetracycline HCl. When induction of the cells was desired, 6 mM melibiose was added to the medium. Incubation temperature was 37 °C, except in the case of cells with a temperature-sensitive carrier, which were incubated at 30 °C.

Low Na^+ medium was prepared in plastic vessels to avoid contamination with Na^+ from the glass. The medium consisted of 100 mM Mops buffer adjusted to pH 7 with tetramethyl ammonium hydroxide, 2 mM $(NH_4)_2SO_4$, 1 mM $MgSO_4$, 1 mM K_2HPO_4, 0.5 μg/ml thiamin and appropriate carbon source. A 20 μl inoculum was placed in 2 ml medium in sterile plastic tubes, which were then incubated on a rotary shaker at 37 °C.

Transport assays

Cells were washed twice in 100 mM Mops adjusted to pH 7 with tetramethyl ammonium hydroxide and resuspended in the same buffer to a density corresponding to 0.6 mg dry wt./ml. NaCl or LiCl was added to 15 mM, if required. The reaction was initiated by the addition of radioactive sugar. Samples (0.2 ml) were taken at given intervals, filtered through 0.65 μm filter discs (Sartorius) and washed with 5 ml buffer The discs were dissolved with Liquiscint and counted in a Beckman scintillation counter. The assay was performed at 22 °C. In all experiments plastic tubes and pipettes were used to eliminate Na^+ contamination from glass.

H^+ movement

For proton uptake measurement the method of

TABLE I

BACTERIAL STRAINS

Δ, deletion, ts, temperature sensitivity

Strain	Genotype (chromosome/F′/plasmid)	Source
W3133	*lacI*$^+$Δ(*ZY*) *melA*$^+$ *B*^{ts}/−/−	from S Luria
RA11	*lacI*$^+$Δ(*ZY*) *melA*$^-$ *B*$^+$/−/−	this laboratory [12]
DW1	*lacI*$^+$Δ(*ZY*) *melΔ*(*AB*)/−/−	this paper
DW1/F′G11	*lacI*$^+$Δ(*ZY*) *melΔ*(*AB*)/*lacI*$^+$ *Z*$^+$Δ*Y*/−	F′G11 from Langridge [13]
DW1/F′G11/pSTY91	*lacI*$^+$Δ(*ZY*) *melΔ*(*AB*)/*lacI*$^+$ *Z*$^+$Δ*Y*/*melA*$^+$ *B*$^+$	pSTY91 Hanatani et al [14]
DW1/pSTY37	*lacI*$^+$Δ(*ZY*) *melΔ*(*AB*)/−/*melB*$^+$	pSTY37 from Tsuchiya
200R/pSTY91	*lacI*$^+$ *Z*$^+$Δ(*Y*)/−/*melA*$^+$ *B*$^+$	200R Franklin and Luria [15]
X71-15	*lacI*$^+$ *Z*$^+$ *Y*$^+$/−/−	this laboratory [16]

West [20] was used. Cells were washed twice in 120 mM choline chloride plus 1 mM 2-mercaptoethanol and were resuspended in the same to a density approximately equivalent to 70 mg dry weight/ml. The suspension was diluted 1/5 in choline chloride containing 10 mM KSCN. The cells (2.5 ml) were placed in a 3 ml plastic vial with the lid cut to fit over an inserted pH electrode. There was a small vent in the lid for the introduction of nitrogen and a second vent for the addition of substrate. The pH was adjusted to pH 6 with dilute HCl. N_2 was passed through one vent over the surface of the suspension, which was stirred with a magnetic stirrer. After 30–40 min the assay was started by introduction of anaerobic sugar solution that had been adjusted to pH 6. The pH values were recorded with a Linear Instruments recorder. The combined pH electrode was from Radiometer, Copenhagen (GK 2321-C).

Na^+ movement

The cells were washed and resuspended as for H^+ movement assay. Cells were then diluted 1/5 into 100 mM Tricine buffer (pH 8) plus 10 mM KSCN. NaCl was added to a concentration 25–250 μM. The 10 ml suspension was placed in a 20 ml plastic vessel with holes in the lid for insertion of the Na^+-selective electrode (Radiometer Copenhagen G502 Na^+) and the calomel electrode (Radiometer Copenhagen K401). The suspension was stirred with a magnetic stirrer and kept anaerobic by introduction of N_2 through a small vent. Anaerobic sugar solution was added through a second vent.

Chemicals

[^{14}C]Lactose was from Amersham. *o*-[^{3}H]Nitrophenyl-β-D-galactopyranoside and D-[^{3}H]galactosamine hydrochloride were from New England Nuclear. The nonradioactive sugars were obtained from Sigma. Mops and Tricine buffers were from Sigma.

Results

Growth experiments

The melibiose carrier (*melB*) of *E coli* was tested for its recognition of several β-galactosides by studying the growth on these sugars of two strains which possess the *melB* gene in the absence of the lactose carrier (*lacY*). These strains were constructed by inserting a plasmid containing the *melB* gene (pSTY91) into cells from which the *lacY* gene had been deleted (Table I). Since β-galactosidase (*lacZ*) is necessary for metabolism of the transported β-galactosides, the *lacZ* gene was either retained on the chromosome (200R/pSTY91) or was inserted on an F-factor (DW1/F'G11/pSTY91). When the two strains were tested for lactose fermentation on MacConkey plates at 37 °C they showed a positive result (red colonies) with 30 and 15 mM sugar concentrations. At the lowest lactose concentration (6 mM) colonies were white with a red center (DW1/F'G11/pSTY91) or completely white (200R/pSTY91) (Table II). The corresponding cells without the plasmid *melB* gene failed to show fermentation at any concentration of lactose.

The same strains were tested for growth on

TABLE II

FERMENTATION AND GROWTH ON LACTOSE

Incubation temperature was 37 °C

Strain	MacConkey lactose plates			Lactose minimal plates		
Lactose (mM)	30	15	6	30	6	2
DW1/F'G11	white	white	white	ng [a]	ng	ng
DW1/F'G11/pSTY91	red	red	red center	2 mm [b]	1 mm	1 mm
200R	white	white	white	ng	ng	ng
200R/pSTY91	red	red	white	1.5 mm	0 5 mm	0 2 mm

[a] No growth.
[b] Colony size

lactose minimal plates containing three different concentrations of sugar. The cells with the melibiose carrier grew well with 30 mM lactose and less well with 6 or 2 mM concentrations of sugar (Table II). The strains without the *melB* gene failed to grow.

In order to determine the cation requirement of the melibiose carrier for lactose transport, the effect of Na^+ and Li^+ on the growth of cells in lactose minimal medium was tested. For this experiment special precautions were taken to exclude Na^+ from the medium since this cation contaminates many salts (particularly potassium phosphate), and in addition it may be leached from glass containers. Cells were placed in plastic tubes containing 2 ml of Mops-buffered minimal medium. Growth of cells in lactose occurred only when Na^+ or Li^+ had been added to the medium (Fig. 1). The rate of growth was slower with Li^+ than with Na^+, but it proceeded to the same extent when cells were exposed to 0.5 or 1 mM Li^+. Slightly less growth was observed with 10 and 25 mM LiCl.

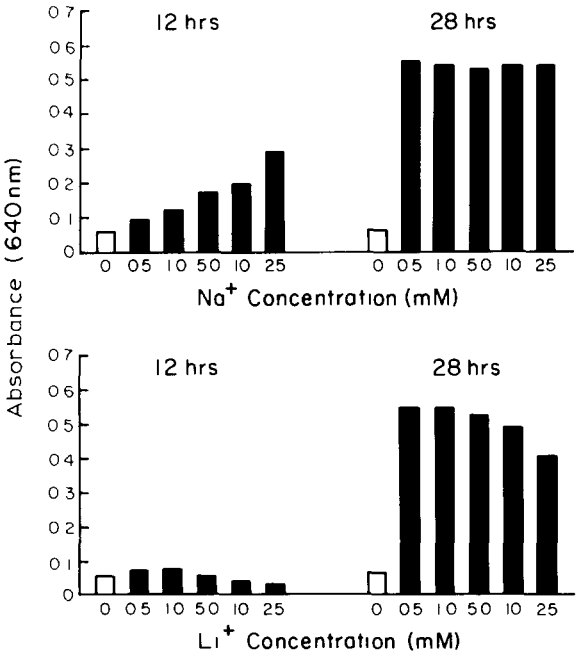

Fig 1 Growth of DW1/F'G11/pSTY91 on lactose minimal medium The low Na^+ medium was prepared as described in Materials and Methods Lactose was added to 6 mM, NaCl or LiCl was added as indicated Tetracycline was 10 μg/ml The cultures were incubated at 37 °C on a rotary shaker Growth was determined by measurement of absorbance of the culture at 640 nm with a Gilford spectrophotometer

The rate of growth of DW1/F'G11/pSTY91 in lactose was compared with the rate of growth in melibiose. No detectable increase in absorbance of cells in lactose medium was observed in the absence of Na^+, whereas the growth rate in the presence of 10 mM Na^+ was comparable to that in melibiose medium, with a generation time of approx. 2 h. It was shown that the Na^+ requirement for lactose growth was not due to a Na^+ requirement for β-galactosidase activity (which is stimulated by Na^+), by growing strain X71-15 (*lacI*$^+$*Z*$^+$*Y*$^+$) in the same medium. Growth of this strain was identical in medium with or without added Na^+.

Two additional β-galactosides were tested for the ability to enter the cell on the melibiose carrier. Growth of DW1/F'G11/pSTY91 occurred on L-arabinose-β-galactoside and on D-fructose-β-galactoside (lactulose) when Na^+ (but not Li^+) was added to the medium. There was no growth in the absence of Na^+.

Effect of cations on radioactive sugar uptake

The accumulation of [^{14}C]lactose was measured in W3133, a strain possessing the melibiose carrier but lacking the transport system for lactose (*lacZY* deletion). The cells were grown on amino acids at 30 °C in the presence or absence of melibiose as inducer. In Fig. 2 it can be seen that induced cells show a 40-fold accumulation of lactose in the presence of Na^+ and more than a 100-fold accumulation with 10 mM Li^+. Only a 2-fold accumulation was observed in the absence of added cations. Uninduced cells showed a very low uptake with or without added Na^+ or Li^+. A strong stimulation of lactose transport by Na^+ and Li^+ was also observed in *lac*-deleted cells containing the melibiose plasmid pSTY91 (data not shown).

In an attempt to determine the affinity of the carrier for lactose relative to another sugar of known K_m, the transport of TMG (K_m = 0.2 mM) [12] was measured in the presence of varying amounts of lactose. Approx. 50% inhibition of TMG transport (0.1 mM) was obtained with a lactose concentration of 30 mM in strain DW1/F'G11/pSTY91.

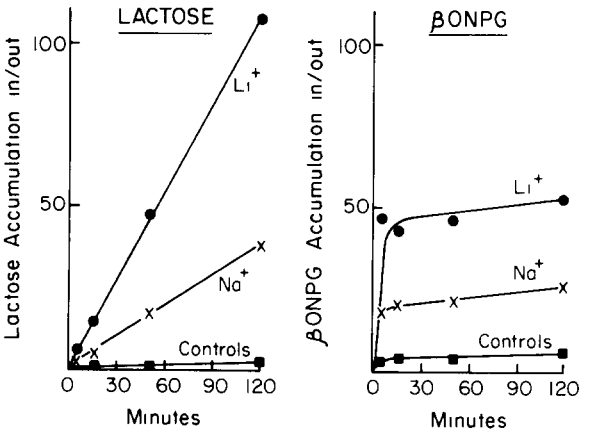

Fig. 2 Uptake of [^{14}C]lactose or [^{3}H]β-ONPG by melibiose-induced W3133 in the presence or absence of 15 mM Na^+ or Li^+ W3133 was grown at 30 °C in M63 with the addition of 1% tryptone + 0 5 μg/ml thiamin Melibiose was added to the growth medium to give a final concentration of 6 mM when induced cells were required. The transport assay was carried out as described in Materials and Methods The assay was initiated by addition of 100 μM [^{14}C]lactose (0 1 μCi/ml) or 50 μM [^{3}H]β-ONPG (0 2 μCi/ml) The controls included melibiose-induced cells without Na^+ or Li^+, and uninduced cells in the presence of Na^+ or Li^+

The accumulation of another β-galactoside, *o*-nitrophenyl-β-galactoside (β-ONPG) was measured in strain W3133. Na^+ and Li^+ caused a marked stimulation of uptake of the sugar (Fig. 2). 50-fold accumulation of β-ONPG was observed in the presence of Li^+, 25-fold accumulation with Na^+ and 5-fold in the absence of added Na^+ or Li^+. Na^+ also stimulated β-ONPG entry into a cell possessing both the melibiose carrier and β-galactosidase (Table III). The uptake of β-ONPG into such a cell (200R/pSTY91) results in intracellular hydrolysis of the sugar to give galactose and *o*-nitrophenol, which is yellow and can be measured photometrically. In this case the entry of sugar is 'down hill', as accumulation does not occur.

An unexpected finding was the transport of D-galactosamine by the melibiose carrier of DW1/pSTY37. In a preliminary experiment, performed at pH 7, accumulation of D-[^{3}H]galactosamine (100 μM) in the presence of 10 mM NaCl reached approx. 11-fold in 10 min. However, the apparent anomaly of a charged molecule entering the cell on a carrier designed for neutral

TABLE III

β-ONPG ENTRY VIA THE MELIBIOSE CARRIER

Strain 200R/pSTY91 (*lac*$Z^+\Delta Y$/plasmid *mel* A^+B^+) was grown on 1% tryptone + 6 mM melibiose + 10 μg/ml tetracycline Washed cells 0 14 mg dry wt /ml) were incubated at 25 °C in Mops/tetramethylammoniumhydroxide buffer (100 mM) (pH 6 9) plus 1 mM β-ONPG, with or without Na^+ The production of *o*-nitrophenol was measured with a Klett-Summerson colorimeter (42 filter) after dilution of the sample in 0.6 M Na_2CO_3 and removal of the cells by centrifugation

Condition	*o*-Nitrophenol produced (nmol/mg dry wt cells)		
	15 min	30 min	60 min
No Na^+	14	20	59
15 mM Na^+	196	353	588

compounds was resolved when the experiment was repeated at three different pH values. At pH 6, where there would be ionization of the amino-group, there was no transport of the sugar. At pH 7, with less ionization, there was 17-fold accumulation in 60 min in the presence of Na^+. At pH 8, where ionization would be suppressed, the accumulation reached 45-fold. Li^+ caused even greater stimulation of uptake of D-galactosamine than did Na^+. A 35-fold gradient of sugar was developed in the cells after 60 min at pH 7 and a 90-fold gradient at pH 8. No accumulation of the amino sugar was observed in the absence of Na^+ or Li^+.

Effect of sugars on Na^+ or H^+ uptake

The uptake of Na^+ or H^+ associated with sugar transport was investigated with the use of ion-specific electrodes. For studies of Na^+ uptake, cells of DW1/pSTY91 were incubated anaerobically in buffer at pH 8 containing 25–250 μM Na^+ and the concentration of this ion in the medium was monitored with a Na^+-specific electrode. On the addition of a small volume of melibiose solution there was a prompt fall in the Na^+ concentration of the external medium as the cation and melibiose entered the cell on the carrier (Fig. 3). Similar stimulation of Na^+ uptake was observed on the addition of the three β-galactosides, lactose, arabinose-β-galactoside and fructose-β-galactoside, when concentrations of approx. 20 mM sugar were used (Fig. 3). The initial rate of Na^+ uptake was slower for lactose than for

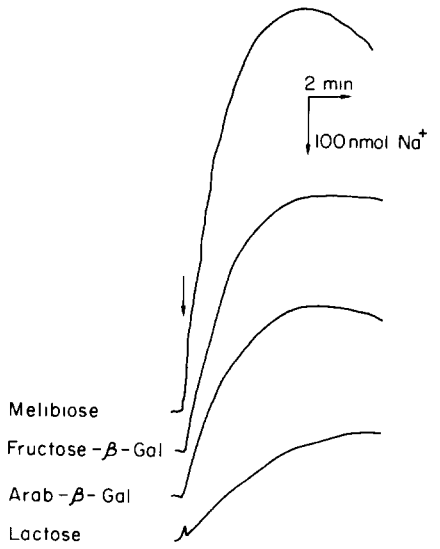

Fig. 3 Na^+ uptake from the medium by DW1/pSTY91 on the addition of melibiose or 3 β-galactosides. Na^+ uptake was measured as described in Materials and Methods The cells were grown without inducer and were suspended in 100 mM Tricine buffer (pH 8) plus 10 mM KSCN to a density of cells 15 mg dry wt/ml. NaCl was added to 250 μM Concentration of sugar added melibiose, 9 mM, D-fructose-β-galactoside, L-arabinose-β-galactoside and lactose, 20 mM. An upward deflection indicates a fall in Na^+ concentration in the incubation medium Strain DW1 without the plasmid showed no Na^+ uptake when tested with these sugars

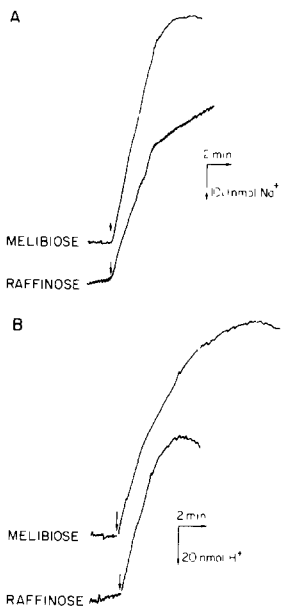

Fig 4 (A) Na^+ uptake from the medium by DW1/pSTY91 on the addition of raffinose or melibiose The cells were grown without inducer, washed twice then suspended in 100 mM Tricine buffer (pH 8) plus 10 mM KSCN Concentration of cells was 15 mg dry wt /ml Concentration of sugar added melibiose, 9 mM, raffinose, 7 mM (B) H^+ uptake from the medium by DW1/pSTY91 on the addition of raffinose or melibiose Cells were grown without inducer, washed twice and then diluted in 120 mM choline chloride/10 mM KSCN, to 13 mg dry wt cells/ml. The cell suspension was adjusted to pH 6 with HCl Raffinose (8 mM) or melibiose (8 mM) was added and H^+ uptake was measured as described in Materials and Methods An upward deflection indicates alkalinization of the incubation medium

the other β-galactosides, presumably because of slower sugar transport It has already been seen (Fig. 2) that the lactose transport rate is very poor.

For the measurement of proton uptake, cells were suspended in an unbuffered solution and the pH was monitored continuously. The cells were made anaerobic to shut off the respiratory chain. Addition of melibiose caused alkalinization of the medium as a result of sugar-H^+ cotransport. However, when the three β-galactosides described above were tested by this method at a concentration of 20 mM, no proton uptake was observed (data not shown).

It was known that transport of the α-galactoside, raffinose, by the melibiose carrier was stimulated by Na^+ [21], but there had been no demonstration of Na^+ uptake during cotransport with this sugar. In Fig. 4(A) is shown the response of DW1/pSTY91 to addition of 7 mM raffinose in the presence of 250 μM NaCl. A distinct Na^+ uptake was seen. Proton uptake was then measured with the same strain in an unbuffered medium. The addition of raffinose caused rapid uptake of protons (Fig. 4(B)).

The only two galactosides previously tested [4] that showed H^+ uptake when transported by the melibiose carrier were the α-galactosides, melibiose and methyl-α-galactoside. The β-form of methyl-galactoside caused uptake of Na^+ only. Another sugar available in both the α- and β-forms is *p*-nitrophenylgalactoside, and a comparison of the cation cotransport of these two isomers was made. The α-compound followed the pattern of the other α-galactosides and stimulated very pronounced H^+ uptake. The β-sugar showed no effect at all on the proton concentration of the medium (Fig. 5(A)). When Na^+ movement was tested at a sugar concentration of 1.5 mM the β-isomer caused a

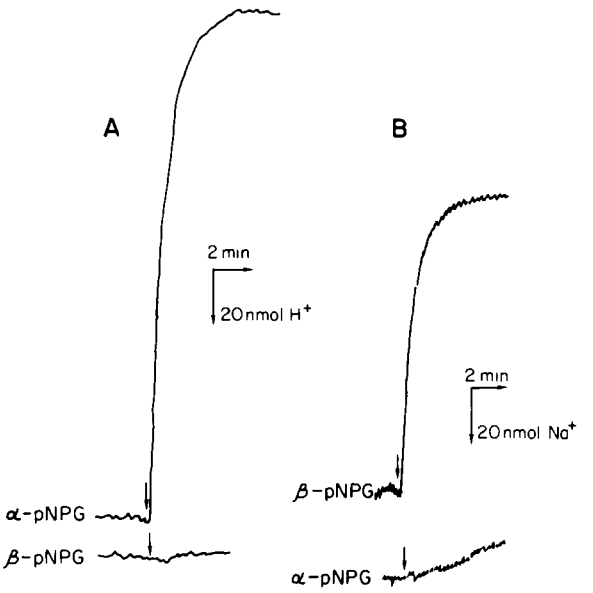

Fig. 5. The uptake of protons (A) and Na^+ (B) by DW1/pSTY91 on addition of α- or β-PNPG The measurement of H^+ or Na^+ uptake is described in Materials and Methods (A) 1 mM α- or β-PNPG was added at the arrow to cells suspended in 120 mM choline chloride/10 mM KSCN (14 mg dry wt cells/ml). (B) 1 mM α- or β-ONPG was added to cells suspended in Tricine buffer (pH 8) containing 50 μM NaCl and 10 mM KSCN.

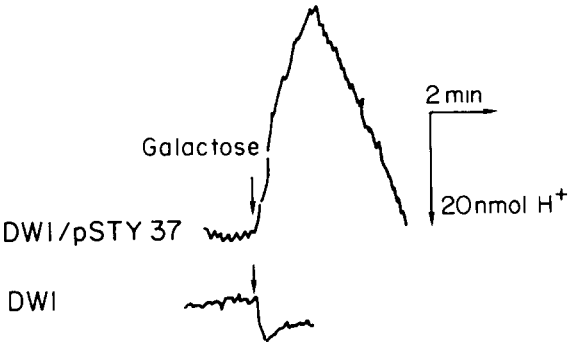

Fig 6 Proton movement after addition of D-galactose to DW1/pSTY37 Cells were grown without inducer, washed and suspended in 100 mM choline chloride/10 mM KSCN (11 mg dry wt cells/ml), then adjusted to pH 6 Galactose (4 mM) was introduced at the arrow

marked Na^+ uptake while the α-sugar had much less effect (Fig. 5(B)). It is known that the K_m for α-PNPG transport is decreased in the presence of 10 mM Na^+ [22] and it seemed that it should be possible to show a substantial Na^+ uptake with this sugar under certain conditions. When the α-PNPG experiments were repeated with a lower sugar concentration (0.125 mM) a distinct Na^+ uptake by the cells was observed (data not shown).

D-Galactose has been shown to enter the cell on the melibiose carrier in cotransport with Na^+ [4]. However, the convincing demonstration of D-galactose cotransport with protons on the *melB* requires a constitutive carrier to avoid the possibility of simultaneous induction of the D-galactose-H^+ cotransport carrier coded for by the *galP* gene [19]. Addition of galactose to strain DW1/pSTY37 grown on amino acids caused alkalinization of the medium, indicating D-galactose-H^+ cotransport by the *melB* gene product (Fig. 6). After 2–3 min the medium became progressively acidified due to metabolism of the D-galactose. When D-galactose was added to the cell that did not contain the plasmid, no alkalinization was observed.

Other monosaccharides related to D-galactose were tested in an attempt to determine whether there was any pattern of cation specificity (either H^+ or Na^+) that could be correlated with changes in structure of the sugar molecule. D-Fucose and L-arabinose both differ from D-galactose with respect to carbon 6 of this sugar. Both of these sugars induced Na^+ uptake (Fig. 7) but no movement of protons (data not shown).

The pentose, D-xylose, the epimer of L-arabinose in the carbon 4 position, does not induce entry of either Na^+ (Fig. 7) or H^+. In the same manner, D-glucose, which is the epimer of D-galactose in the 4 position, causes no movement of Na^+ and no apparent H^+ uptake. Acid production is rapid from glucose-exposed cells, which could possibly mask H^+ uptake. However, acid is produced from D-galactose also (and from melibiose when the cell contains α-galactosidase) without concealing the initial proton uptake.

A sugar differing from D-galactose in the 2 position is 2-deoxy-D-galactose. This sugar does not promote either Na^+ or H^+ uptake. However, the addition of D-galactosamine (2-deoxy-2-amino-D-galactose) to DW1/pSTY91 caused marked Na^+ uptake at pH 8 (Fig. 8). This observation is consistent with the data given earlier in this paper that Na^+ stimulates uptake of this sugar at pH 8. There was no proton uptake seen when measurements were made with the pH elec-

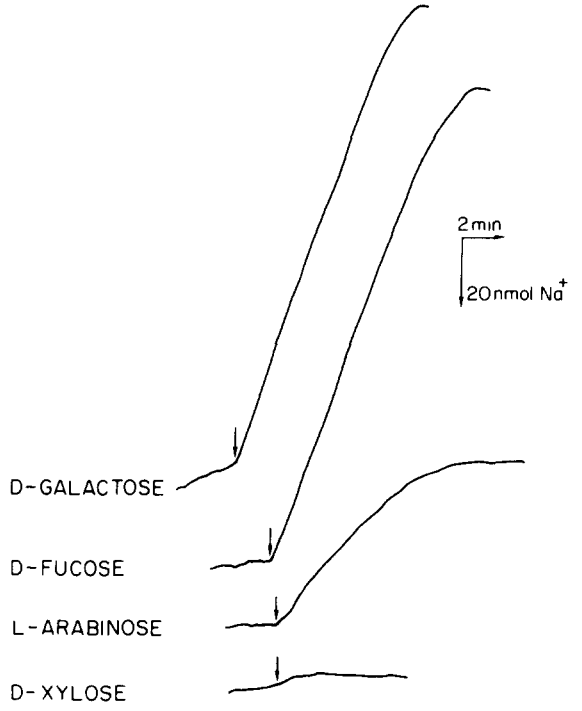

Fig. 7 Na^+ uptake on addition of monosaccharides to DW1/pSTY27. Sugars (10 mM) were added to the suspension of cells (12 mg dry wt/ml) in Tricine buffer (pH 8) containing 50 μM NaCl and 10 mM KSCN

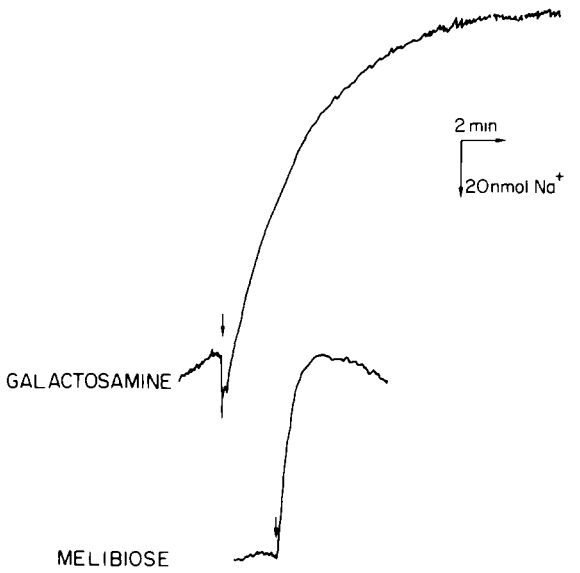

Fig 8 Na^+ uptake on addition of D-galactosamine to DW1/F'G11/pSTY37 Sugars (5 mM) were added to the cell suspension (13 mg dry wt/ml) in Tricine containing 50 μM NaCl and 10 mM KSCN.

trode after addition of D-galactosamine to cells at pH 6. Since it is likely that the failure of the sugar to be transported at pH 6 is due to the charged state of the molecule, the derivative *N*-acetyl-D-galactosamine (which does not ionize) was tested in this system. However, this substitution resulted in failure of uptake of Na^+ at pH 8 as well as showing the same lack of H^+ cotransport at pH 6.

Two more compounds related to D-galactose were tested for possible cotransport with H^+ or Na^+ on the melibiose carrier. The keto-sugar corresponding to D-galactose, D-tagatose, showed no Na^+ or H^+ uptake when added to *melB*$^+$ cells. Similarly, there was no movement of ions when the alcohol, galactitol, was added to the system.

Discussion

The α-galactoside carrier of *E. coli* has a broader substrate recognition than previously believed. Of special interest is a group of β-galactosides which have been found to be transported by this carrier. It has been determined by growth experiments and by measurement of radioactive sugar transport that lactose (which has long been thought not to be a substrate [4,5,23]) is in fact transported by this carrier, although the affinity for the sugar is poor. Lactose does not induce the melibiose operon, but growth on lactose can be observed with a *lacZ*$^+$ *Y*^{del} strain containing a constitutive *melB* gene on the plasmid. Growth on this β-galactoside, or uptake of [^{14}C]lactose by the *melB* gene product depends on the presence of Na^+ or Li^+. Uptake of lactose is stimulated to a greater extent in the presence of Li^+ than with Na^+, but growth is slower when Li^+ is the available cation. This apparent contradiction can be explained by the results of Umeda et al. [24], who showed that the growth rate of *E. coli* is inhibited by Li^+ when sugars constitute the sole carbon source. The toxicity of Li^+ was due to inhibition of pyruvate kinase I; Na^+ had little effect on this enzyme.

Na^+ cotransport was observed with lactose and to a more marked extent with two additional β-galactosides, L-arabinose-β-galactoside and D-fructose-β-galactoside. No H^+ cotransport could be detected with any of these β-galactosides, and

this was consistent with the requirement for Na^+ for growth on these sugars.

A correlation between the chemical structure of the sugar and the cation specificity has been observed. β-Galactosides are cotransported with Na^+ while α-galactosides may use either H^+ or Na^+ for cotransport. The previous data of Tsuchiya and Wilson [4] had shown that melibiose and methyl-α-galactoside cause H^+ uptake when added to cells possessing the melibiose transport protein. In the present work it is demonstrated that two additional α-galactosides, raffinose and α-PNPG, have the property of cotransport with protons. In contrast, the seven β-galactosides did not show the ability to effect H^+ movement under the conditions employed.

Among the monosaccharides tested, D-galactose was the only one to show uptake with H^+ as well as with Na^+. For this experiment the constitutive plasmids obtained from Dr. Tsuchiya were particularly valuable in obviating the necessity for induction of the transport system with melibiose Galactose is one of the products of hydrolysis of melibiose, and this in turn might conceivably induce the galactose carrier (*galP*), a H^+-sugar cotransport system [25]. The constitutive *melB* plasmid strains show that galactose-H^+ transport is present without induction and is due to the *melB* product, as strains without the plasmid do not have this property.

D-Fucose and L-arabinose differ from D-galactose at carbon 6. D-Fucose is the 6-deoxy derivative of D-galactose, while L-arabinose is a pentose that forms the same pyranose ring as D-galactose but lacks the carbon in the 6 position. Both of these sugars stimulated Na^+ uptake in the same manner as D-galactose but failed to cause proton movement. It is interesting that the loss of a hydroxyl group in the 6 position (or even the loss of the carbon 6 moiety as a whole) should cause loss of only the proton cotransport, whereas Na^+ cotransport is still present. D-Fucose, in fact, shows very similar movement of Na^+ to that seen with D-galactose (Fig. 7). The effect with L-arabinose, however, (where carbon 6 is missing) is considerably decreased. Isomerization of L-arabinose in the carbon 4 position gives D-xylose, which does not promote movement of either cation. Since the epimer of D-galactose in the 4 position (D-glucose) also does not stimulate Na^+ uptake or cause proton uptake, it would seem that the configuration around carbon 4 is of importance for specificity for both types of cotransport.

The loss of the OH group at carbon 2 (2-deoxy-D-galactose) results in a sugar that does not stimulate cotransport of either H^+ or Na^+. However, when the hydroxyl is replaced with an amino group (D-galactosamine), there is cotransport of this sugar with Na^+ at pH 8. At this pH the compound would not be charged and the carrier appears to accept the unionized NH_2 group in place of the hydroxyl. At pH 6 there is no transport of D-galactosamine and no proton uptake can be demonstrated. It was thought that the derivative *N*-acetyl-D-galactosamine (which does not ionize) might be able to enter on the carrier at pH 6 with protons. However, the addition of the acetyl group altered the specificity so that there was no uptake of either cation.

These studies with monosaccharides have shown that whereas changes in the hydroxyl group at carbon 2 or 4 positions of galactose affect both the Na^+ and H^+ pathways for cotransport, changes in the carbon 6 hydroxyl affect only the proton coupled transport. A similar situation occurs in the case of the disaccharides where the change of configuration from α- to β-galactoside affects the H^+ cotransport only. It therefore appears that the sugar specificity requirements for H^+ cotransport are more exacting than those for Na^+ cotransport.

Acknowledgements

We wish to thank Dr. Tomofusa Tsuchiya for the generous gift of the plasmids pSTY91 and pSTY37. Thanks are also due to Dr. Donna Seto-Young for the P_1 lysate of cells containing TN10. This work was supported by the National Institutes of Health Research grant DK-05736 and the National Science Foundation grant DMB-8414848.

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