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Uptake and Catabolism of D-Xylose in *Salmonella typhimurium* LT2

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Salmonella typhimurium LT2 grows on D-xylose as sole carbon source with a generation time of 105 to 110 min. The following activities are induced at the indicated time after the addition of the inducer, D-xylose: D-xylulokinase (5 min), D-xylose isomerase (7 to 8 min), and D-xylose transport (10 min). All other pentoses and pentitols tested failed to induce isomerase or kinase. Synthesis of D-xylose isomerase was subject to catabolite repression, which was reversed by the addition of cyclic adenosine monophosphate. Most of the radioactive counts from D-[^{14}C]xylose were initially accumulated in the cell in the form of D-xylose or D-xylulose. D-Xylose uptake in a mutant which was deficient in D-xylose isomerase was equal to that of the wild type. The apparent K_m for D-xylose uptake was 0.41 mM. Some L-arabinose was accumulated in D-xylose-induced cells, and some D-xylose was accumulated in L-arabinose-induced cells. D-Xylitol and L-arabinose competed against D-xylose uptake, but D-arabinose, D-lyxose, and L-lyxose did not. Osmotic shock reduced the uptake of D-xylose by about 50%; by equilibrium dialysis, a D-xylose-binding protein was detected in the supernatant fluid after spheroplasts were formed from D-xylose-induced cells.

Among the pentoses, only L-arabinose, D-ribose, and D-xylose are used as sole sources of carbon by *Salmonella typhimurium* LT2 and several strains of *Escherichia coli* (6), whereas *Aerobacter aerogenes* can utilize seven out of eight aldopentoses and all four of the pentitols (22, 23). Catabolism of pentoses (except D-ribose) in gram-negative bacteria involves the transport of the aldopentose into the cell and the isomerization of the aldopentose to a ketopentose, which is then phosphorylated. This phosphorylated derivative is converted into D-xylulose-5-phosphate by epimerases and then channeled into the pentose phosphate pathway. In *A. aerogenes* (23) and in *E. coli* K-12 (7), the presence of three inducible activities, D-xylose transport, D-xylose isomerase, and D-xylulokinase, has been demonstrated.

This study is a description of the nature of induction of the D-xylose catabolic enzymes in *S. typhimurium* LT2 and partial characterization of the D-xylose transport system. An accompanying paper (19) discusses the genetics and regulation of D-xylose catabolism.

MATERIALS AND METHODS

Strains. The majority of studies were done with *S. typhimurium* LT2 (from the *Salmonella* Genetic Stock Centre, University of Calgary) or with *E. coli* K-12 (obtained from the Department of Microbiology, University of Alberta); both are prototrophic Xyl$^+$ strains. The origin of the D-xylose isomerase-deficient strain, SA1963 (*xylA118*), is described in the accompanying paper (19). SA792 is a multiply-mutant strain of *S. typhimurium* (*purC7 proA46 ilv-461 rpsL rha-461 fla-56 fim mtlB121*). All strains were maintained at −76°C in saline solution containing 15% glycerol.

Chemicals. Most of the chemicals were purchased from Sigma Chemical Co., St. Louis, Mo. D-[^{14}C]xylose and L-[^{14}C]arabinose were obtained from Amersham Corp., Arlington Heights, Ill., whereas D-xylulose was purchased from Koch-Light Laboratories, Colnbrook, England.

Growth media. Minimal salts medium, modified from Davis and Mingioli (8), contained per liter: 7 g of K_2HPO_4; 3 g of KH_2PO_4; 0.1 g of $MgSO_4 \cdot 7H_2O$; 1 g of $(NH_4)_2SO_4$. To prepare minimal agar, 15 g of agar (Difco) was added to the above medium. Routinely, 0.2% of the carbon source was added. The other growth media included the following: minimal agar with D-glucose, minimal agar with D-xylose, liquid minimal medium with D-glucose, and liquid minimal medium with D-xylose. For the growth of amino acid auxotrophs, appropriate amino acids were added to a final concentration of 50 μg/ml.

Measurement of bacterial growth. Bacterial turbidity was measured by use of a Klett-Summerson colorimeter with a 660-nm filter. One Klett unit corresponded to approximately 10^7 cells per ml. The generation time was the time required for doubling the turbidity while 10 ml of cell suspension in a 250-ml side-arm flask was shaking at 150 rpm in a New Brunswick reciprocating shaker.

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Preparation of cells for enzyme assays. About 1 liter of bacterial culture was grown in shake cultures using a New Brunswick gyratory shaker at 250 rpm on minimal medium supplemented with 0.2% of the carbon source (unless otherwise specified). When carbon sources other than D-xylose were used, cells were induced for synthesis of D-xylose catabolic enzymes by the addition of 0.1% D-xylose. The cells were harvested in late logarithmic phase (150 Klett units), washed in minimal salts medium, centrifuged for 10 min at 10,000 × *g*, and stored at −76°C. Frozen cells stored for 4 months maintained over 95% of the activities of D-xylose isomerase and D-xylulokinase compared to fresh, unfrozen cells.

About 0.3 g (wet weight) of cells was thawed, suspended in 6 ml of 50 mM Tris-hydrochloride (pH 7.5) containing 1 mM dithiothreitol, and sonicated with a Bronson Sonifier model S75 for 2 min in 15-s bursts, cooling the samples for 1 min between bursts. The cell suspension was centrifuged at 15,000 × *g* for 15 min to remove cell debris, and the supernatant (crude extract) was used in enzyme assays. The protein concentration was about 8 to 10 mg/ml.

D-Xylose isomerase. The rate of formation of D-xylulose from D-xylose was determined. The reaction mixture consisted of 40 μmol of Tris-maleate buffer at pH 7.5, 0.7 μmol of $MnSO_4$, and 7.0 μmol of D-xylose, adjusted to a 0.7 ml volume with distilled water. The assay was performed at 37°C, started by the addition of substrate, and stopped by the addition of trichloroacetic acid to a final concentration of 2%. The suspension was centrifuged at 8,000 × *g* for 15 min, and the supernatant was assayed for D-xylulose by the cysteine-carbazole method (9). One unit of the enzyme is the amount which produces 1 μmol of D-xylulose per min.

D-Xylulokinase. D-Xylulokinase was assayed by two methods. (i) Disappearance of D-xylulose: the reaction mixture consisting of 40 μmol of Tris-hydrochloride (pH 7.5), 10 μmol of $MgCl_2$, 4 μmol of freshly prepared cysteine, 10 μmol of ATP, 1 μmol of NaF, and 1 μmol of D-xylulose was adjusted to a final volume of 0.5 ml with distilled water. The assay was performed at 37°C, started by the addition of D-xylulose, and stopped by the addition of 3.0 ml of ethanol and 0.2 ml of 1 M barium acetate. The assay tubes were chilled in an ice bucket for 10 min and centrifuged at 8,000 × *g* for 10 min, and the supernatant was assayed for D-xylulose by the cysteine-carbazole method (9). (ii) Appearance of ADP: ADP formed as a result of the D-xylulokinase reaction was coupled with the pyruvate kinase reaction as follows:

$$\text{D-xylulose} + \text{ATP} \xrightarrow{\text{D-xylulokinase}} \text{D-xylulose-5-P} + \text{ADP}$$

$$\text{ADP} + \text{phosphoenolpyruvate} \xrightarrow{\text{pyruvate kinase}} \text{pyruvate} + \text{ATP}$$

$$\text{pyruvate} + \text{NADH} \xrightarrow{\text{lactate dehydrogenase}} \text{D-lactate} + \text{NAD}^+$$

The reaction mixture consisted of the following: 20 μmol of Tris-hydrochloride (pH 7.5), 2 μmol of ATP, 0.2 μmol of phosphoenolpyruvate, 3 μmol of reduced glutathione, 150 μg of lactate dehydrogenase, 150 μg of pyruvate kinase, 0.1 μmol of NADH, 2 μmol of $MgCl_2$, 5 μmol of KCN, 5 μmol of NaF, and 1 μmol of D-xylulose, adjusted to a volume of 1.3 ml with distilled water. The reactions were carried out in 1-cm light-path cuvettes and started by the addition of D-xylulose, and the rates were measured by following the change in absorbance at 340 nm using a Unicam SP 1800 spectrophotometer with a Unicam AR 25 linear recorder. The change in optical density of the reaction mixtures containing the above compounds, except D-xylulose, was ascribed to the activity of NADH oxidase. Net activity of D-xylulokinase was obtained as the difference between total activity and NADH oxidase levels. The NaF and KCN inhibited ATPase and NADH oxidase activities, respectively, which were present in the crude extract, but did not affect activity of D-xylulokinase. One unit of D-xylulokinase is the amount of enzyme which phosphorylates 1 μmol of D-xylulose per min.

Protein estimation. Protein estimation was by the method of Lowry et al. (14), with bovine serum albumin as standard.

Transport assay. Flasks were inoculated to approximately 2 Klett units with cells grown on minimal medium containing 0.2% glycerol and 0.4% D-xylose and grown to 50 Klett units turbidity in the same medium. (For growth of uninduced cells, D-xylose was not added to the growth medium.) Cells were centrifuged at 10,000 × *g* for 10 min, washed, and suspended to 50 Klett turbidity units in minimal medium containing 0.2% glycerol and 150 μg of chloramphenicol per ml.

The cells were incubated at 37°C with shaking in the presence of radioactive ^{14}C-labeled sugar (D-xylose or L-arabinose) (specific activity 0.1 μCi/mol, unless otherwise specified) with different concentrations of unlabeled sugar. The volume of the assay mixture was 1.0 ml. In routine assays, after 5 min of uptake, cells were collected on membrane filters (Millipore Corp.; HAWP, 25 mm diameter, 0.45 μm pore size), and the filters were placed in scintillation vials and dried overnight at 45°C. A 10-ml volume of scintillation fluid containing toluene (1,000 ml), 2,5-diphenyloxazole (4 g), and 1,4-bis-[2-(4-methyl-5-phenyloxazolyl]-benzene (100 mg) was added to the vials, and radioactivity was determined in a Packard Tri-Carb model 3375 liquid scintillation spectrometer. One unit of D-xylose transport activity transports 1 μmol of D-xylose per min.

Chromatography. Samples of D-xylose and D-xylulose (25 μg) were spotted on cellulose chromatographic thin-layer chromatography sheets (Kodak) or on Whatman no. 1 filter paper. The sugars were separated by chromatography in a solvent system containing butanol, pyridine, and water (10:3:3, vol/vol/vol). The sheets were sprayed with napthoresorcinol reagent (50 ml of 0.2% napthoresorcinol in 100% ethanol, 50 ml of 0.25 N HCl, and 5 ml of orthophosphoric acid). D-Xylose produced a blue color, whereas D-xylulose and D-xylulose-5-phosphate produced a brown color, with R_f values of 0.26, 0.35, and 0.01,

respectively. After a run using radioactive substrates, the sheets were cut into strips and the cellulose was scraped off and counted for radioactivity. A 25-μg amount of standard unlabeled substrates was chromatographed along with the radioactive substrates.

Release of periplasmic proteins. (i) Cold shock treatment. A 100-ml volume of cell suspension was harvested at logarithmic phase and subjected to cold shock by the method described by Neu and Heppel (12, 15). Cold-shocked cells were suspended in minimal medium (no carbon source) and used in transport assays. The supernatant or shock fluid was a source of periplasmic proteins.

(ii) Preparation of spheroplasts. A 1-liter volume of cell suspension was grown in minimal medium plus 0.4% D-xylose and 0.2% glycerol until it reached 120 Klett units. The cells were centrifuged at 10,000 $\times$ *g* for 10 min, washed in 30 mM Tris-hydrochloride (pH 8.0), suspended in 100 ml of the same buffer containing 20% sucrose, 10 mM EDTA, and 250 μg of lysozyme per ml, and stirred at room temperature with a magnetic stirrer. When greater than 80% of the cells had formed spheroplasts, as observed through a phase-contrast microscope (after about 20 min), the suspension was centrifuged at 27,000 $\times$ *g* for 20 min. The supernatant, presumed to contain the periplasmic proteins, was concentrated in an Amicon ultrafiltration unit with a PM-10 membrane at 0°C, under 3.7 kg of nitrogen per cm^2, to a final protein concentration of about 5 mg of protein per ml. The concentration of the sucrose in the protein solution was decreased by diluting with 50 ml of 50 mM Tris-hydrochloride (pH 8.0) and reconcentrating the solution by Amicon ultrafiltration to about 5 mg of protein per ml. The protein solution was dialyzed against 0.05 M Tris-hydrochloride (pH 7.5) for about 10 h.

Equilibrium dialysis. Binding proteins were assayed by equilibrium dialysis in a multichamber apparatus (10). A 0.1-ml volume of the protein solution was introduced into one of the wells through the addition port, and, into the well below the protein, 0.09 ml of 50 mM Tris-hydrochloride (pH 7.5) and 0.01 ml of radioactive ^{14}C-labeled substrate (0.2 μCi/mg) were added. The apparatus was mechanically rotated for 10 h, 25-μl aliquots were removed from the protein and substrate wells, and radioactivity was determined.

RESULTS

Growth on pentoses as sole carbon source. Both *S. typhimurium* LT2 and *E. coli* K-12 utilize L-arabinose, D-ribose, and D-xylose as sole carbon sources, although the generation time on D-xylose for both was nearly double that on D-glucose (Table 1). Colony formation on minimal agar containing D-xylose required 2 days rather than 1 day as on minimal agar containing D-glucose. All other pentoses and pentitols tested, including D-arabinose, D- and L-lyxose, D- and L-arabitol, ribitol, L-ribose, L-xylose, and xylitol, were not utilized as sole sources of carbon by *S. typhimurium*.

Induction of catabolic activities. When cells were grown on minimal medium containing glycerol or Casamino Acids as the sole sources of carbon, no activity of D-xylose isomerase or D-xylulokinase was detected; there was a very low level of D-xylose transport activity (Table 2). However, when cells were induced by growth in D-xylose in addition to glycerol, the activity increased by at least 140-, 350-, and 50-fold in D-xylose isomerase, D-xylulokinase, and D-xylose transport, respectively. L-Arabinose, D-arabinose, D-lyxose, D-ribose, and D-xylitol did not induce detectable D-xylose isomerase or D-xylulokinase activities. Growth of strain LT2 on minimal medium plus D-xylose or on minimal medium plus D-xylose plus Casamino Acids or glycerol all gave about the same specific activities for D-xylose isomerase and D-xylulokinase (data not shown). The specific activities of D-xylose isomerase and D-xylulokinase in *E. coli* K-12 were comparable to those of *S. typhimurium* LT2, but the rate of transport of D-xylose in *E. coli* was nearly double that of *S. typhimurium*. The specific activities observed are similar to those reported earlier for *E. coli* K-12 (Table 2) (1, 7).

TABLE 1. *Growth on different carbon sources*

Carbon source[a]	Generation time (min)[b]	
	E. coli K-12	*S. typhimurium* LT2[c]
D-Glucose	45–50	55–60
Glycerol	55–60	60–65
L-Arabinose	65–70	90–95
D-Ribose	75–80	85–90
D-Xylose	80–90	105–110
Sodium succinate	150	150

[a] Cells were grown in liquid minimal medium supplemented with 0.2% of the carbon source.

[b] Generation time was determined as described in Materials and Methods.

[c] *S. typhimurium* LT2 cannot grow on D-arabinose, D- and L-lyxose, D- and L-arabitol, ribitol, L-ribose, L-xylose, or xylitol as sole sources of carbon.

Activities of D-xylose isomerase and D-xylulokinase were induced in minimal medium with D-xylose levels as low as 0.01% (0.66 mM), but no activities were detected in cells grown on glycerol supplemented with 0.001% D-xylose (66 μM). The failure to detect activity with the lower concentration of D-xylose could be due to rapid utilization of the inducer.

The kinetics of induction was studied to determine if the catabolic activities responded as a unit to the presence of the inducer. *S. typhimurium* LT2 was grown on glycerol-minimal medium, and D-xylose was added at logarithmic phase. Enzyme assays (Fig. 1) indicate sequential induction of the catabolic activities at the following intervals after addition of the inducer:

TABLE 2. *Inducibility of D-xylose isomerase, D-xylulokinase, and D-xylose transport activities in S. typhimurium and E. coli*

Strain	Cells grown on minimal glycerol containing:	Concn of sugar (%)	Sp act[a]		
			D-Xylose isomerase	D-Xylulokinase	D-Xylose transport
S. typhimurium LT2 (wild type)	D-Xylose	0.2	13.0–15.3	33.5–37.0	30.3–35.0
		0.1	13.5	34.5	NT[b]
		0.01	14.2	33.9	NT
		0.001	<0.1	<0.1	NT
	L-Arabinose	0.2	<0.1	<0.1	35.3[c]
	D-Arabinose	0.2	<0.1	<0.1	NT
	D-Lyxose	0.2	<0.1	<0.1	NT
	D-Ribose	0.2	<0.1	<0.1	NT
	Xylitol	0.2	<0.1	<0.1	NT
	No addition		<0.1	<0.1	0.6
S. typhimurium SA792	D-Xylose	0.2	NT	NT	30.0
S. typhimurium SA1963 (*xylA118*)	D-Xylose	0.2	NT	NT	32.8
E. coli K-12	D-Xylose	0.2	16.2	39.5	75–80
E. coli χ289[d]	D-Xylose	0.015	18.6	103	70
E. coli K-12[e]	D-Xylose	0.2	25.4	NT	43.8

[a] The cells were prepared and the assays were done as described in the text.
[b] NT, not tested.
[c] Refer to section on specificity of D-xylose transport for details.
[d] Data of David and Weismeyer (7).
[e] Data of Ackerman et al. (1).

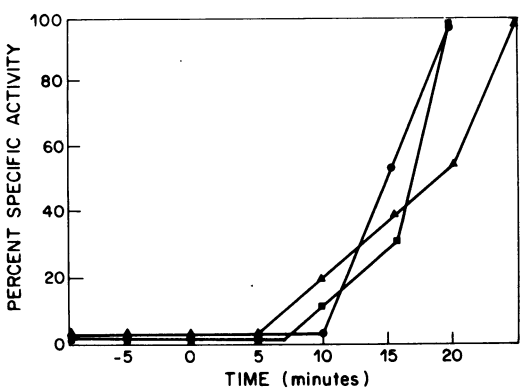

FIG. 1. *Kinetics of induction of D-xylose transport and catabolic activities. The inducer (0.2% D-xylose) was added at time zero. Samples were collected at intervals and assayed for activities. For enzyme activities, 200 ml of cell suspension was immediately mixed with ice and sodium azide (final concentration 30 mM), washed, and sonicated. For transport of D-xylose, 10-ml volumes were immediately filtered through membrane filters, and the cells were washed in ice-cold minimal glycerol medium containing 150 μg of chloramphenicol per ml. 100% specific activities were as follows: D-xylose isomerase (■), 12 μmol/min per g of protein; D-xylulokinase (▲), 30 μmol/min per g of protein; and D-xylose transport (●), 35 μmol/min per g (dry weight) of cells.*

D-xylulokinase, 5 min; D-xylose isomerase, 7 to 8 min; D-xylose transport activity, 10 min.

Repression of D-xylose isomerase by D-glucose and its reversal with cAMP. The presence of D-xylose induced D-xylose isomerase activity, but the addition of D-glucose reduced this activity by nearly 75% (Table 3). This resembles catabolite repression (17, 20) which is caused by D-glucose or one of its catabolic intermediates. Upon the addition of 5 mM cAMP to the growth medium containing D-xylose and D-glucose, the specific activity of D-xylose isomerase recovered to about 75% of original levels (Table 3).

Transport of D-xylose. Induced *S. typhimurium* LT2 cells accumulated 30 to 50 times more D-xylose than uninduced cells under the same assay conditions (Table 2, Fig. 2). Accumulation of D-xylose in strain SA792 (Xyl^+) and in SA1963, a D-xylose isomerase-deficient mutant, was comparable to that of wild-type LT2. Accumulation of D-xylose by D-xylose-induced LT2 or SA1963 cells was linear up to 10 min and then leveled off. Kinetic studies of D-xylose transport indicated only one transport system with an apparent K_m of 0.41 mM (Fig. 3), whereas in *E. coli* K-12 our data show two transport systems with apparent K_m values of 110 and 24 μM.

The fate of D-xylose subsequent to uptake was determined by allowing D-xylose-induced strain LT2 cells to accumulate D-[^{14}C]xylose (1 mM, 0.5 μCi/μmol) for 15 min in a standard transport assay. The membrane filters on which cells were collected were washed and shaken vigorously in

TABLE 3. *Repression of D-xylose isomerase by D-glucose and reversal of the effect with cAMP*[a]

Addition			D-Xylose isomerase (U/g of protein)
D-Xylose (0.2%)	D-Glucose (0.5%)	cAMP (5 mM)	
+			15.8
	+		<0.1
+	+		4.0
+	+	+	12.0

[a] Cells of *S. typhimurium* LT2 were grown in minimal medium plus glycerol and then inoculated and grown for 6 h to late logarithmic phase in the media shown.

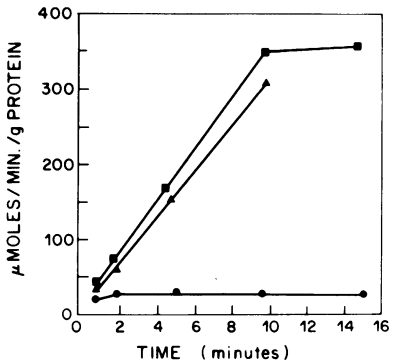

FIG. 2. *Transport of D-xylose in S. typhimurium. Uptake of D-[^{14}C]xylose was measured in LT2 cells grown in minimal medium plus 0.2% glycerol induced with 0.4% D-xylose (■); without D-xylose (●) (uninduced); or in SA1963 cells in minimal medium plus glycerol plus D-xylose (▲).*

0.5 ml of a mixture of acetone–chloroform (1:1, vol/vol), and the solution was centrifuged at 8,000 × *g* for 10 min. The supernatant fluid was partially evaporated, and then spotted and chromatographed on thin-layer chromatography sheets precoated with cellulose (see Materials and Methods). Nine percent of the radioactive counts remained at the origin (corresponding to D-xylulose-5-phosphate or its products), 36% were in fraction 3 (corresponding to D-xylose), 47% were in fraction 4 (corresponding to D-xylulose), whereas none of the other three fractions had more than 3% of the total counts. Thus, 15 min after initial uptake, 83% of the ^{14}C label was in D-xylose or D-xylulose, suggesting that initial uptake is D-xylose and that in vivo D-xylulose is the first product of D-xylose metabolism.

D-Xylose accumulation was about the same in LT2 cells that were induced with D-xylose or with L-arabinose (Table 4). The accumulation of L-arabinose was much greater in L-arabinose-induced than in D-xylose-induced cells.

Specificity of D-xylose transport. Competition with D-xylose transport in *S. typhimurium*

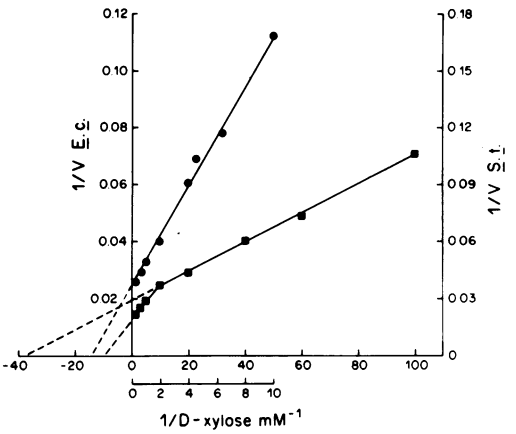

FIG. 3. *K_m for D-xylose transport in S. typhimurium and E. coli. Double reciprocal plot of D-xylose uptake in wild-type cells; V is expressed as micromoles of D-xylose accumulated per minute per gram (dry weight) of cells. X-axis: top line, E. coli; bottom line, S. typhimurium. Symbols: ■, E. coli; ●, S. typhimurium.*

TABLE 4. *Accumulation of D-xylose and L-arabinose in D-xylose- and L-arabinose-induced cells*

Inducer[a]	Accumulation of pentoses[b]	
	D-Xylose	L-Arabinose
D-Xylose	38.0	3.5
L-Arabinose	35.3	173.5
None	0.8	0.6

[a] Cells of strain LT2 were grown on minimal medium plus 0.2% glycerol with or without 0.2% D-xylose or L-arabinose.

[b] Initial rate of uptake in micromoles per minute per gram of cells. Concentration of pentoses, 1 mM.

was tested by adding competing sugars to a reaction mixture containing 1 mM D-[^{14}C]xylose (Table 5). The observed uptake where nonradioactive D-xylose was the competing substrate was in the expected range. Accumulation of radioactive D-xylose was not significantly reduced by D-arabinose, D-lyxose, or L-lyxose, indicating that these pentoses do not compete with D-xylose transport. Competition with xylitol and L-arabinose reduced D-xylose uptake by about 15 and 25%, respectively.

Effect of osmotic shock on D-xylose transport. When *S. typhimurium* cells were subjected to osmotic shock, they showed impairment of some amino acid and sugar transport systems (12). Normal induced cells of *S. typhimurium* transported D-xylose at the rate of 35 μmol/min per g (dry weight) of cells, but osmotically shocked cells lost 55 ± 7% of their ability to transport D-xylose (in three separate

TABLE 5. *Accumulation of D-xylose in the presence of other sugars*

Competing substrate[a]	% Accumulation		
	Expt 1[b]	Expt 2[b]	Expt 3[c]
None[d]	100	100	100
D-Arabinose		106	
L-Arabinose	76.7	72	73.4
D-Lyxose	94.6		100.8
L-Xylose		97.1	
Xylitol	84.3		82.9
D-Xylose	29.5	28.2	16.2

[a] D-Xylose-induced cells suspended in minimal medium + 0.2% glycerol were added to 1 mM D-[^{14}C]-xylose. After 5 min of uptake, the rate obtained in the presence of the competing substrate was compared to the accumulation in the absence of the competitor. Assays were performed in duplicate.

[b] Concentration of competing substrate, 3 mM.

[c] Concentration of competing substrate, 5 mM.

[d] 100% uptake in the absence of competitor is 30 to 35 μmol/min per g of cells.

experiments), presumably due to the loss of a periplasmic binding component.

Equilibrium dialysis chambers containing the supernatant fluid from spheroplast formation from uninduced LT2 cells gave no increase of D-[^{14}C]xylose counts over chambers containing substrate, whereas the dialysis chambers containing comparable supernatants from D-xylose-induced cells had 6.5 ± 1% more D-xylose than the substrate chambers in three separate tests. As a test of our assay system, a chamber containing supernatant from spheroplast formation from D-galactose-induced cells of LT2 accumulated 6.2% more D-[^{14}C]glucose than the substrate chamber; the D-galactose binding protein can bind D-galactose and D-glucose (4).

DISCUSSION

D-Xylose, L-arabinose, and D-ribose can be used as sole carbon sources for *S. typhimurium* LT2 and *E. coli* K-12. All other pentoses and pentitols tested, including L-lyxose, could not be used (Table 1), although tests using a different method showed L-lyxose utilization (11).

The catabolic pathway for D-xylose utilization in *S. typhimurium* appears to be identical to that of *A. aerogenes* (23) and *E. coli* (7). The activities of D-xylose transport, D-xylose isomerase, and D-xylulokinase were inducible, and no sugars other than D-xylose induced synthesis of isomerase and kinase. However, transport activity for D-xylose was induced by L-arabinose in addition to D-xylose. In *A. aerogenes*, D-xylose isomerase and D-xylulokinase are induced by D-xylose and to a limited extent by D-lyxose (23); in addition, D-xylulokinase is induced by xylitol and D-arabitol.

In *S. typhimurium*, since the activity of D-xylulokinase in D-xylose isomerase-deficient (*xylA*) mutants is comparable to levels present in wild-type cells (19), D-xylose and not a subsequent metabolite is the inducer for the D-xylose catabolic enzymes. The level of D-xylose necessary for induction of the D-xylose catabolic activities appears to be between 66 and 660 μM; 1 to 10 μM L-arabinose was necessary for induction of L-arabinose isomerase in *E. coli* (18). D-Xylose induction of D-xylose catabolic activities shows that D-xylulokinase appeared 5 min after inducer addition, D-xylose isomerase at 7 to 8 min, and D-xylose transport at 10 min. This tentatively suggests sequential induction of a single operon, but genetic data (19) show that the genes for isomerase (*xylA*) and kinase (*xylB*) are close to but in a different operon from that for transport (*xylT*).

Synthesis of D-xylose isomerase (and presumably D-xylulokinase and transport) was repressed by D-glucose, and this repression was reversed by added cAMP, suggesting that this effect is due to lowering of intracellular cAMP levels. It is likely that cAMP regulates expression of the D-xylose isomerase gene by a positive mechanism analogous to control of the *lac* and *gal* genes (17).

D-Xylose accumulated in *S. typhimurium* via an inducible transport system. There was at least a 40-fold concentration of D-xylose inside cells when compared to the outside medium, assuming that 80% of the total cell weight is water.

Chromatographic analysis of accumulated D-[^{14}C]xylose indicates that, at steady state, most radioactive label was still in a form with the chromatographic mobility of D-xylose or D-xylulose. This, together with the observation that uptake of D-xylose in a mutant lacking D-xylose isomerase was as great as in the wild type, indicates that D-xylose is taken up in unaltered form, before being converted to D-xylulose.

The substrate specificity of the transport system was tested by determining the accumulation of D-xylose in reaction mixtures containing unlabeled pentose and pentitol substrates as well as radioactive D-xylose (Table 5); reduction in D-xylose uptake is evidence that the added substrate competes with D-xylose uptake. Slight inhibition was observed when L-arabinose and xylitol were present in the reaction mixture, whereas D-arabinose, D-lyxose, and L-xylose did not compete. L-Arabinose and xylitol are sterically similar to D-xylose (second, third, and fifth hydroxyl groups are identical); this suggests a stereospecific transport activity.

D-Xylose is transported through the D-xylose-induced transport system; small but significant levels of L-arabinose are also transported in D-

xylose-induced cells (Table 4). In addition, D-xylose accumulates in L-arabinose-induced cells, presumably through the L-arabinose transport system. Similar data were obtained in *E. coli* B/r (16). This could be due to the steric similarities of L-arabinose and D-xylose, which permit uptake of L-arabinose through the D-xylose-induced system and D-xylose uptake through the L-arabinose-induced system; alternatively, they could be due to induction of the L-arabinose transport system by D-xylose, and of the D-xylose transport system by L-arabinose. In a mutant of *E. coli* K-12, able to utilize D-lyxose as sole source of carbon, transport of D-lyxose was via the D-xylose system (F. J. Stevens, and T. T. Wu, Fed. Proc. **35**:1660, 1976).

When D-xylose-induced cells were subjected to osmotic shock, the rate of accumulation of D-xylose was decreased by about 50%. Osmotic shock also reduced L-arabinose uptake by 50% and an arabinose binding protein was detected in the supernatant (13); in most systems a greater reduction in transport after osmotic shock is seen (3). These results suggest that a binding protein was released from induced cells upon osmotic shock, and the presence of this binding protein in the periplasmic fraction was demonstrated by equilibrium dialysis against D-xylose. An attempt to demonstrate the presence of this binding protein in periplasmic proteins from D-xylose-induced cells and its absence from uninduced cells by polyacrylamide gel electrophoresis was not successful (data not shown).

L-Arabinose and D-ribose are transported via two transport systems, a "low-affinity" and a "high-affinity" transport system (2, 5, 21). In both cases, binding proteins were associated with the high-affinity transport system, since binding proteins were released by osmotic shock, with a simultaneous reduction in transport activity towards L-arabinose and D-ribose and loss of the high-affinity system. Our kinetic studies on D-xylose transport suggest that there is only one transport system for D-xylose in *S. typhimurium* with a K_m of 0.41 mM but two in *E. coli*, although studies using L-arabinose-induced cells suggested a role for the L-arabinose transport system in D-xylose transport. The role of the binding protein in the transport process remains unclear. The D-galactose binding protein and possibly other sugar binding proteins recognize D-xylose (24); these might be induced under the growth conditions used.

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