Biochimica et Biophysica Acta, 646 (1981) 365–367
Elsevier/North-Holland Biomedical Press

BBA Report

BBA 71532

L-SORBOSE PHOSPHORYLATION IN *ESCHERICHIA COLI* K-12

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(Received May 18th, 1981)

Key words: L-Sorbose; Phosphotransferase; Glucose transport; Fructose transport; (E. coli)

L-Sorbose is phosphorylated by *Escherichia coli* by two distinct Enzymes II of the phospho*enol*pyruvate-dependent phosphotransferase system. The glucose Enzyme II (specified by the gene *ptsG*) phosphorylates L-sorbose with an apparent K_m of 0.08 ± 0.03 mM and V of 31.8 ± 3.5 nmol · mg^{-1} · min^{-1} whilst the fructose Enzyme II (specified by the gene *ptsF*) phosphorylates it with an apparent K_m of 28.9 ± 2.7 mM and V of 20.2 ± 0.8 nmol · mg^{-1} · min^{-1}. L-Sorbose induces neither of these Enzymes II, but sorbose inhibits the growth of strains expressing either of these functions constitutively. Mutants that have lost their sensitivity to L-sorbose are found to have lost either the glucose or the fructose phosphotransferase Enzyme II.

L-Sorbose has long been considered an isostere of fructose [1–3] and L-sorbose 1-phosphate is an isostere of D-glucose 6-phosphate [4]. L-Sorbose is a substrate [5] for the phospho*enol*pyruvate-dependent sugar phosphotransferase of *Escherichia coli* [6]. The growth of mutants that express the fructose-specific Enzyme II of the phosphotransferase constitutively (genotype *fruC*) is inhibited by L-sorbose [7] because of the accumulation of a non-metabolisable phosphorylated sugar derived from L-sorbose (see Ref. 8 for a review). We observed by chance that certain strains of *E. coli* which either lacked the fructose-specific Enzyme II (genotype *ptsF*) or expressed it only after induction by fructose (genotype *fruC*$^+$) were nevertheless sensitive to inhibition by L-sorbose. In the present paper, we show that this inhibition is the result of the expression of the glucose-specific Enzyme II and that L-sorbose is a better substrate for this Enzyme II than it is for the fructose specific Enzyme II.

Three lines of evidence show that L-sorbose is a substrate of the Enzyme II specified by the gene *ptsG*. First, the growth on acetate minimal medium [9] plates, after replica-plating from nutrient agar, of stain HK743 (Table I) was inhibited by 10 mM L-sorbose whereas the growth of strain HK742 and HK737 was not. Since these strains, being constructed by phage P1-mediated transduction [10], are isogenic except for the genes indicated in Table I, the constitutive expression of the *ptsG* gene (*umgC*) is required for the cell to be sensitive to L-sorbose. Secondly, a spontaneous sorbose-resistant mutant of strain HK743 was isolated. This strain (AS1) has lost the ability to grow with glucose as sole carbon source and could not accumulte ^{14}C when incubated with methyl α-D-[^{14}C]glucoside [11]. Bacteriophage P1-mediated transduction [10] showed that the lesion was approx. 15% cotransducible with the gene *pyrC*, as expected for a mutation in the gene *ptsG* [12]. This excludes the possibility that L-sorbose resistance is the effect of a hitherto unrecognised gene linked to the gene *ptsG*. Thirdly, suspensions of cells that constitutively form the glucose Enzyme II (genotype *umgC*) took up 0.1 mM L-[^{14}C]sorbose; this uptake

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TABLE I

STRAINS OF *E. COLI* USED, AND THEIR SENSITIVITY TO L-SORBOSE DURING GROWTH ON ACETATE

The following symbols indicate presence (+) or absence (–) of function: *ptsF* = Enzyme II activity for fructose; *fruC* = inducibility of PtsF activity; *ptsG* = principal Enzyme II activity for glucose; *umgC* = inducibility of PtsG activity; *ptsM* = secondary Enzyme II activity for glucose. N.T = not tested; I = inducible; C = constitutive; S = sensitive; R = resistant.

Strain	Genotype					Phenotype		
	ptsF	*fruC*	*ptsG*	*umgC*	*ptsM*	PtsF	PtsG	L-Sorbose sensitivity
HK 737	+	+	–	–	–	I	–	R
HK 742	+	+	+	+	–	I	I	R
HK 743	+	+	+	–	–	I	C	S
KN 6	+	+	+	+	–	I	I	N.T.
AS 1	+	+	–	–	–	I	–	R
JM 2086	+	–	–	–	–	C	–	S
C600Xt1^{S}	+	–	+	+	+	C	I	S

of sorbose was virtually abolished by the presence of non-radioactive glucose. Moreover, when the uptake of [^{14}C]sorbose had reached its steady-state level, the addition of unlabelled glucose led to a rapid loss of the ^{14}C that had been taken up (Fig. 1). This result further showed that, although a phosphorylated derivative of L-[^{14}C]sorbose had presumably accumulated in the cells, it was not further metabolised but could be displaced from the cells, as can other such non-catabolisable analogues of glucose, by glucose taken up through the *ptsG*-specified system [13]. Thus the *ptsG*-specified system, like the *ptsM*-specified system [14], can phosphorylate ketoses as well as aldoses.

The kinetic parameters of L-sorbose phosphorylation by the *ptsG*-specified Enzyme II were determined using glucose-grown cells of strain HK743 rendered permeable with toluene [15]. Experimental points were fitted to an hyperbola [16] with the aid of an IBM370 computer. The apparent K_m was 0.08 ± 0.03 mM and the apparent V 31.8 ± 3.5 nmol (mg dry mass)$^{-1}$ · min^{-1}. These values may be compared with those for glucose phosphorylation by this system: glucose has a V of approx. 100 nmol (mg dry mass)$^{-1}$ · min^{-1} and a K_m of less than 0.01 mM (Hunter, I.S., personal communication).

The kinetic parameters of L-sorbose phosphorylation by the *ptsF*-specified Enzyme II were similarly determined by using fructose-grown cells of the *ptsG*-strain HK737. The apparent K_m was 29.8 ± 2.7 mM and the apparent V 20.2 ± 0.8 nmol (mg dry mass)$^{-1}$ · min^{-1}. The corresponding values for fructose (calculated from Fig. 5 of Ref. 8) were 0.04 mM and approx. 50 nmol (mg dry mass)$^{-1}$ · min^{-1}. This

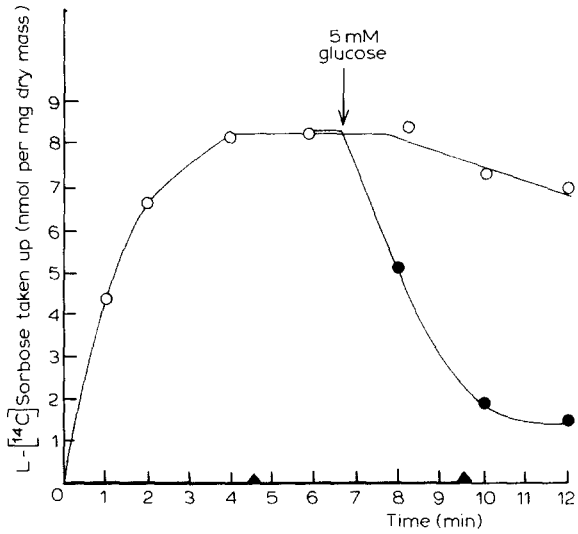

Fig. 1. Effect of unlabelled glucose on the retention of [^{14}C]sorbose taken up by *E. coli*. To each of two cultures of acetate-grown HK743 (0.4 mg dry mass · ml^{-1}; 37°C) was added L-[^{14}C]sorbose (200 μM; 0.6 μCi); samples (0.1 ml) were taken as indicated and their radioactivity measured as described in [8,17]. Between minutes 6 and 7, glucose to 5 mM was added to one flask (●) and an equal volume of phosphate buffer to the other (○). For comparison, [^{14}C]-sorbose was also added (▲) to an acetate-grown culture of strain HK 737 (genotype *ptsG umgC*).

very high value for the K_m for sorbose might suggest that our samples of this hexose are not themselves substrates for the phosphotransferase system, but might contain an impurity that can be phosphorylated. Two lines of evidence show that this is not the case. First, a sorbose-sensitive derivative of strain HK737 was constructed by phage P1-mediated transduction using strain C600Xt1^s [7] as the donor. This strain (JM2086) was of genotype *ptsF*$^+$ *fruC*. However, sorbose-resistant mutants derived from it had lost the ability to grow with fructose as sole carbon source. This result not only confirms the finding of Reiner [7] that, under some circumstances, the acquisition of tolerance to L-sorbose may be achieved by mutation of the gene *ptsF* but also implicates the fructose phosphotransferase in the response of our strains of organism to our sample of L-sorbose. Secondly, neither the uptake of glucose nor that of fructose, measured as in [8,17], was significantly inhibited by a 400-fold excess of L-sorbose. This shows that neither of these sugars constitutes a significant impurity in the L-sorbose, and suggests that the K_m of the *ptsG*-specified system for glucose must be considerably lower than 0.01 mM.

Although we confirm the previous report [7] that L-sorbose is a substrate for the fructose phosphotransferase of *E. coli* K-12, it is a very poor one; our present evidence shows that it is transported and phosphorylated much better by the glucose phosphotransferase specified by the gene *ptsG*.

We thank Dr. P.J.F. Henderson, Mr. A.J.S. Macpherson and Mrs. K. Brown for helpful advice, Drs. A.M. Reiner and K. Nyberg for the gift of bacterial strains and The Science Research Council for support under Grant No. GR/A22482.

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