INVOLVEMENT OF THE PHOSPHOTRANSFERASE SYSTEM IN GALACTOSE TRANSPORT IN *SALMONELLA TYPHIMURIUM*

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1. Introduction

At least four different transport systems for galactose are known in *Escherichia coli* [1]. Two of those, TMG I and TMG II, are the lactose and melibiose transport systems, respectively, while a third, the β-methylgalactoside system, involves the galactose binding protein and is induced by D-fucose. The fourth system, the galactose permease, seems to be rather specific for galactose (and glucose) as compared to the first three systems. In all cases galactose is accumulated as the free sugar and the first step in its metabolism is a phosphorylation to galactose-1-phosphate, catalyzed by galactokinase.

It has been reported that galactose can also be phosphorylated in vitro to galactose 6-phosphate [2,3]. This reaction is catalyzed by the phosphoenolpyruvate-dependent phosphotransferase system (PTS)*. Since no pathway for galactose 6-phosphate metabolism is known in *E. coli*, it is not clear whether this reaction plays a role in galactose metabolism.

I here report data that suggest that the membrane-bound Enzyme II of the PTS is able to catalyze the transport of galactose in a strain of *Salmonella typhimurium* which lacks all other galactose transport systems [4,5]. The soluble components of the PTS, Enzyme I and HPr, are not involved in this process.

*Abbreviations: PTS, phosphoenolpyruvate: sugar phosphotransferase system; TMG, thiomethylgalactoside; man, mannose; αMG, α-methylglucoside.

2. Methods

All strains were derived by diethylsulfate mutagenesis from *Salmonella typhimurium* SB 3509 *(trpB223)*. Cells were grown in a minimal salts medium containing 0.2% carbon source and 20 μg tryptophane per ml, and harvested as described in [6]. After breaking cells by passing them through a French pressure cell [6], membranes and high-speed supernatant were obtained by centrifuging for 60 min at 200 000 *g*. PTS and galactokinase activity were determined as described in [6] and [7] using 5 mM ^{14}C-labelled sugar. The high-speed supernatant of extracts of SB 2226 *(ptsH38 trpB223)* and SB 1690 *(ptsI34 trpB223)* were used as a source of Enzyme I and HPr, respectively, while membranes of SB 2950 *(trzA-ptsHI-crr49 trpB223)* were used as a source of Enzyme II [6]. Oxygen consumption was measured with a Clark-type oxygen electrode. Generation times were measured as described in [8], using 1% carbon source.

3. Results

Since a possible contribution of the PTS to galactose transport in *S. typhimurium* will be obscured by the presence of the three other known systems (*S. typhimurium* lacks TMG I) experiments were done with a point mutant, *mem-1*. As will be shown elsewhere [5], this mutant lacks a large number of membrane-bound proteins, including the Enzymes II of the PTS, the melibiose transport system (TMG II), the β-methylgalactoside transport system and the

Table 1
Growth rates of mutant strains on mannose and galactose

Strain	Relevant genotype	Generation time (min)	
		Mannose	Galactose
SB 3509	*trpB223*	48	54
SB 2138	*trpB223 mem-1*	>600	>600
SB 3605	*trpB223 mem-1 man⁺-24*	79	144
PP 135	*trpB223 mem-1 man⁺-24 pts I401*	>600	129

Cells were pregrown in 0.2% lactate. Growth on 1% mannose or 1% galactose was followed by measuring the optical density at 600 nm.

specific galactose permease. The defect is possibly due to an impaired integration of a number of membrane proteins [9].

The mutant, *mem-1*, is unable to grow on galactose (table 1) Since none of the galactose transport systems can be induced. Uptake of TMG, β-methylgalactoside or galactose is absent in either fucose or galactose induced cells [5]. The observation that introduction of the *lac* operon into *Salmonella* by means of a *F'lac o^c* episome results in a cell which can grow not only on lactose but also ferments galactose again, shows that the defect in *mem-1* is not due to impaired metabolism of galactose after transport. Table 2 shows that the membrane-bound Enzymes II of the PTS for α-methylglucoside (and glucose), mannose and fructose are lacking in *mem-1*, in addition to galactose phosphorylation. Specific revertants of *mem-1* can be obtained which can grow only on mannose and are different from general revertants which regain the wild-type phenotype [9]. The mannose-specific revertant, SB 3605, regained Enzyme II activity for all sugars mentioned, including galactose (table 2). As is clear from 1, a man⁺ revertant can grow also on galactose, although not at wild-type rates. Out of 34 man⁺ revertants of *mem-1* isolated, 11 were specific revertants whereas 23 were general revertants. All 11 specific man⁺ revertants were able to grow again on galactose.

It could be shown that none of the known galactose transport systems is restored in the man⁺ revertant, SB 3605. It is unable to grow on melibiose or to transport TMG and β-methylgalactoside.

We considered the possibility that Enzyme II of the PTS is able to catalyze galactose transport without concomitant phosphorylation. To test this hypothesis, a mutant which lacks Enzyme-I activity (table 3) was isolated from the man⁺ revertant. It is shown in table 1

Table 2
Membrane-bound PTS activity in various mutants

Strain	Relevant genotype	Phosphorylation by membranes			
		αMG	mannose	fructose	galactose
SB 3509	*trpB223*	53	122	78	51
SB 2138	*trpB223 mem-1*	2.5	5	3	1
SB 3605	*trpB223 mem-1 man⁺-24*	25	60	37	–
SB 3606	*trpB223 mem-1 man⁺-25*	39	58	43	32

Cells were grown in minimal salts medium containing 0.2% lactate. Membranes were prepared as described in Methods. PTS assays were performed with [^{14}C]sugars as substrate and phosphoenolpyruvate (PEP) as phosphate donor, in the presence of excess HPr and Enzyme I. Specific activity is expressed as nmoles sugar phosphorylated/min per mg protein at 37°C.

Table 3
HPr and Enzyme I activity

Strain	HPr	Enzyme I
SB 2138	35	20
SB 3605	31	20
PP 135	29	>0.1

HPr and Enzyme I activity were measured in the high-speed supernatant of lactate-grown cells in the presence of an excess of the other component. Membranes of SB 2950 were added as a source of Enzyme II. As a substrate 5 mM [^{14}C]αMG was used. Specific activity is expressed as nmoles αMG phosphorylated/min per mg protein at 37°C.

that this mutant, PP 135, is now impaired in its growth on mannose (and also glucose and fructose) but that it retains its ability to grow on galactose. This result demonstrates that in this case Enzyme I-dependent phosphorylation is not obligatorily linked to the translocation of galactose.

Another indication that galactose is able to cross the membrane comes from induction studies. While it is impossible to induce galactokinase in *mem-1* [5], the man$^+$ revertant and the Enzyme I mutant derived from it can synthesize galactokinase again (table 4).

Table 4 shows also the oxidation of galactose by various strains. The results parallel the growth studies. Consistent with the galactose being transported by Enzyme II is the observation that α-methylglucoside and glucose (in PP 135) inhibit galactose oxidation in the man$^+$ revertants but have no effect on the wild type. This observation eliminates the possibility that also the galactose permease has been restored in the man$^+$ revertant. Whereas 10 mM αMG inhibits in the wild type the transport of 0.1 mM galactose to about 20% (data not shown) and has no effect on galactose oxidation (table 4), αMG does inhibit galactose oxidation in the man$^+$ revertant. Rotman et al. [1] have similarly found that αMG does not inhibit the galactose permease. The affinity of galactose for Enzyme II is very low, the apparent K_M for oxidation being about 10 mM. These results are also found when uptake of ^{14}C-labelled galactose is measured (not shown). On the other hand, the K_M for galactose uptake in both wild type and galactose-specific revertants (which regain the specific galactose permease [9]) is in the order of 0.2 mM, similar to the value found by Rotman et al. [1].

Table 4
Galactose oxidation and galactokinase in various mutants

Strain	Oxygen uptake			Galactokinase
	Glucose	Galactose		
		−αMG	+αMG	
SB 3509	0.32	0.27 (0.27)	0.27	400
SB 2138	0.07	0.005(0.01)	–	33
SB 3605	0.21	0.06 (0.09)	0.02	157
PP 135	0.005	0.12 (0.29)	0.025	164

Cells were grown in minimal salts medium containing 0.2% lactate *plus* 0.2% galactose. Oxygen consumption was measured in a medium containing 50 mM potassium phosphate buffer, pH 7.1 6 mM glucose (saturating) and 6 mM galactose were used as substrates, except in the case of SB 3605, 18 mM galactose. The values between brackets are the maximal oxidation rates at saturating galactose concentration. If present, 18 mM αMG was used except with SB 3605, 24 mM αMG. The activity is expressed in natoms 0 taken up/min per mg dry weight at 25°C. Galactokinase activity was determined in the high-speed supernatant. Specific activity is expressed in nmoles galactose phosphorylated/min per mg protein at 37°C.

4. Discussion

Galactose transport in *E. coli* and *S. typhimurium* can be catalyzed by a number of systems. The common characteristic is the accumulation of free galactose and its subsequent phosphorylation to galactose 1-phosphate, catalyzed by galactokinase [1,10]. We have examined the possibility that the PTS is involved in galactose transport and metabolism in a mutant of *S. typhimurium* which lacks all known galactose-transport systems. It is known that isolated membranes are capable of phosphorylating galactose to galactose 6-phosphate, a reaction dependent on the soluble PTS enzymes, Enzyme I and HPr, and PEP [2,3]. No catabolic pathway is known for galactose 6-phosphate in *E. coli* and *S. typhimurium*.

Two conclusions can be drawn from the results presented here.

(i) The Enzyme II system of the PTS is able to catalyze the transport of galactose in cells which have lost all other (known) galactose-transport systems. Although it is likely that the man$^+$ revertant has regained Enzyme II-B (since activities for α-methyl-glucoside, fructose and mannose return at the same time and Enzyme II-B is thought to be common to all three sugars [11]) we do not know whether any of the II-A enzymes (cf. [7]) is involved.

(ii) This transport is not coupled to PTS-mediated phosphorylation of galactose, since further loss of Enzyme I has no effect on galactose metabolism, and probably represents facilitated diffusion.

Although it could be argued that the specific mannose revertant regains also one of the known galactose transport systems, this is unlikely for the following reasons:

(i) *All* specific mannose revertants can grow again on galactose. It is very unlikely that in all cases two mutations occur.

(ii) No TMG or β-methylgalactoside transport can be observed in the man$^+$ revertant. Galactose transport has about a hundred-fold lower affinity in the man$^+$ revertant compared to the wild type.

The man$^+$ revertant lacking in Enzyme I (PP 135), was unable to utilize PTS sugars such as glucose, fructose, and mannose. This is an expected result, since the PTS is required for both the translocation and phosphorylation of these sugars [11]. An unanswered question in the mechanism of action of the PTS, however, is whether or not the sugar-specific Enzyme II complexes are capable of catalyzing facilitated diffusion in the absence of functional Enzyme I or HPr. If the rate of such process is sufficient and an alternate mechanism exists for phosphorylation of the sugar internally, then sugar utilization could occur independent of Enzyme I and/or HPr. The published data on this question do not clearly answer this question. In *Staphylococcus aureus*, the rate of facilitated diffusion of a lactose analogue in an Enzyme I mutant was very low [12] or negligible. However, the same mutant, which was unable to ferment a large number of sugars showed slow fermentation on glucose. Similarly, a *S. typhimurium* Enzyme I mutant unable to grow on a number of PTS sugars did grow slowly on high concentrations of glucose, and a similar mutant containing elevated levels of a mannofructokinase (which also phosphorylates glucose) grew on all three PTS sugars [13]. These results suggest that at least some PTS sugars can cross the membrane by a facilitated diffusion process, catalyzed by the corresponding Enzyme II complexes, and this is suggested to be the route by which galactose is taken up in the present experiments. The affinity of the Enzyme II complex for galactose is low, but at high concentrations of the sugar the rate of uptake in conjunction with the elevated levels of the inducible galactokinase permit growth on this sugar. If this conclusion is substantiated, it is important in that it shows that a portion of the PTS can be used, the sugar-specific proteins, independent of Enzyme I and HPr, for the uptake of sugars.

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