PtsX: A GENE INVOLVED IN THE UPTAKE OF GLUCOSE AND FRUCTOSE BY *ESCHERICHIA COLI*

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1. Introduction

The uptake by *Escherichia coli* of a variety of carbohydrates necessarily involves the activity of a PEP-dependent phosphotransferase (PT) system [1], containing a number of components. Two of these, a small, histidine-containing protein (HPr) and an enzyme (Enzyme I) that catalyses the transfer of phosphate from PEP to HPr, are required for the utilization of all carbohydrates that are taken up via the PT-system [2–6]. In contrast, the membrane-linked multicomponent fractions that effect the transfer of phosphate from the phosphorylated HPr to sugars (collectively termed Enzymes II) are generally considered to be specific for individual sugars, although the uptake of any one sugar may involve the functioning of more than one Enzyme II. Thus, for example, the uptake by *E. coli* of fructose at low concentration (< 2 mM) involves predominantly an Enzyme II, specified by the *ptsF* gene (located at min. 41 on the *E. coli* genome) that catalyses the formation of fructose-1-phosphate, whereas at higher concentrations (> 2 mM) fructose is phosphorylated also to fructose 6-phosphate by the action of a different Enzyme II, specified by the *ptsX* gene (located at min. 35.5 on the *E. coli* genome) [7–10].

It is the main purpose of this paper to present evidence that the Enzyme II specified by the *ptsX* gene is involved also in the uptake and phosphorylation of glucose. Mutants of *E. coli* devoid of an Enzyme II that catalyses the phosphorylation of methyl-α-glucoside, specified by the *umg* gene (located at min. 24 on the *E. coli* genome, [11]), are markedly impaired in glucose utilization but still grow slowly on that sugar: we here show that *umg ptsX* double mutants do not. It is thus likely that the *ptsX* marker corresponds to that designated *gptB* [12,13], which specifies a second Enzyme II for glucose and was first described by Curtis and Epstein [12].

2. Experimental

Cultures of *E. coli* were grown, and their growth was measured, as previously described [14]. The incorporation of [^{14}C]glucose by cultures growing on a mixture of glucose and some other carbon source was determined as also previously described [15,16]. The genetical methods employed were those compiled by Miller [17]. Strains of *E. coli* used are listed in table 1.

3. Results

When cultures of *E. coli*, grown either on sugars taken up via the PT-system (such as fructose) or on carbohydrates not taken up via that system (such as lactose) are suspended in growth media containing that carbon source and also containing glucose, the subsequent growth of the organisms occurs largely at the expense of the glucose. This preferential use of glucose is not seen with mutants that lack an Enzyme II for the uptake (and phosphorylation) of methyl-α-glucoside (and hence designated Umg$^-$) [15,16,18], which implies that the entry of glucose into the metabolic routes of *E. coli* is effected largely via this Umg-system. This behaviour is illustrated in fig.1. A culture of strain K2.1t incorporated approx. 3 μmol

Table 1
Strains of *E. coli* used in this work

Organism	Genetic markers	Mating type	Reference and source
KL16.21	*ptsF ptsX kdgR*^{c}	Hfr	[7]
KL16.23	*ptsF*	Hfr	[9]
K2.1t	*his argH thr leu pps str*	F^{-}	[19]
K2.1.22a	*his argH thr leu pps umg str*	F^{-}	[11]
K2.21.22	*his argH thr leu ptsX umg str*	F^{-}	[KL16.21 × K2.1.22a]Pps^{+} Str^{R}.

of carbon from glucose into cell components per increase in dry mass of 1 mg. when the glycerol-grown cells continued to grow on a mixture of 20 mM-glycerol and 5 mM[^{14}C]glucose. In contrast, the mutant K2.1.22a derived from it [11], which differed from K2.1t in lacking Umg-activity, incorporated only 0.8 μmol of glucose carbon per increase in dry mass of 1 mg. It thus appears that some 70% of the glucose

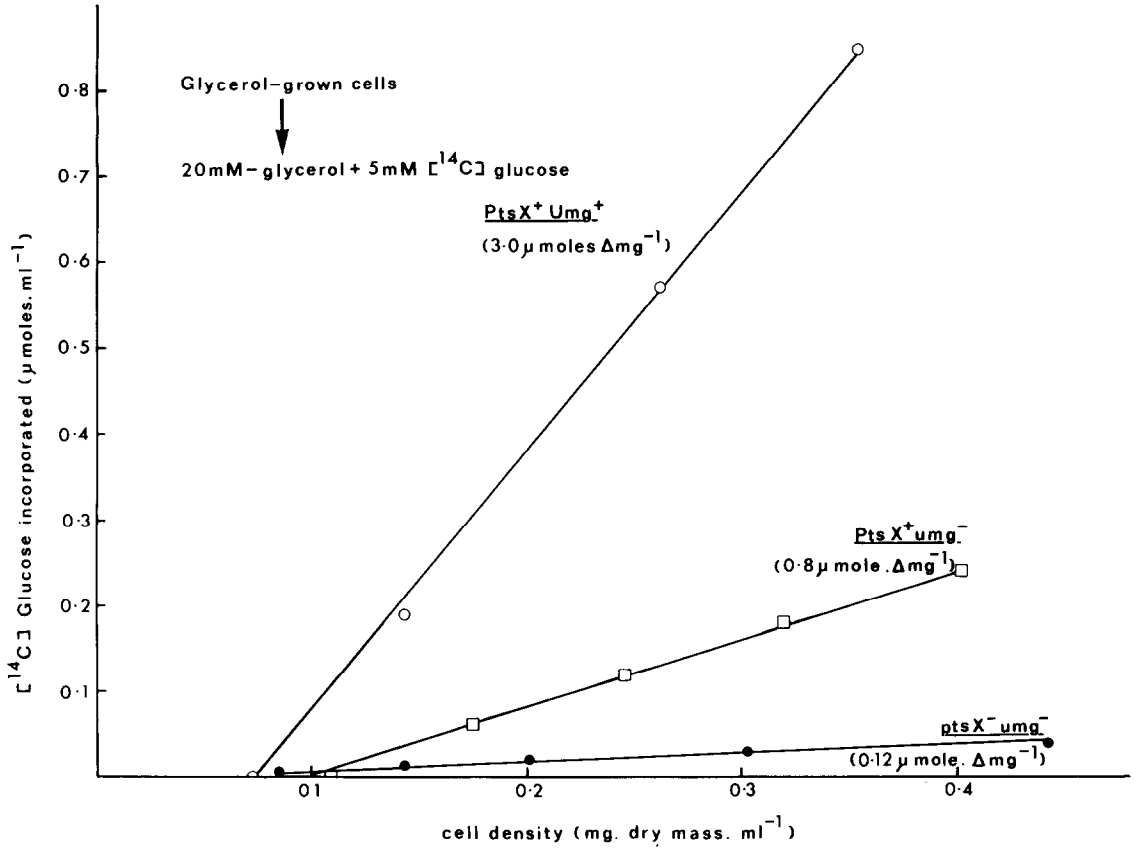

Fig.1. Incorporation of [^{14}C]glucose by strains of *E. coli* growing on 20 mM-glycerol plus 5 mM[^{14}C]glucose. The organisms used were (○) K2.1t, (□) K2.1.22a, and (●) K2.21.22. Samples (0.2 ml) of the growing cultures were filtered through Millipore filters and washed. The radioactivity of the cells was assayed as previously described [22].

that is utilized by Umg$^+$-organisms under these conditions of growth enters the cells via the Umg-system. That the majority of the glucose still taken up by Umg$^-$-mutants enters via the PtsX-system is also shown in fig.1: the *ptsX umg-* double mutant K2.21.22 grew readily on the mixture of glycerol and [^{14}C] glucose but incorporated into cell components only 0.12 μmol of glucose carbon per increase in dry mass of 1 mg, which is but 4% of the amount incorporated by its wild-type ancestor.

These differences in the proportionate utilization of glucose are reflected also in the rates at which these organisms grow at 37°C aerobically on media containing 10 mM-glucose as sole carbon source (fig.2). Whereas strain K2.1t doubles every hour under these conditions, the Umg$^-$-mutant takes approx. 3 hr to double, and the Umg$^-$ PtsX$^-$-double mutant requires over 18 hr: indeed, growth of the *ptsX umg*-mutant on plates of this medium solidified with agar is so poor that it can, by this property, be readily distinguished from *ptsX*$^+$ *umg*-mutants.

The use of glucosamine as an alternative carbon source to screen for *ptsX umg*-mutants was suggested by the work of S. J. Curtis and W. Epstein, which was generously communicated to us, prior to publication, by Dr Epstein. These workers found that two genes were involved in glucose uptake. One (*gptA*) corresponds to *umg*; the other, originally designated *gptB* [12,13] and now termed *mpt* (for mannose phosphotransferase), specifies an Enzyme II that catalyses the phosphorylation of a number of hexoses including glucose, glucosamine and mannose. The *gptB* (*mpt*) marker was found to be co-transducible with *kga*. Since *ptsX* has similarly been found to be co-transducible with this marker [10], it is likely that these genes correspond to each other. This conclusion is strengthened by the observation (fig.3) that the *ptsF ptsX*-double mutant KL16.21 does not grow on glucosamine, and grows poorly on mannose, although both these carbon sources are utilized by the related organisms KL16.23 (*ptsF* PtsX$^+$) and KL16 (PtsX$^+$ PtsF$^+$). Similarly, *ptsX umg*-double mutants do not grow on glucosamine, although PtsX$^+$ *umg* mutants do.

This inability of the Umg$^+$-strain KL16.21 to utilize glucosamine as sole carbon source appears to be associated with the inducibility of the Umg-system. Strains that form the Enzyme II specified by the *umg*$^+$-gene constitutively [20,21] grow on glucosamine even when they are *ptsX*, strains that (like KL16.21) form the Umg-system iducibly, do not. Moreover when glucose-grown cultures of strain KL16.21 (in which the Umg-system has therefore been induced) are transferred to medium containing glucosamine as carbon source, growth begins but continues at a steadily decreasing rate. This suggests that glucosamine,

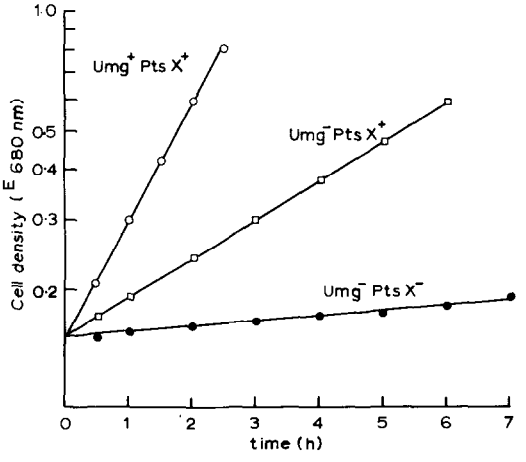

Fig.2. Aerobic growth at 37°C of *E. coli* strains (○) K2.1t, (□) K2.1.22a and (●) K2.21.22 on medium containing 10 mM-glucose as sole carbon source. Cell density was measured as previously described [14].

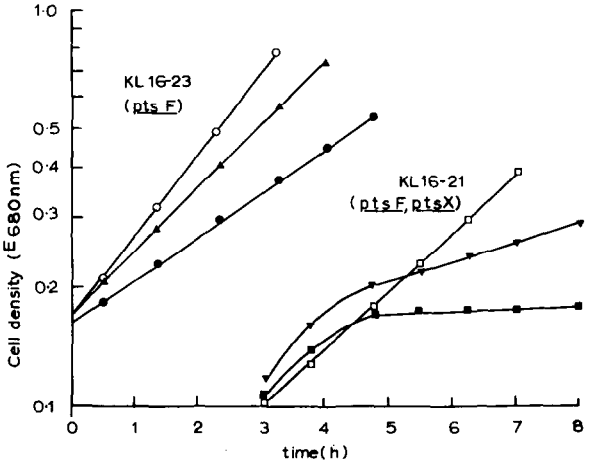

Fig.3. Growth of *E. coli* strains KL16.23 (*ptsF*) and KL16.21 (*ptsF ptsX*) on media containing (○,□) 20 mM glycerol, (▲,▼) 10 mM mannose, and (●,■) 10 mM glucosamine as sole carbon source.

which can be transported by the Umg-system, can neither induce that system nor maintain its induction.

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