The roles of HPr and FPr in the utilization of fructose by *Escherichia coli*

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A mutant impaired in FPr activity was isolated. The altered gene (*fpr*), which was located near min. 44 on the *E. coli* genome, was transferred by phage-mediated transduction to appropriate recipients that lack HPr (*ptsH*), or Enzyme II^{man} (*ptsM*), or neither. The rates of growth on fructose of such transductants indicate that phosphate from PEP is transferred predominantly via FPr to fructose that enters the cells by Enzyme II^{fru}, but that HPr can play a role in transferring phosphate to fructose taken up via Enzyme II^{man}

(E.coli) *Fructose transport* *PT-system* *FPr* *HPr* *Enzyme II^{fru}* *Enzyme II^{man}*

1. INTRODUCTION

The PEP-dependent phosphotransferase (PT-) system effects the uptake and concomitant phosphorylation of a number of carbohydrates by *E. coli*. This system contains components specific to individual substrates, and elements common to all. Among the former are the membrane-bound Enzymes II and membrane-associated factors III; among the latter are a small, histidine-containing carrier protein HPr and the Enzyme I that catalyses the transfer to it of phosphate from PEP (for review, see [1]). As expected from this arrangement, mutants that lack Enzyme I activity (*ptsI*) do not grow on any PT-sugar; similarly, mutants that lack HPr (*ptsH*) would not be expected to grow on all these substrates. However, this latter expectation is not fulfilled: whereas *ptsH* mutants indeed do not grow on a wide range of PT-sugars, they will grow readily on fructose [2,3]. This indicates that a protein other than HPr acts as a carrier of phosphate between the phosphorylated Enzyme I and the Enzyme II^{fru} that is specified by *ptsF* [4,5]. Indeed, Waygood et al. [6] have provided evidence that such a protein (FPr) exists and can be phosphorylated by PEP in vivo. However, it is not known whether HPr can also play a role in fructose utilization, and what are the relative contributions of HPr and FPr to this process. It is the purpose of this paper to describe the isolation and properties of a mutant impaired in FPr and its use to answer these questions.

This paper is dedicated to S. Prakash Datta

2. MATERIALS AND METHODS

All organisms used were derivatives of *E. coli* K12. Relevant genetic markers are listed in table 1. The procedures used for the growth of bacteria, for measurements of doubling times and for phage P1-mediated transductions, were as previously reported [7]. Other experimental details are given in the table legends or in the text.

3. RESULTS AND DISCUSSION

3.1. *Isolation of* fpr *mutants*

The mutant HK 876 carries the marker *fruC* and thus forms constitutively [8] the components of the fructose uptake system; these are Enzyme II^{fru} specified by *ptsF* and FPr, specified by *fpr*, as well as fructose-1-phosphate kinase, specified by *fpk*. This has two consequences. In the first place, it

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Table 1

Strains of *E. coli* used in this work

Strain	Relevant genotype
HK 803	*ptsH1 trp his argHBCE thr leu*
HK 876	*fruC ptsH2 ptsM Tn10::zee argHBCE thr leu*
HK 881	*fruC ptsH2 ptsM Tn10::zee fpr argHBCE thr leu*
HK 1011	*uhpC trp his argHBCE thr leu*
HK 1013	*uhpC ptsM Tn10::zee fpr argHBCE thr leu cysA*
HK 1016	*uhpC Tn10::zee fpr trp his argHBCE thr leu*
HK 1038	*fpr trp his argHBCE thr leu cysA*
HK 1040	*fpr ptsH1 trp his argHBCE thr leu*

renders the organism susceptible to inhibition of growth by xylitol, since this pentitol is taken up and phosphorylated by the fructose-specific components of the PT-system, but the xylitol phosphate formed cannot be catabolized. Secondly, although this mutant lacks the HPr that is normally required for the uptake of sugars such as glucitol and glucose, the over-production of FPr in this *ptsH fruC* strain enables the cells to grow on these sugars, albeit somewhat more slowly than do *ptsH*$^+$ strains of *E. coli*.

When a culture of strain HK 876 was plated on 2% (w/v) casein hydrolysate agar supplemented with 5 mM xylitol, isolated colonies appeared after 2 days at 37°C. These xylitol-resistant mutants were likely to be of two main types. They either would not take up xylitol because they were impaired in the fructose (and xylitol)-specific Enzyme II^{fru} [8], or they would be impaired in FPr activity. In the former case, they would still form FPr constitutively and would thus still grow upon sugars taken up via other Enzymes II, such as glucitol or glucose; in the latter case, the uptake of all PT-sugars would now no longer occur and such *fpr* mutants would be expected to be pleiotropically affected in sugar utilization. After screening the xylitol-resistant mutants, one organism (HK 881) with the latter properties was chosen for further study.

3.2. *Characterization of the* fpr *mutant*

In order to confirm that the mutant HK 881 was impaired in FPr function, cultures of it, and of its *fpr*$^+$ parent HK 876, were grown on nutrient broth overnight and tested for their ability to catalyse the formation of [^{14}C]fructose phosphate from [^{14}C]fructose and PEP. Whereas the extract from strain HK 876 readily catalysed this phosphorylation of fructose, the extract derived from strain

Table 2

PEP-dependent phosphorylation of fructose by extracts of *E. coli*

Components of assay mixture			[^{14}C]Fructose phosphate formed (nmol·min^{-1}·ml^{-1})
Cytosol from	Membrane from	Other	
HK 876	HK 876	–	3.9
HK 876	HK 881	–	4.0
HK 881	HK 881	–	0.1
HK 881	HK 876	–	0.2
HK 881	HK 881	FPr	4.2

E. coli strains HK 876 and its xylitol-resistant derivative HK 881 were grown overnight at 37°C, each in 100 ml of double-strength nutrient broth. The cells were harvested, washed, suspended in 50 mM sodium/potassium phosphate buffer, pH 7.2, at 50 mg dry mass·ml^{-1}, and sonicated on ice 8 times in 15-s bursts at 1 min intervals in an MSE sonic oscillator. The extracts were centrifuged lightly (800 × *g* for 5 min) at 4°C to remove cell debris and then at 100000 × *g* for 2 h. The supernatant (cytosol) fraction, containing approx. 12 mg protein·ml^{-1}, was decanted from the pelleted (membrane) fraction; this latter, containing approx. 8 mg protein·ml^{-1}, was washed with the phosphate buffer and resuspended in the original volume of that buffer. Assays of PEP-dependent phosphorylation of [^{14}C]-fructose were performed by the method of Waygood et al. [6], 0.1–0.2 mg protein being used for each assay, and extra Enzyme I being added to all incubation tubes as recommended by these authors. When appropriate, 10 μg of partially purified FPr was also added

HK 881 did not, even when supplemented with a membrane fraction derived from HK 876 and known to be rich in Enzyme II^{fru} activity (table 2). This shows that the mutant HK 881 was not affected in Enzyme II^{fru} but lacked a component of the cytosol (FPr and/or factor III^{fru}) that was necessary for fructose phosphorylation. Since the mutant HK 881 was impaired in its growth on a variety of sugars that, in this *ptsH* background, would necessitate high FPr activity, it was likely that the missing component is FPr. Indeed, the addition to the HK 881 extract of a protein fraction, derived from wild-type *E. coli*, that was enriched in FPr and possibly factor III^{fru} (a gift from Dr E.B. Waygood), restored its ability to phosphorylate fructose.

3.3. *Location of* fpr *on the chromosome*

In order to locate the affected gene on the *E. coli* chromosome, phage P1 were grown on the mutant HK 881 and used to infect a recipient strain, HK 1011. In addition to the altered *fpr* gene, the donor strain carried the transposon Tn10 integrated at min. 44 into the genome (*Tn10::zee*); transductants were therefore selected on nutrient agar supplemented with tetracyclin ($10\ \mu g \cdot ml^{-1}$). Of 48 such transductants screened, 20 were significantly impaired in their rates of growth on agar plates containing 2.5 mM fructose as sole carbon source, although growth on glucitol, glucose and gluconate was normal. One such transductant (HK 1016) was grown overnight at 37°C in liquid medium containing 10 mM fructose or 10 mM fructose 1-phosphate as carbon sources. As illustrated in fig.1, growth on glucose was rapid, after a short lag. Growth on fructose 1-phosphate was also rapid as the strain HK 1011 carried the *uhpC* marker that permits the entry and utilization of fructose 1-phosphate [9]. It is therefore evident that HPr function, and fructose-1-phosphate kinase activity, were normal in the transductant. However, growth on fructose was severely retarded: the doubling time was 210 ± 10 min (4 experiments) instead of the approx. 65 min usually observed with wild-type *E. coli*. The *fpr* gene had thus been transferred concomitantly with *Tn10::zee* (approx. 40% linkage) and was therefore located close to the *ptsF* and *fpk* genes at min. 44.5 on the genome of *E. coli* [10].

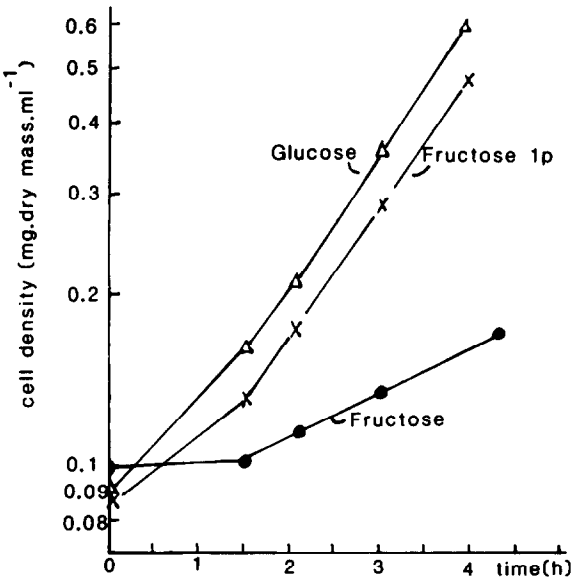

Fig.1. Growth of the *E. coli* mutant strain HK 1016 (*uhpC fpr*) at 37°C on media containing 10 mM glucose (Δ), fructose (●) or fructose 1-phosphate (×) as sole carbon source.

3.4. *Relative roles of HPr and FPr in fructose uptake*

Although the xylitol-resistant mutant HK 881 was unable to grow upon fructose, the transductant HK 1016 derived from it grew slowly on this hexose (fig.1). This might indicate either that HK 881 was a multiple mutant from which only one lesion had been transferred to the phage recipient HK 1011 or (since HK 876 was *ptsM* but HK 1011 was $ptsM^+$) it might suggest that part of

Table 3

Effect of mutations in HPr or FPr on the growth of *E. coli* at 37°C

Strain	Relevant genotype	Doubling time (min) on			
		Glucose	Glucitol	Fructose	Gluconate
HK 640	–	60	60	65	60
HK 803	*ptsH*	>500	>500	70	60
HK 1038	*fpr*	60	60	210	60
HK 1013	*ptsM fpr*	65	60	>500	60
HK 1040	*ptsH fpr*	>500	>500	>500	60

the fructose taken up entered the cells via the Enzyme II^{man} specified by *ptsM*$^+$ [11] using HPr as phosphate carrier. That this latter explanation is the more likely is shown by the experiments summarized in table 3. Strain HK 803 is *ptsH*: as previously reported [3], this lesion virtually abolishes growth on glucose but not on fructose. Strain HK 1038 is *fpr* but *ptsM*$^+$*ptsH*$^+$: only growth on fructose is impaired. Strain HK 1013 is *fpr ptsM*: growth on fructose is now abolished, though growth on glucose and glucitol is unimpaired. As expected, growth on all PT-sugars is virtually abolished by the simultaneous absence of HPr and FPr function, as in strain HK 1040.

The results indicate that the utilization of fructose by *E. coli* occurs by two main mechanisms: via Enzyme II^{fru} and FPr and, at fructose concentrations higher than 2.5 mM [11], also via Enzyme II^{man} and HPr. The uptake of fructose at low external concentrations of this hexose occurs predominantly by the former route.

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