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Fructose Transport by Escherichia coli

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Fructose transport by *Escherichia coli*

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The utilization of fructose by *Escherichia coli* involves, as first step, the uptake of the sugar, normally via the phosphoenolpyruvate-dependent phosphotransferase system (PTS). This fructose-specific PTS differs in several ways from that effecting the uptake of other sugars that also possess the 3,4,5-D-*arabino*-hexose configuration: these differences are discussed. Mutants that lack the genes *ptsI* and *ptsH*, which specify components of the PTS common to most PT-sugars, can mutate further to regain the ability to utilize fructose when this is present in relatively high concentration (i.e. greater than 2 mM) in the medium. Some of the properties of this unusual uptake system as discussed.

1. Introduction

Like other sugars possessing the 3,4,5-D-*arabino*-hexose configuration, D-fructose is taken up by *Escherichia coli* via the phosphoenolpyruvate-dependent phosphotransferase (PT) system (Fraenkel 1968). There are, however, several factors that distinguish the fructose-PT system from the systems that effect the uptake of, e.g. glucose, mannose, *N*-acetylglucosamine, mannitol, glucitol, or β-glucosides, and that make it valid to consider the mechanism of fructose uptake separately.

2. The role of HPr

An essential component of all glycose-PT systems, including that for fructose at concentrations greater than 2 mM, is HPr, a small histidine-containing carrier protein that effects the transfer of phosphate from phosphoenolpyruvate (PEP) to the incoming glycose. It does so by acting as carrier of a phosphate group between phosphorylated enzyme I and the relevant sugar-specific membrane-associated enzyme III or, if no separate enzyme III is involved, the enzyme III-like portion of enzyme II (figure 1).

On the other hand, the utilization of fructose, at concentrations less than 2 mM, proceeds readily in the absence of HPr. As was first shown by Saier *et al.* (1970), mutants devoid of HPr (designated *ptsH*) grow on fructose with doubling times only slightly different from those of wild-type organisms, though they do not grow on other PT-sugars. Previous growth on fructose allows *ptsH*-mutants also to utilize other PT-sugars for a while but this ability is diluted out during subsequent growth, which then ceases. Clearly, the cellular component that functions in lieu of HPr is induced by fructose but can act pleiotropically only if present in adequate cellular concentration.

It has now been established (Geerse *et al.* 1989) that, in *Salmonella typhimurium*, phosphate is transferred from PEP, via the enzyme I that is common to all PT-systems, to an HPr-like region of a 39.6 kDa protein that also serves as a fructose-specific enzyme III (figure 1). The DNA

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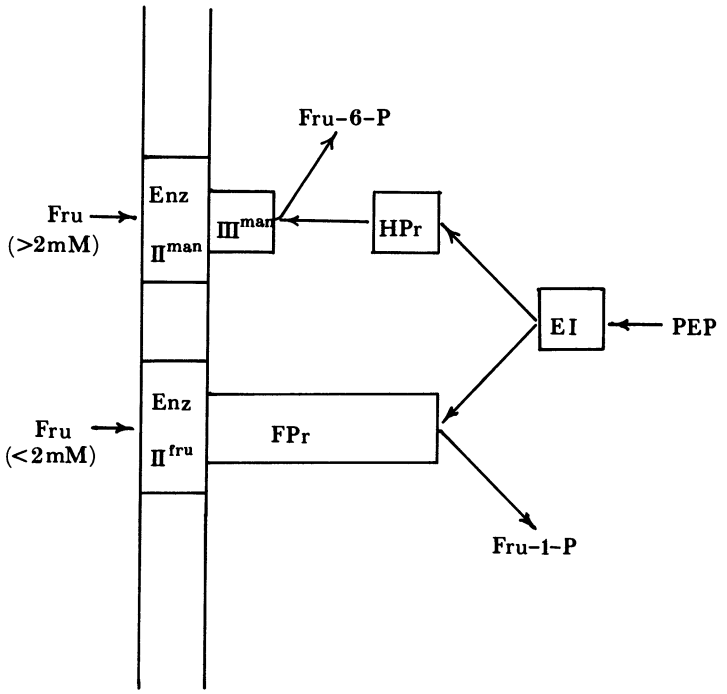

FIGURE 1. Uptake and concomitant phosphorylation of fructose mediated by the PEP-dependent phosphotransferase system. At low external concentrations of fructose (i.e. less than 2 mM), fructose readily enters *E. coli* via the membrane-spanning enzyme II^{fru} and appears inside the cell as fructose 1-phosphate; at higher external concentrations, it can also enter via enzyme II^{man} and appear inside the cell as fructose 6-phosphate. In the former route, the phosphoryl moiety of PEP is transferred to the incoming ketose via a 39.6 kDa protein FPr; in the latter, a 9 kDa protein HPr effects this transfer initially to a portion of EII^{man} that is accessible from the cytoplasmic surface of the membrane.

corresponding to this protein has been sequenced: it codes for a polypeptide of 376 amino acids, the N-terminal region of which exhibits 45% homology with the cytoplasmic domain of the membrane-spanning enzyme II for mannitol, and the C-terminal region of which is 35% homologous to the HPr both of Gram-positive and Gram-negative bacteria. These findings also suggest that the physical separation of proteins with enzyme III and with pseudo-HPr activities, and the identification of pseudo-HPr as a protein of molecular mass 8 kDa (Waygood *et al.* 1984) or 9 kDa (Sutrina *et al.* 1988), may have been the result of some proteolysis of the 39.6 kDa protein that is now designated FPr and that is specified by a gene formerly designated *fpr* (Kornberg 1986), but better designated *fruF* (Geerse *et al.* 1989).

The physiological roles of HPr and FPr as mediators of phosphate transfer between PEP and the appropriate receptor proteins are manifested by the rates at which relevant *E. coli* mutants grow on PT-sugars (table 1).

Lack of HPr abolished growth on all sugars except fructose. Growth was restored if a sufficiency of FPr was maintained by mutating the repressor that regulates the expression of the fructose operon (*fruR*; Geerse *et al.* 1986; Kornberg & Elvin 1987). The sugars tested include glucose and mannose, in which the formation of the phosphate esters involves both membrane-spanning enzymes II and separate enzymes III, and *N*-acetylglucosamine, for which the enzyme II contains a C-terminal domain that shows homology with the enzyme III for glucose (Peri & Waygood 1988), and which thus has no separate enzyme III.

TABLE 1. EFFECT OF MUTATIONS IN HPr AND IN COMPONENTS OF THE FRUCTOSE OPERON ON THE GROWTH OF *ESCHERICHIA COLI* AT 37 °C

strain	relevant genotype	doubling time (min) on glucose	mannose	*N*-acetylglucosamine	fructose
640	—	60	60	60	65
1344	*fruR*	60	60	60	60
803	*ptsH*	> 500	> 500	> 500	70
994	*ptsH fruR*	75	75	75	65
1038	*fruF*	60	65	60	205
1038 P	*fruF pfkA*	> 500	> 500	> 500	> 500
1013	*ptsM fruF*	60	> 500	60	> 500
1040	*ptsH fruF*	> 500	> 500	> 500	> 500

In contrast, *ptsH*$^+$ *fruF*-mutants grew on all PT-sugars, including fructose. However, the rates of growth on fructose were profoundly affected by the external concentration of that sugar over the range tested (2–20 mM; figure 2), which suggested that fructose entered the cells via the enzyme II for mannose (PtsM), rather than via that for fructose (FruA). It is known that the former can effect the uptake and phosphorylation of fructose to fructose 6-phosphate (Ferenci & Kornberg 1971), but that the apparent K_m for this process is greater than 2 mM (Ferenci & Kornberg 1974) (figure 1). This is supported by the finding that abolition of fructose 6-phosphate kinase activity (*pfkA*, Morissey & Fraenkel (1968, 1972); Kornberg & Smith 1970), also abolished growth on fructose at all concentrations tested. It is thus evident that under physiological conditions and in the absence of FPr, HPr cannot mediate the transfer of phosphate from enzyme I to the fructose-specific enzyme II, although (if present in adequate amounts) FPr can substitute for HPr. This apparent paradox is resolved by recognition of the dual function of the FPr protein: the *fruF* mutants of *E. coli* described by Kornberg (1986) lack both pseudo-HPr and enzyme III fructose activities, and in such mutants, HPr alone clearly cannot provide both these functions.

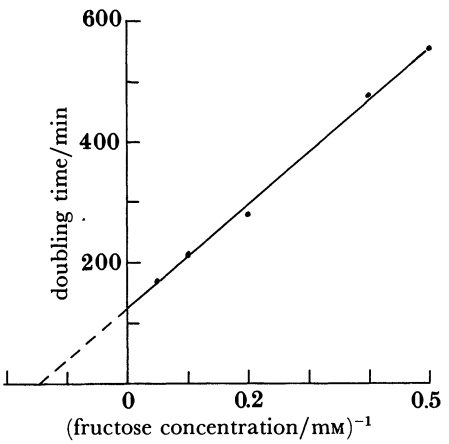

FIGURE 2. Growth of a *fruF*-mutant of *E. coli* on medium containing various concentrations of fructose as sole carbon source.

3. THE ENZYME II FOR FRUCTOSE

The enzyme II for fructose differs from other enzymes II of the PT-system in at least two major ways. One such difference is apparent from size comparisons. In general, enzymes II

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that have a built-in-enzyme III component have molecular masses that range from that for β-glucosides (66.5 kDa) to that for *N*-acetylglucosamine (68.7 kDa), with that for mannitol (67.9 kDa) in between. As might be expected, enzymes II that co-operate with separate enzymes III have smaller molecular masses, but such enzymes II fall into two broad classes. Thus the enzymes II for glucose (50.6 kDa) and for glucitol (54.0 kDa) are clearly much larger than the two membrane-spanning components of the mannose enzyme II, designated PtsM(M) (31.0 kDa) and PtsM(P) (27.6 kDa). However, the molecular masses of the respective enzymes III are in inverse relation to this; those for glucose (18.2 kDa) and glucitol (13.3 kDa) are much smaller than the two that interact with the PtsM(M) and PtsM(P), each of which has molecular mass of 35 kDa.

The fructose enzyme II fits into neither class. It has a molecular mass (calculated from the deduced amino acid sequence; Prior & Kornberg (1987)) of 57.3 kDa; the enzyme III domain of the 39.6 kDa FPr protein (of *S. typhimurium*) is calculated to be of molecular mass *ca.* 32 kDa. The remarkable uniformity of mass of all the enzymes II that have no separate enzyme III, or the sums of [enzyme II + enzyme III] of those that have, was noted by Saier *et al.* (1985, 1988) but clearly breaks down in the case of fructose. If, as seems likely, the FPr of *E. coli* is similar in size to that of *S. typhimurium*, the sum of the masses of the fructose-specific enzyme II and the enzyme III domain of FPr would amount to nearly 90 kDa, which is far out of the range of all the others (60–68.7 kDa).

A second major difference between the enzyme II for fructose and all other enzymes II of the PT-system is seen in the portion of the molecule that is presumed to encompass the membrane-spanning domain. The hydropathic profile of the protein specified by the *fruA*$^+$-gene (Prior & Kornberg 1988) (figure 3) indicates that there are at least eight well-defined alternating hydrophilic and hydrophobic regions towards the carboxy-terminal region. If the implication of this observation is correct, that it is the carboxy-terminal portion of the protein that spans the membrane and the *N*-terminal region that interacts with the cytoplasm, the enzyme II for fructose would differ fundamentally from all other enzymes II, in which it is the N-terminal regions that form the membrane-spanning domains.

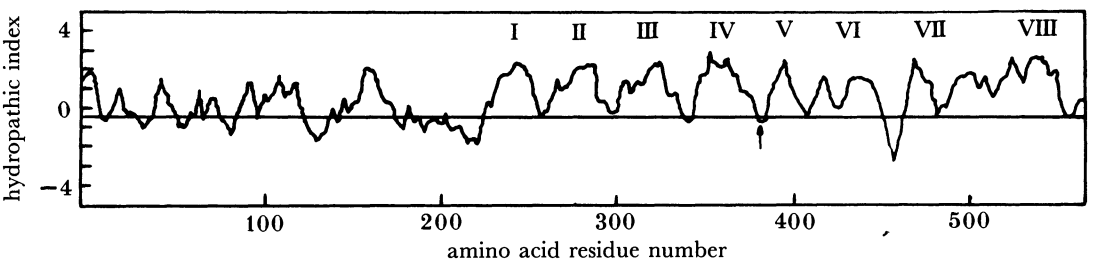

FIGURE 3. Hydropathy plot of the deduced amino acid sequence of enzyme II^{fru}. The plot was generated by using a span of 11 residues, plotted every 3 residues. The horizontal line drawn at −0.4 indicates the average hydropathy of a soluble protein. The histidine residue (381) postulated to be involved in phosphate transfer from FPr to the incoming sugar is indicated by the arrow. (Reproduced from Prior & Kornberg (1988), by permission of the editors of the *Journal of general Microbiology*.)

Comparison of the amino acid sequence of the fructose-specific enzyme II with those of several other enzymes II, such as those for mannitol, glucose, mannose, glucitol, β-glucosides and *N*-acetylglucosamine, as well as the enzymes III for glucose, mannose and glucitol, reveals very little homology and further emphasizes the apparent uniqueness of the fructose transport

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protein. Only in those regions concerned with the transfer of phosphate, which is an activity common to all enzymes II of the PT-system, does there appear to be a marked commonality of amino acid sequence. Thus there is a high degree of similarity in the regions of many enzymes II that contain a histidine residue that is probably involved in phosphorylation. The regions surrounding such histidine residues in the enzymes II for mannitol (His195), glucose (His211), and β-glucosides (His306) have been shown (Bramley & Kornberg 1987; Saier *et al.* 1988) to correlate with the region adjacent to His15 of HPr, which is known to be phosphorylated (Weigel *et al.* 1982). The region surrounding His381 of the fructose-specific enzyme II also exhibits this conservation of amino acid sequence. Marked homology is observed also with the regions that surround His309 in the enzyme II for sucrose, His190 in the enzyme II for glucitol and His189 in that for *N*-acetylglucosamine, which suggests that all these histidine residues are involved in phosphate transfer.

Analogous conclusions can be drawn from comparison of amino acid regions that flank cysteine residues thought to be functionally significant in various enzymes II (Nuoffler *et al.* 1988; Pas & Robillard 1988). Homologies are most apparent in these sequences as they appear in the enzymes II for mannitol and for mannose (P), and in the enzymes II for *N*-acetylglucosamine, glucose, and one of the cysteines (Cys24) of the β-glucoside uptake protein; a second cysteine (Cys387) shows considerable homology with the region adjacent to Cys112 of the enzyme II for fructose.

4. The role of enzyme I

Escherichia coli and *S. typhimurium* mutants that lack enzyme I of the PT-system (*ptsI*) do not grow on fructose as sole carbon source, and suspensions of such mutants do not take up [^{14}C]fructose (Postma & Roseman 1976). This shows that enzyme I plays as important a role in the utilization of fructose as it does in that of other PT-sugars. However, such *ptsI*-mutants can mutate further to regain their ability to grow on fructose, by mechanisms that either result in routes of uptake and metabolism totally different from the fructose-PT system, or that enable the missing enzyme I activity to be replaced by a modified protein ('enzyme I*'; see Chin *et al.* (1987); Sutrina *et al.* (1988)) that, in *S. typhimurium*, is specified by a gene mapping between *ptsI* and *cysA*.

The former option was first described by Saier *et al.* (1971). From strains of *S. typhimurium* that lack both enzyme I and HPr, further mutants were derived that could utilize fructose and mannose, but not other PT-sugars. Such organisms were found to contain elevated levels of manno(fructo)kinase activity, which catalyses the reactions:

$$\text{fructose} + \text{ATP} \rightarrow \text{fructose 6-phosphate} + \text{ADP} \quad (1)$$

and

$$\text{mannose} + \text{ADP} \rightarrow \text{mannose 6-phosphate} + \text{ADP} \quad (2)$$

But, for this enzyme to effect growth on fructose or mannose, those sugars must first cross the membrane. The work of Postma & Stock (1980) clarified this point only to some extent; growth on mannose appeared to necessitate constitutive synthesis of galactose permease, although, surprisingly, elevated intracellular manno(fructo)kinase activity appeared to suffice for growth on fructose.

Our attempts to isolate similar mutants of *E. coli* have so far proved unsuccessful. Instead,

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we have, with several different strains of organisms that lack enzyme I activity, either alone or in conjunction with HPr, repeatedly obtained further mutants that grew on fructose and, at lesser rates, on mannose; they grew only very poorly on glucose. Such organisms exhibited some of the characteristics of the enzyme I* mutants of *S. typhimurium* first described by Chin *et al.* (1987), but there appear to be some significant differences in their properties that merit more detailed consideration.

Isolation and properties of Fru$^+$-strains of E. coli *lacking enzyme* I *and HPr activities*

A mutant of *E. coli*, designated TP 2811, was a gift from Dr A Danchin (Institut Pasteur, Paris). It carried a kanamycin-resistance transposon inserted into the genome at min. 52, which resulted in a deletion extending from *ptsI*, through *ptsH*, into the *crr* gene. In consequence, this organism did not grow on any PT-sugar, even when the media were supplemented with 3′,5′-cyclic AMP. However, when a suspension of strain TP 2811 was spread on agar plates containing required amino acids, kanamycin (15 μg ml^{-1}) and 20 mM fructose as sole carbon source, colonies appeared after 2–3 days' incubation at 37 °C. These pseudo-revertants grew in liquid culture on fructose at rates related to the concentration of fructose in the medium. At 37 °C and with 50 mM fructose, they doubled in number in less than 3 h, whereas, with 5 mM fructose, more than 9 h were required. However, the specific activities of fructo(manno)kinase (Sebastian & Asensio 1972) were low, and were not significantly different in extracts of the nutrient-broth grown strain TP 2811, in similar extracts of the Fru$^+$-pseudo revertants, and in extracts of the Fru$^+$-cells when those cells had been grown on fructose. This showed these organisms to be fundamentally different from those described by Saier *et al.* (1971) and by Postma & Stock (1980).

Bacteriophage P1 grown (Miller 1972) on strain TP 2811, and on one of the Fru$^+$-derivatives (designated strain 1318) were used to transduce the *ptsH fruR*-recipient strain HK 994, kanamycin-resistant transductants being selected on nutrient agar containing this antibiotic at 15 μg ml^{-1}. As expected, none of the transductants obtained from the cross [P1(TP 2811) × HK 994] grew on glucitol, glucose, mannose or fructose as carbon sources, although all grew well on medium containing 5 mM gluconate. Similarly, the cross [P1(1318 × HK 994)] yielded kanamycin-resistant transductants, none of which grew on glucitol or glucose within 72 h; however, *ca.* 40 % of such transductants grew within 36 h on fructose: their growth on fructose continued without lag when they were maintained on fructose medium. This shows that, as in similar mutants of *S. typhimurium* (Chin *et al.* 1987), the activity that permits *E. coli* Fru$^+$–[*ptsI*…*crr*]$^\Delta$ mutants to grow on fructose (or, at a lesser rate, on mannose) is specified by a gene or genes linked to, but not part of, the *ptsHI* operon at min. 52 on the *E. coli* genome. The location of this gene is clearly very similar to that reported by Chin *et al.* (1987) for *S. typhimurium*, as phage P1, grown on strain 1318 and crossed with a *cysA fruR* – strain of *E. coli*, yielded *cysA*$^+$ transductants virtually all of which were kanamycin-resistant and unable to grow readily on any PT-sugar except fructose. It is also probable that the gene that confers this Fru$^+$ phenotype on [*ptsI*…*crr*]$^\Delta$ mutants specifies a protein, as reported for *S. typhimurium* by Sutrina *et al.* (1988), as [*ptsI*…*crr*]$^\Delta$ mutants can be transformed to grow on fructose (but not on glucose or glucitol) with DNA subcloned from the F32-plasmid (Prior 1988); this plasmid is believed (McFall 1967) to carry genes located on the *E. coli* genome from *ca.* min. 44–min. 52.

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The role of the fructose operon

That the components of the fructose operon, specified by the genes *fruF fruK* and *fruA* located at min. 45.8 and under the control of the repressor specified by *fruR*$^+$ at min. 2, are necessary for the growth on fructose of Fru$^+$–[*ptsI*...*crr*]$^\Delta$ *fruR* mutants of *E. coli* can be demonstrated in a variety of ways. For example, this growth is concentration-dependent, with an apparent K_m for the overall process of 5–8 mM if the enzyme II for fructose or that for mannose, or both, are functional; if both are absent or impaired, very slow growth may still be observed (tables 2, 3) but the apparent K_m rises to 25 mM (table 2).

TABLE 2. EFFECT OF MUTATIONS IN ENZYMES II FOR FRUCTOSE (FruA) AND FOR MANNOSE (PtsM) ON THE GROWTH OF Fru$^+$ [*ptsI*...*crr*]$^\Delta$ *fruR* MUTANTS OF *ESCHERICHIA COLI*

	genotype		doubling time/(min) on fructose/mM				
strain	*fruA*	*ptsM*	5	10	20	50	K_m (apparent)/mM
1322	−	−	−	680	444	278	25
1337	+	−	435	315	240	194	8
1336	+	+	342	225	165	135	5
1334	+	+	540	375	249	180	7
1350	−	+	380	255	215	203	8

With *ptsM*-mutants that are *fruR* [*ptsI*...*crr*]$^\Delta$ and Fru$^+$, it is also easy to show that the product of fructose uptake is fructose 1-phosphate. Abolition of fructose 6-phosphate kinase activity (*pfkA*) makes little difference to growth on fructose, but abolition of fructose 1-phosphate kinase activity (*fruK*) also abolishes growth on fructose (table 3). Similarly, derivatives of Fru$^+$ *ptsM fruR* [*ptsI*...*crr*]$^\Delta$ strains that lack FPr activity (*fruF*) do not grow on fructose at any concentration even if such strains are *ptsH*$^+$.

TABLE 3. DOUBLING TIMES OF Fru$^+$ *ptsM fruR* [*ptsI*...*crr*]$^\Delta$ MUTANTS OF *E. COLI* GROWING ON 50 mM FRUCTOSE

genotype			
pfkA	*fruK*	*fruF*	doubling time/min
+	+	+	190
−	+	+	205
+	−	+	∞
+	+	−	> 600

All the strains used for these experiments were *fruR* because *fruR*$^+$-mutants that were otherwise similar, when transferred to fructose from, e.g. nutrient broth, exhibit much longer lag times before growth commences. The relatively poor affinity for fructose of the pseudo-enzyme I–FPr–enzyme II system, that is indicated by the relatively high apparent K_m of the overall process, suggests that it is the coupling of the pseudo-enzyme I to the proteins of the fructose operon that is inefficient; it may be that external fructose can enter this sequence, which effects its ultimate conversion to fructose 1,6-*bis*phosphate, at all readily only if the fructose-specific components are formed abundantly in amounts characteristic of de-repressed cells.

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