ROLE OF FRUCTOSE-1,6-DIPHOSPHATASE IN FRUCTOSE UTILIZATION BY *ESCHERICHIA COLI*

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1. Introduction

The major pathway for the utilization of fructose by *Escherichia coli* involves the formation of fructose-1-phosphate (reaction 1), catalysed by the phosphopyruvate phosphotransferase (PT) system [1], followed by an ATP-dependent phosphorylation of this product (reaction 2) to fructose-1,6-diphosphate [1, 2].

fructose + PEP → fructose-1-phosphate + pyruvate (1)

fructose-1-phosphate + ATP → fructose-1,6-diphosphate + ADP (2)

It would be expected from this sequence that the biosynthesis of carbohydrates, such as glycogen, pentoses or the *N*-acetylglucosamine moieties of cell walls, would require the formation of fructose-6-phosphate from fructose-1,6-diphosphate, and that this step would involve fructose-1,6-diphosphatase (reaction 3)

fructose-1,6-diphosphate + H_2O → fructose 6-phosphate + Pi (3)

However, although *E. coli* mutants devoid of fructose diphosphatase activity do not grow on substrates such as glycerol, lactate, acetate, or C_4-acids, such mutants grow at virtually wild-type rates on fructose [1, 3]. This suggests that either fructose, or the fructose-1-phosphate derived from it, can yield fructose-6-phosphate directly and in a manner that by-passes reaction (3).

It is the purpose of this paper to present evidence that reaction(s) associated with the PT-system can effect the formation of fructose-6-phosphate from fructose at the high concentrations normally used in media containing fructose as carbon source for growth, and that any direct formation of fructose-6-phosphate from fructose-1-phosphate does not occur at a significant rate.

2. Experimental

The strains of *E. coli* used in this work are listed in table 1. Procedures for the growth of organisms and for the preparation of extracts have been previously published [2]. Fructose diphosphatase activity was assayed by the method of Fraenkel and Horecker [1]. Genetic procedures were as previously described [4]. The assay of the fructose–PT-system is described in table 3.

3. Results and discussion

Although the mutant K2-19, which is devoid of fructose diphosphatase activity (table 2), grew on media containing 20 mM fructose at a rate indistinguishable from its wild-type parent, K2, this growth was considerably slower when media containing lower concentrations of fructose were used: the striking diminution of growth rate observed was not exhibited by the parent organism. When the least mean doubling times for the growth of these organisms on

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

Strain	Genetic markers*	Mating type	Source
K2	*his,arg,thr,leu,trp,str*	F^-	[4]
K2-19	*his,arg,thr,leu,trp,fdp,str*	F^-	EMS**-mutant of K2
K2.1.5^c.7	*his,thr,leu,pgi,iclR,str*	F^-	[5]
K10	–	Hfr	D. Fraenkel
AB674	–	Hfr	E.A. Adelberg
KL16	–	Hfr	B. Low
KL16-21	pts_F	Hfr	[2]

* The abbreviations used indicate a requirement for *his* = histidine, *arg* = arginine, *thr* = threonine, *leu* = leucine, *trp* = tryptophan; *fdp* = absence of fructose diphosphatase; *pgi* = absence of phosphoglucoseisomerase, *iclR* = constitutive formation of glyoxylate cycle enzymes, pts_F = absence of enzyme II for fructose phosphorylation via phosphopyruvate phosphotransferase system, *str* = resistance to streptomycin.

** EMS = ethylmethanesulphonate. Mutagenesis [6] and selection with penicillin [7] for inability to grow on glycerol or lactate, whilst retaining ability to grow on glucose, was by the general procedures previously described [4].

Table 2
Fructose diphosphatase activity of *E. coli* strains used.

Organism	C source for growth	Specific activity of fructose diphosphatase*
K2	fructose	12
K2	glycerol	14
K2-19	fructose	< 0.6
K2-19	glycerol	no growth

* Specific activity is here expressed as nmoles of NADP reduced × min^{-1} × mg $protein^{-1}$ in the assay of Fraenkel and Horecker [3].

media of various fructose concentrations, i.e. the reciprocals of the maximal growth rates attained, were plotted against the reciprocal of these fructose concentrations, the results obtained were similar to those given by Lineweaver-Burk plots of enzyme kinetics (fig. 1). As will be apparent from this figure, the maximal growth rate achievable on fructose ("V_{max}") is not greatly affected by the absence of fructose diphosphatase; however, half this maximal rate ("K_m") was attained by the mutant only at approx. 1.8 mM fructose, whereas in the wild-type organism approx. 0.16 mM sufficed.

This behaviour was shown also by recombinants of K2-19 obtained from two genetic crosses. The Hfr strain AB674 transfers its genome to recipient (F^-) cells in the order *O–argH–fdp*.....; strain K10 injects in the reverse order, *O–leu–thr–fdp*..... .

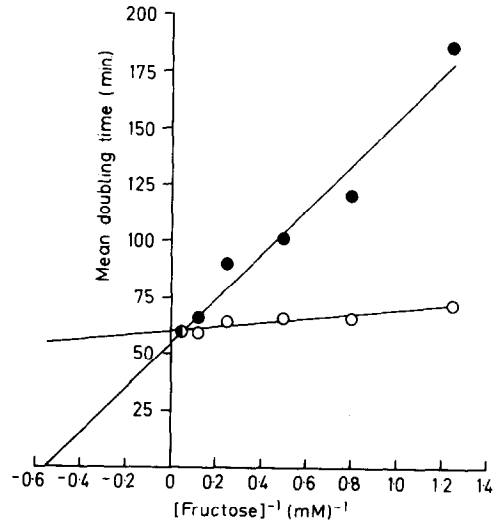

Fig. 1. Growth of the wild-type of *Escherichia coli* K2 (○) and of its mutant K2-19 (●) devoid of fructose diphosphatase activity, on media containing fructose as sole carbon source. The data are expressed as the reciprocals of the maximum growth rates attained at 37° at various concentrations of fructose, plotted against the reciprocals of those fructose concentrations.

Both strains were crossed with the mutant K2-19, and, after periodic interruption of conjugation, recombinants were selected on plates containing (a) glucose as carbon source for arg^+ and thr^+leu^+ respectively, and (b) glycerol as carbon source for fdp^+. In all cases, the male phenotype was counterselected by inclusion

Table 3
Fructose 6-phosphate formation from 50 mM-fructose by toluene-treated *E. coli.*

Organisms	C source for growth	Assay system*	Rate of fructose-6-phosphate formation**	Activity (%)
K2.1.5^{c}.7	fructose	complete	4.1	100
		−PEP, + ATP	0.3	7
		$-Mg^{2+}$	1.8	45
		−fructose, + F1P	0	0
K2.1.5^{c}.7	glucose	complete	1.1	26
K2.1.5^{c}.7	glycerol	complete	0.7	17
K2-19	fructose	complete	2.8	–
KL16	fructose + glycerol	complete	2.5	–
KL16.21	fructose + glycerol	complete	< 0.6	–

* The complete system contained in 1 ml, in μmoles: $MgCl_2$ 1, PEP 1.25, fructose 50, potassium phosphate pH 7.5 50, and toluene-treated cells [11], 1–1.5 mg dry wt. Where indicated, ATP (1 μmole) and fructose-1-phosphate (1 μmole) replaced the PEP and fructose respectively. After 20 min at 37°, the reactions were stopped by millipore filtration and samples (0.5 ml) of the filtrates were assayed for glucose-6-phosphate and fructose-6-phosphate: these assays involved the spectrophotometric determination at 340 nm of the reduction of NADP (0.6 μmole) in the presence of iminazole pH 7.2 100 μmoles, and crystalline glucose-6-phosphate dehydrogenase 6.25 μg and (for fructose-6-phosphate assay) phosphoglucoseisomerase 6.25 μg.
** nmoles of fructose-6-phosphate formed × min^{-1} × mg dry wt of $cells^{-1}$.

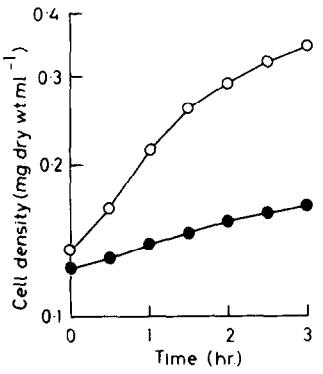

Fig. 2. Growth of the wild-type strain of *Escherichia coli*, K2 (○) and of the mutant K2-19 (●), devoid of fructose diphosphatase activity, on media containing 5 mM fructose-1-phosphate as sole carbon source. Inocula for these experiments were grown on glucose-6-phosphate [2].

of streptomycin in these plates. In confirmation of previous findings [8], the *fdp*$^{+}$-allele was found to enter 6 min from the *arg* and 6 min from the *thr,leu* markers. All of the 195 *fdp*$^{-}$-recombinants tested manifested the phenotype of K2-19: they grew on plates containing 20 mM fructose but failed to grow readily on plates containing 0.5 mM fructose as carbon source. In contrast, all of the 75 *fdp*$^{+}$-recombinants grew readily on either concentration of fructose.

These observations suggested that, at low concentrations of fructose (< 2 mM), fructose diphosphatase plays an important role in the generation of fructose-6-phosphate from the growth substrate but that, at higher fructose concentrations, fructose-6-phosphate can arise by reaction(s) that by-pass the diphosphatase, either directly or from fructose-1-phosphate.

The latter possibility is rendered unlikely by two findings. Firstly, and in confirmation of the work of Fraenkel [1], we fail to find any in vitro conversion of fructose-1-phosphate to fructose-6-phosphate, by cell-free extracts of fructose-grown *E. coli*. Secondly, the in vivo occurrence of such a conversion is ruled out by the inability of K2-19 to grow on fructose-1-phosphate (fig. 2). As was previously reported [2], wild-type strains of *E. coli* can grow on fructose-1-phosphate as sole carbon source if the system that effects the uptake of hexose phosphates is first induced by prior growth on glucose-6-phosphate. Strain K2 manifested this behaviour: when cultures of this organism were transferred from glucose-6-phosphate

to a medium containing 5 mM fructose-1-phosphate, this latter compound served as a good growth substrate for more than 1 doubling. However, although the mutant K2-19, devoid of fructose diphosphatase, also grew readily on glucose-6-phosphate, cells thus grown did not utilize fructose-1-phosphate. Thus, fructose-6-phosphate could not arise from fructose-1-phosphate in K2-19, although it could in K2: hence, fructose diphosphatase activity is necessary for the utilization of fructose-1-phosphate as carbon source for growth, under conditions where it is not necessary for the utilization of fructose.

A direct formation of fructose-6-phosphate from fructose could be catalysed either by an ATP-linked kinase, such as the fructo(manno)kinase known [9] to be present in *E. coli,* or by the PT-system. Our evidence favours the latter for two main reasons.

In the first place, *E. coli* mutants devoid of enzyme I of the PT-system [10] or of the enzyme II specific for fructose [2] do not grow on fructose, nor do suspensions of such mutants take up ^{14}C-labelled fructose, even when this is supplied at high concentrations. This implies that the PT-system is necessarily involved in fructose utilization, and that any process of transport of fructose via any other system is of negligible significance. It is thus probable that the growth of K2-19 on fructose necessarily involves the operation of the PT-system.

Secondly, and more directly, we show (table 3) that toluene-treated *E. coli* [11] can effect the net formation of fructose-6-phosphate from added fructose at high concentrations of the sugar; this process requires Mg^{2+} and PEP (the phosphate-donor in the PT-system) and does not occur if ATP replaces PEP. In the complete system, which contained 50 mM fructose, both fructose-1-phosphate and fructose-6-phosphate were formed; when low concentrations of fructose were used, such as the 0.04 mM employed by Fraenkel [1] to demonstrate fructose-1-phosphate formation via the PT-system, fructose-6-phosphate formation was not detected.

The possibility that small amounts of glucose, present in the fructose solutions used, might have given rise to glucose-6-phosphate and then to fructose-6-phosphate was ruled out by demonstrating this process with a mutant K2.1.5^{c}.7 that lacked phosphoglucoseisomerase activity: toluene-treated suspensions of the fructose-grown cells formed approx. 4 nmoles of fructose-6-phosphate $\times$ min^{-1} $\times$ mg dry wt^{-1}, and this rate was maintained linearly over at least 20 min. Suspensions of the mutant grown on glycerol or glucose catalysed this reaction at only 17–26% of this rate, which further argues for its specific, and probably inducible, nature. As expected, mutants that lacked the enzyme II component of the fructose PT-system [2] failed to form either the 1- or 6-phosphate ester.

The results here presented resolve the paradox [1, 3] that *E. coli* mutants devoid of fructose diphosphatase grow on fructose although the major pathway of fructose conversion to fructose diphosphate is, in essence, a gluconeogenic process. It remains to be investigated why similar mutants of *Aerobacter aerogenes* [12] behave differently.

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References

[1] D.G. Fraenkel, J. Biol. Chem. 243 (1968) 6458.
[2] T. Ferenci and H.L. Kornberg, FEBS Letters 12 (1971) 127.
[3] D.G. Fraenkel and B.L. Horecker, J. Bacteriol. 90 (1965) 837.
[4] C.B. Brice and H.L. Kornberg, Proc. Roy. Soc. London Ser. B. 168 (1967) 281.
[5] H.L. Kornberg, in: Metabolic Regulation and Enzyme Action, eds. A. Sols and S. Grisolia (Academic Press, London, New York, 1970) p. 5.
[6] E.C.C. Lin, S.A. Lerner and S.E. Jorgensen, Biochim. Biophys. Acta 60 (1962) 422.
[7] L. Gorini and H. Kaufman, Science 131 (1960) 604.
[8] M.T. Yu, A.R. Kaney and K.C. Atwood, J. Bacteriol. 90 (1965) 1150.
[9] J. Sebastián and C. Asensio, Biochem. Biophys. Res. Commun. 28 (1967) 197.
[10] S. Roseman, J. Gen. Physiol. 54 (1969) 138s.
[11] G. Gachelin, Biochem. Biophys. Res. Commun. 34 (1969) 382.
[12] V. Sapico, T.E. Hanson, R.W. Walter and R.L. Anderson, J. Bacteriol. 96 (1968) 51.