PATHWAY OF FRUCTOSE UTILIZATION BY *ESCHERICHIA COLI*

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Received 14 December 1970

1. Introduction

Escherichia coli grows readily on fructose as sole source of carbon. It was long assumed that the manner in which this sugar is utilized is analogous to that by which glucose enters the main metabolic pathways of the cells; this would imply the occurrence of an initial enzymatic phosphorylation by ATP of fructose to fructose-6-phosphate and ADP, followed by a second phosphorylation to fructose-1,6-diphosphate. Enzymes catalysing both these reactions have been demonstrated to be present *E. coli* [1, 2]. However, recent studies in several laboratories have cast doubt on this view, and have suggested that fructose is initially phosphorylated to fructose-1-phosphate (reaction 1) with concomitant conversion of phospho*enol*pyruvate (PEP) to pyruvate. Fructose-1-phosphate is then further phosphorylated to fructose-1,6-diphosphate, through the agency of an ATP-linked fructose-1-phosphate kinase (reaction 2) distinct from the action of the phosphofructokinase that catalyses the phosphorylation of fructose-6-phosphate to fructose-1,6-diphosphate.

$$\text{fructose} + \text{PEP} \rightarrow \text{fructose-1-phosphate} + \text{pyruvate} \quad (1)$$

$$\text{fructose-1-phosphate} + \text{ATP} \rightarrow \text{fructose-1,6-diphosphate} + \text{ADP} \quad (2)$$

The main evidence for this alternative route of fructose utilization may be summarized as follows:

(i) although a fructo(manno) kinase, which catalyses the conversion of fructose to fructose-6-phosphate with concomitant cleavage of ATP to ADP, has been isolated from *E. coli* [1], its activity is low and appears to be insufficient to account for the rate of fructose utilization by cells growing on this hexose;

(ii) *E. coli* mutants devoid of phosphofructokinase activity, which do not grow on glucose, grow at virtually wild-type rates on fructose [2, 3];

(iii) the pathway summarized in reactions (1) and (2) has been shown to occur in another member of the Enterobacteriaceae, *Aerobacter aerogenes* [4];

(iv) the product of the PEP-linked phosphotransferase reaction [5] with fructose as substrate has been identified as fructose-1-phosphate [6];

(v) *E. coli* inducibly forms fructose-1-phosphate kinase when exposed to fructose [6].

Thus, as Fraenkel [6] rightly concludes, "on balance, most of the available data on fructose metabolism in *E. coli* and *A. aerogenes* favor the *major* pathway being via fructose-1-phosphate. But, in order to accommodate all the data more mutant analysis and dissection of the enzymes will be necessary". It is the main purpose of this paper to describe the properties of a mutant of *E. coli* that provide direct evidence for the occurrence of this pathway.

2. Experimental

The mutant KL 16–21 was derived from the Hfr strain KL 16 of *E. coli* K12, by treatment with ethyl methane sulphonate [7] and was selected with penicilin [8] for its inability to grow on medium containing salts [9] and fructose as carbon source, whilst retaining the ability to grow normally on similar medium containing glucose as carbon source. The mutant was purified by repeated single-colony isolation; it grew at wild-type rates also on glucose-6-phosphate, fructose-6-phosphate, gluconate and glycerol. The organism was stored on slopes of Oxoid

nutrient agar; liquid cultures were grown at 37° aerobically on a Gallenkamp incubator shaker. Growth was measured as ΔA_{680nm}; under our conditions $\Delta A_{680nm} = 1.0$ was found to be equivalent to an increase of 0.68 mg dry wt of cells ml^{-1} of culture

2.1. *Enzyme assays*

The activity of the phosphotransferase system that catalyses reaction (1) was measured as the fructose-dependent release of pyruvate from PEP concomitant with the enzymic phosphorylation of fructose to fructose-1-phosphate. Cultures of *E. coli* were harvested, washed in 1% (w/v) KCl and suspended at 30–40 mg dry wt/ml in 0.1 M potassium phosphate buffer pH 7.5. The suspension was stored at −20° overnight and thawed at room temperature [10]. The assay systems, incubated at 37° for 0, 3 and 6 min, respectively, contained in a final volume of 1.0 ml (μmoles): fructose 1; PEP 1; potassium phosphate pH 7.5, 20; sodium arsenite 10; potassium fluoride 100; magnesium chloride 0.5. The reaction was started by the addition of thawed cells (1–2 mg dry wt) and stopped by the addition of 0.33 ml of 0.1% (w/v) 2,4-dinitrophenylhydrazine in 2 N HCl. The pyruvate 2,4-dinitrophenylhydrazone content was measured [11] after centrifugation to remove cell debris. Each assay was read against a blank, incubated as above with all components of the complete system except fructose, which by itself gives negligible reaction.

For assays of fructose-1-phosphate kinase activity, cultures of *E. coli* were harvested from appropriate growth media, washed in 1% (w/v) KCl and suspended, at 1–5 mg dry wt ml^{-1}, in buffer, pH 7.5, containing 10 mM tris, 10 mM $MgCl_2$ and 2 mM mercaptoethanol The cells were disrupted at 0° by exposure for 2 min to the output of a MSE 100W sonicator and cell debris was removed by centrifugation for 1hr at 30,000 *g*. The enzyme was assayed in a system containing in a final vol. of 1 ml, a mixture of (μmoles): imidazole buffer, pH 7.2, 67; magnesium chloride 10; fructose-1-phosphate 2.5; ATP 1.25; NADH 0.15; and crystalline aldolase 12,5 μg; triosephosphate isomerase + α-glycerolphosphate dehydrogenase, 20 μg. Under these conditions, the phosphorylation of 1 μmole of fructose-1-phosphate is taken to be equivalent to the oxidation of 2 μmoles of NADH, which is measured as ΔA_{340nm}. All measurements were corrected for the small residual NADH-oxidase activity of the sonic extracts.

3. Results and discussion

Since the mutant KL 16–21 grew at wild-type rates on glucose, glucose-6-phosphate and fructose-6-phosphate, its inability to utilize fructose indicated a dysfunction in either the formation of fructose-1-phosphate (reaction 1) or its phosphorylation to fructose-1,6-diphosphate (reaction 2). The experiments illustrated by fig. 1 and table 1 rule out the latter possibility.

Studies with wild-type cells showed [12] that, although fructose-1-phosphate did not normally support the growth of *E. coli*, it could serve to a limited extent as a carbon source for the growth of cells that had been previously grown on hexose phosphates. Under these conditions, the bacteria inducibly form a transport system that specifically effects the uptake of hexose phosphates [13–15]: fructose-1-phosphate is a substrate transported by this system, but is not an inducer of it. As expected from this property, cultures of the wild-type KL16, grown on medium containing 10 mM glucose-6-phosphate as sole carbon source, grew rapidly for about 1 generation when the harvested cells were transferred to medium containing

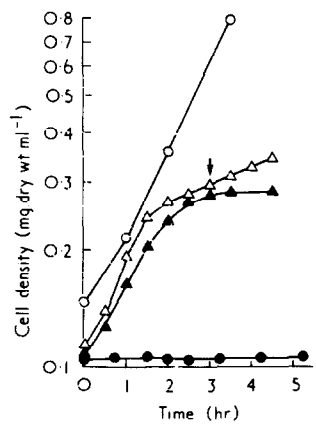

Fig. 1. Growth of KL 16 (open symbols) and its mutant KL 16–21 (closed symbols), previously grown on 10 mM glucose-6-phosphate, after transfer to 5 mM fructose (○, ●) or 5 mM fructose-1-phosphate (△, ▲) as carbon sources. A further portion of fructose-1-phosphate, sufficient to raise its concentration in the medium by 2.5 mM, was added at the arrow.

Table 1
Inducible formation of fructose-1-phosphate kinase in KL 16 and KL 16–21.

Growth substrate	Specific activity* of fructose 1-phosphate kinase in			
	KL 16	Increase (fold)	KL 16–21	Increase (fold)
Glucose-6-phosphate	14	–	4	–
Fructose-1-phosphate	111	8	39	9
Fructose	138	10	n.g.	n.g.

* Specific activity = nmoles of fructose-1-phosphate phosphorylated. min^{-1} mg of $protein^{-1}$.
n.g. = no growth on fructose; hence this assay could not be done..

5 mM fructose-1-phosphate; thereafter, the rate of growth decreased sharply and was not increased by further addition of fructose-1-phosphate (fig. 1). The slow growth now observed was presumably due to the slow hydrolysis of fructose-1-phosphate in the medium, to yield fructose which, as shown in fig. 1, is an excellent growth substrate for KL 16. Cultures of the mutant KL 16–21, previously grown on glucose-6-phosphate, also grew rapidly on fructose-1-phosphate for about 1 generation: thereafter, growth ceased, as any fructose produced from the hydrolysis of fructose-1-phosphate could not be utilized by this mutant (fig. 1).

That this rapid growth on fructose-1-phosphate involved the activity of fructose-1-phosphate kinase is shown in table 1. The specific activity of this enzyme in extracts of cells grown on glucose-6-phosphate was low, but was 8–9 fold higher in extracts of cells which had been allowed to grow for one generation on fructose-1-phosphate. The similar induction of fructose-1-phosphate kinase after growth of the wild-type KL 16 for 1 generation on fructose is further evidence [6] for the role of this enzyme in fructose metabolism.

Two main findings indicate that the inability of the mutant KL 16–21 to grow upon fructose is associated with a lesion in the complex system [4, 5] that effects the formation of fructose-1-phosphate. This system contains at least three components: a histidine-containing protein (HPr), phosphorylated by PEP in the presence of an enzyme (enzyme I: ref. 5) to yield HPr-P, and a second enzyme (Enzyme II) presumably specific for the transfer of the phosphate from HPr-P to the 1-position of fructose. Mutants defective in the formation of HPr or enzyme I are pleiotropic, and cannot utilize a variety of sugars including fructose [16]; a mutant devoid only of the ability to utilize fructose might be expected to lack enzyme II activity. This is borne out by the results of a genetic cross between the mutant KL 16–21 and a recipient pleiotropically affected in hexose utilization [17]. About 30% of the recombinants from such a cross grew on all hexoses, and had therefore received the functional allele specifying HPr and/or enzyme I without also receiving the defective allele for fructose utilization; the remaining recombinants grew on glucose but not upon fructose and thus had the phenotype of KL 16–21. It is therefore likely that KL 16–21 which is not impaired in either HPr or enzyme I, lacks enzyme II; it is also likely that the gene specifying enzyme II is fairly closely linked to the gene specifying the pleiotropic defect. Evidence to this effect is provided also by measurements of the time of entry of the marker for fructose utilitzation, from the wild-type KL 16 which injects its genome in the order *O-thy-his*, into a F^- mutant K2.1.21, which carried the defective allele specific for fructose utilization and also required thymine and histidine for growth. The results showed that the ability to grow upon fructose was acquired by the recipient approx. 13 min after the entry of *thy* and approx. 3 min before *his*; this would place the marker for enzyme II of the fructose system at about 42 min on the *E. coli* linkage map [18], and about 4 min from the marker specifying the pleiotropic defect in sugar utilization [19].

A dysfunction of enzyme II should have two consequences. In the first place, mutants thus defective should not be able to effect the phosphorylation of fructose (reaction 1). This was found to be so: whereas frozen and thawed suspensions of the wild-type KL 16, which had been grown on glycerol plus fructose, catalysed the PEP-dependent formation of 21 nmoles of fructose-1-phosphate from fructose min^{-1} mg dry

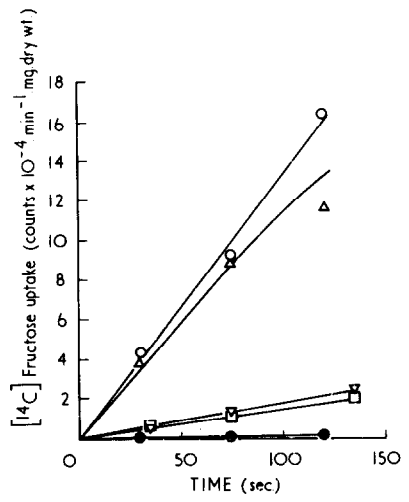

Fig. 2. Uptake of U-^{14}C-fructose by KL 16 (open symbols) and its mutant KL 16–21 (closed symbols). Cultures of KL16 had been grown on fructose (○), fructose-1-phosphate (△), glucose (▽) or glycerol (□); those of KL 16–21 had been grown on fructose-1-phosphate (●). The uptake of ^{14}C-fructose (0.1 mM; 0.29 μCi ml^{-1}) by washed suspensions of cells (0.3 mg dry wt ml^{-1}) thus grown, was measured as previously described [21].

wt^{-1} of cells, suspensions of KL 16–21, similarly grown, phosphorylated less than 1 nmole of fructose under these conditions. Secondly, the phosphorylation reaction (1) has been shown to be necessarily associated with uptake of hexoses by *E. coli* (for review, see [20]). It would thus be expected that a mutant lacking enzyme II activity would be impaired in the uptake of labelled fructose. This is shown in fig. 2. Whereas washed suspensions of the wild-type KL16 inducibly formed a system effecting the uptake and incorporation of ^{14}C-fructose after growth on either glycerol plus fructose or on fructose-1-phosphate, suspensions of KL 16–21 were grossly impaired in this uptake, even when the mutant had been grown on those carbon sources that acted as good inducers in the wild-type.

The results thus show that the growth of *E. coli* on fructose necessitates the occurrence of the pathway summarized in reactions (1) and (2). The mutant KL 16–21 here described probably lacks the enzyme II component of reaction (1); the gene specifying this defect is located about 4 min from that specifying the HPr and/or enzyme I components of this reaction [19].

Acknowledgements

We thank Mrs. Janet Smith for assistance in the genetic analysis, Mr. G. Asquith for the preparation of the figures, and the Science Research Council for support through Grant B/SR/7246. This work was performed during the tenure by T.F. of a Postgraduate Studentship of the Science Research Council.

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