ISOLATION AND PROPERTIES OF A REGULATORY MUTANT IN THE HEXOSE PHOSPHATE TRANSPORT SYSTEM OF *ESCHERICHIA COLI*

T.FERENCI, H.L.KORNBERG and Janet SMITH
Department of Biochemistry, School of Biological Sciences, University of Leicester, Leicester LE1 7RH, England

Received 24 December 1970

1. Introduction

Escherichia coli can utilize a variety of hexose phosphates without first hydrolysing them to the free sugars [1]. The system that effects the transport of these esters into the cells is inducible [1, 2] and common to them all: growth in the presence of glucose-6-phosphate, or fructose-6-phosphate, or mannose-6-phosphate, elicits the formation of the uptake system [3], and mutants that lack the ability to take up one of these hexose phosphates also lack the ability to take up the others [3, 4]. The gene specifying this uptake system, designated *uhp*, is cotransducible with *pyrE* and is thus located at about 72 min [4] on the *E. coli* linkage map [5].

We recently observed [6] that *E. coli* can grow on fructose-1-phosphate as sole carbon source. However, rapid growth occurred only if the cells were previously exposed to hexose-6-phosphates; the rate of growth on fructose-1-phosphate by cells thus induced decreased sharply after 1–1½ doublings. This suggested that fructose-1-phosphate could be transported by the hexose phosphate uptake system but was not an inducer of that system. We now confirm this interpretation, and utilize it to select mutants that grow readily on fructose-1-phosphate without prior exposure to hexose-6-phosphates, and whose rate of growth on this substrate remains constant for many doublings. Such mutants form the uptake system for hexose phosphates constitutively; their genotype is designated *uhp*^{c}. Analysis by conjugation and by phage-mediated transduction, establishes *uhp*^{c} to be closely linked to *uhp* and to be also cotransducible with *pyrE*; this raises the possibility that the uptake of hexose phosphates by *E. coli* may be regulated as an operon [7].

2. Experimental

The procedures used for the growth of *E. coli*, for measurement of the rates of uptake of labelled substrates by washed cell suspensions, and for genetic procedures, were as previously described [6, 8, 9]. Methods for the isolation and detection of *uhp*^{c}-mutants are discussed in the following section.

3. Results and discussion

The observation [6] that an *E. coli* mutant devoid of the ability to grow upon fructose can grow upon fructose-1-phosphate shows that this phosphorylated compound can enter *E. coli* without prior hydrolysis to fructose. However, this ability to utilize fructose-1-phosphate is manifested only by cultures that have been exposed previously to substances that induce the uptake system for hexose-6-phosphates (UHP), and persists for only about 1–1½ doublings on fructose-1-phosphate [6]. This implies that fructose-1-phosphate is not an inducer of the UHP-system but can be transported by it. The results shown in fig. 1 support this view. Washed suspensions of the wild-type strain KL 16, which had been grown on glucose-6-phosphate as sole carbon source, rapidly incorporate isotope when exposed to 0.1 mM ^{14}C-glucose-6-phosphate, as expected [3, 4] from a transport system common to all hexose-6-phosphates. This incorporation is sharply reduced if unlabelled hexose-6-phosphates, such as the fructose-6-phosphate shown in fig. 1, are also added. Although unlabelled fructose-1-phosphate also dilutes the uptake of ^{14}C-glucose-6-phosphate, it does so less effectively than does fruc-

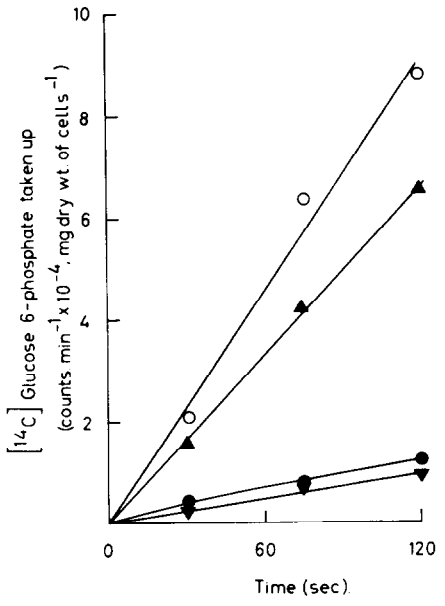

Fig. 1. Uptake of ^{14}C-glucose-6-phosphate by the wild-type *E. coli* strain KL 16. Washed suspensions (0.3 mg dry wt/ml) of the organism grown on glucose-6-phosphate were incubated with 0.1 mM ^{14}C-glucose-6-phosphate (0.3 μCi/ml) alone (○) or in the presence of 1 mM fructose-1-phosphate (▲) or fructose-6-phosphate (▼). The uptake of ^{14}C-glucose-6-phosphate by cells grown for approx. 1½ doublings on fructose-1-phosphate is shown as ●.

tose-6-phosphate: this indicates that fructose-1-phosphate is a substrate, but a poor one, for the UHP system. Growth on fructose-1-phosphate is not sufficient to maintain the induction of this uptake system and, as shown in fig. 1, the ability of the cells to take up ^{14}C-glucose-6-phosphate had decreased to a low level by the time that rapid growth on fructose-1-phosphate also ceased. Measurements (not shown) of the rates of ^{14}C-glucose-6-phosphate uptake by cultures transferred from growth on glucose-6-phosphate to growth on fructose-1-phosphate confirm that the UHP-system is indeed 'diluted out' under these conditions.

Since fructose-1-phosphate is not an inducer of the UHP-system, it would be predicted that mutants able to grow on this substance for more than 1–1½ doublings would differ from their parents in one of two ways: they would either be able to effect the rapid hydrolysis of the phosphate ester [10], and thus in effect grow on the fructose produced thereby, or be derepressed for the UHP-system. A mutant of the latter type, KL 16-11^{c}-21, was isolated from the Hfr strain KL 16-21: this strain is not capable of utilizing free fructose [6]; selection of mutants of the latter type would thus be favoured. KL 16-11^{c}-21 was obtained by spreading cultures of KL 16-21 on minimal salts–agar plates containing 2 mM fructose-1-phosphate as carbon source: the colonies that appeared after 48–72 hr at 37° were picked and purified by single-colony isolation. In liquid media, such clones grew rapidly on fructose-1-phosphate, although they had not been previously exposed to inducers of the UPH-system, and growth continued for many doublings; they did not grow on fructose.

That this ability constitutively to utilize fructose-1-phosphate was associated with an altered regulation

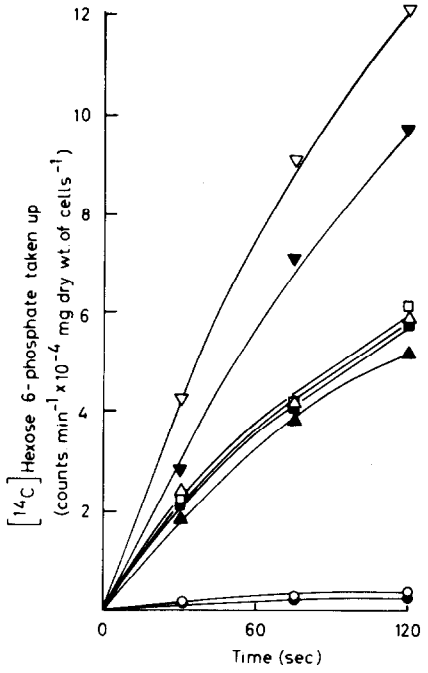

Fig. 2. Uptake of ^{14}C-glucose-6-phosphate (open symbols) and ^{14}C-fructose-6-phosphate (closed symbols) by suspensions of KL 16-21 grown on glycerol (○, ●) or glucose-6-phosphate (△, ▲) and by suspensions of KL 16-11^{c}-21 grown on glycerol (▽, ▼) or glucose-6-phosphate (□, ■).

of the UHP-system was demonstrated in three ways. KL 16-11^c-21 and its parent KL 16-21 were grown on minimal salts–agar plates containing 5 mM glucose and 20 μM ^{14}C-fructose-6-phosphate (0.05 μCi/ml), the colonies were adsorbed onto Whatman no. 40 filter paper, and this paper left in contact with Kodak 'Blue-Brand' X-ray film for 1–2 days [11]. The film was considerably more blackened by contact with the KL 16-11^c-21 than with its parent KL 16-21 colonies; this indicated that the former mutant could take up labelled fructose-6-phosphate under conditions where the latter could not, and, hence, that the UHP-system was now derepressed. This was confirmed by direct measurement of the ability of washed suspensions of the two organisms to take up ^{14}C-glucose-6-phosphate and ^{14}C-fructose-6-phosphate. As shown in fig. 2, KL 16-21 took up either of these substances when the UHP-system was induced by prior growth on glucose-6-phosphate, but not when the cells had grown on glycerol. In constrast, KL 16-11^c-21 took up ^{14}C-glucose-6-phosphate very rapidly, and ^{14}C-fructose-6-phosphate slightly less rapidly, even after growth on glycerol: indeed, these rates of uptake were considerably higher than those observed with either strain after growth on glucose-6-phosphate.

The third procedure involved transfer of the *uhp*^{c}-allele from KL 16-11^c-21 to suitable recipients. When the Hfr strain KL 16-11^c-21 was incubated with a F$^-$ strain K2.1t.11i., which carried the UHP$^-$-allele (and was thus devoid of the ability to take up hexose phosphates) and which required isoleucine and valine for growth [4], all the recombinants selected for the ability to grow on glucose in the absence of isoleucine and valine were found (by the film technique) to be either still unable to utilize glucose-6-phosphate (UHP$^-$) or to have acquired the *uhp*^{c}-character: this indicated a very high degree of linkage between *uhp* and *uhp*^{c}. This was also indicated by the results of phage-mediated transfer of the *uhp*^{c}-marker. For this, phage P1-kc were grown [8, 9] on KL 16-11^c-21 and were used to infect a strain of *E. coli* which was unable to utilize free fructose and which carried the *pyrE*-marker: in consequence of this latter lesion, it required uracil for growth. Transductants were selected on minimal salts–agar plates containing glucose but no uracil. Tests, by the film technique, showed that of 182 uracil$^+$-transductants, 93 had remained inducible for the UHP-system but 89 had acquired the *uhp*^{c}-allele (fig. 3). This distribution is very similar to the degree of linkage between *uhp* and *pyrE* [4]. Moreover, when such UHP-inducible and UHP-constitutive transductants were grown on glucose and transferred to media containing fructose-1-phosphate, the latter transductants grew rapidly and virtually without lag, whereas the former did not. As expected, both types of transductant grew on glucose-6-phosphate (fig. 4).

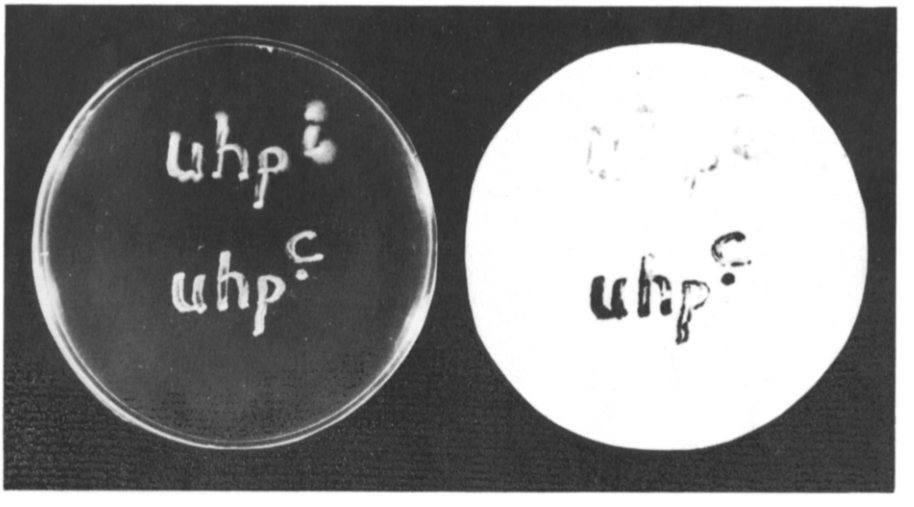

Fig. 3. Identification of uracil$^+$-transductants inducible (*uhp*^{i}) and constitutive (*uhp*^{c}) for the uptake of hexose phosphates. *Left*: organisms after growth on 5 mM glucose plus 20 μM ^{14}C-fructose-6-phosphate; *right*: blackening of X-ray film after exposure to these organisms absorbed on filter paper. For experimental details, see text.

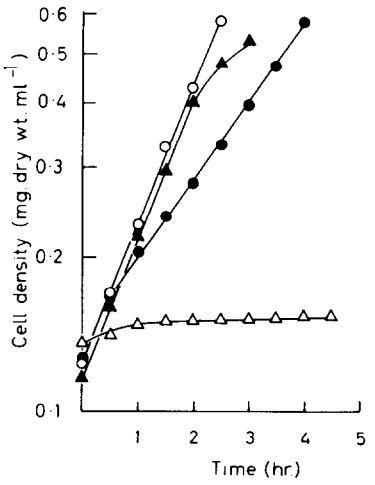

Fig. 4. Growth of UHP-inducible (open symbols) and UHP-constitutive (closed symbols) transductants on 5 mM glucose-6-phosphate (○, ●) or 5 mM fructose-1-phosphate (△, ▲) as carbon sources.

The results reported in this paper thus show that mutants selected for rapid growth on fructose-1-phosphate are also derepressed in their ability to take up hexose phosphates; conversely, recombinants or transductants selected for their constitutivity of hexose phosphate uptake are also able to grow rapidly and continuously on fructose-1-phosphate. Besides elucidating the manner in which fructose-1-phosphate enters *E. coli*, these findings provide a means for isolating mutants with altered regulation of hexose phosphate transport. Such mutants will be useful for investigating the mechanism of induction of the UHP-system [10, 12].

The results further show that the ability to effect the uptake of hexose phosphates, and the regulation of that ability, are specified by closely linked genetic markers. It is not yet known whether this system behaves as an operon.

Acknowledgements

We thank Mr. G.Asquith for preparing the diagrams and The Science Research Council for support under Grant B/SR/7246. This work was performed during the tenure by T.F. of a Research Studentship of the Science Research Council.

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