GENETIC CONTROL OF GLUCOSE UPTAKE BY *ESCHERICHIA COLI*

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1. Introduction

The uptake by *Escherichia coli* of a variety of carbohydrates necessarily involves the activity of a PEP-dependent phosphotransferase (PT)-system [1], in which at least three components participate. Two of these – a small, histidine-containing protein (HPr) and an enzyme (Enzyme I) that catalyses the transfer of phosphate from PEP to HPr – are required for the utilization of all carbohydrates that are taken up via the PT-system: mutants devoid of either component are pleiotropically impaired in the uptake of all such sugars [2, 3]. However, the membrane-linked multi-component fractions that effect the transfer or phosphate from the phosphorylated HPr to sugars (collectively termed Enzyme II) are specific for individual sugars. Thus, for example, *E. coli* mutants devoid of an Enzyme II for fructose do not grow or take up this sugar, although such mutants are unimpaired in their ability to grow on other hexoses and on fructose 1- and 6-phosphates [4]. The gene (*pts*F) specifying this Enzyme II is located at about 43 min [4] on the *E. coli* linkage map [5].

It is the main purpose of this paper to report the isolation of *E. coli* mutants deficient in an Enzyme II for the uptake of glucose and of its non-catabolizable analogue, methyl-α-glucoside (αMG). The genetic marker specifying this uptake system (*umg*) is located at approx. 23.5 min on the linkage map and is co-transducible with the *pur*B marker.

2. Experimental

2.1. *Organisms used*

The organisms are listed in table 1; with the exception of *umg*, the abbreviations for genetic markers are those given in [5]. The procedures used for the growth of bacteria, and for genetic transfers, by periodic interruption of conjugation and by phage P1kc-mediated transduction, have been previously described [6]; the method used for measurements of the activity of the PT-system, and the rates of uptake of methyl-α[^{14}C]-glucoside, by washed bacterial suspensions are reported elsewhere [8].

Table 1
Organisms used in this study.

Strain	Derived from	Genetic markers	Response to streptomycin	Mating type
K2	laboratory stock [6]	*trp, his, arg, thr, leu*	R	F$^-$
K2.2w	K2	*trp, his, arg, thr, leu, umg*	R	F$^-$
1075	(a)	*pyr C*	R	F$^-$
0144	(a)	*pur*B	R	F$^-$
0.144.W	0144	*pur*B, *umg*	R	F$^-$
B11	(b)	*met*	S	Hfr
K1	laboratory stock [6]	*met, thy*	S	Hfr
AT2571	(c)		S	Hfr

(a) Gifts from Prof. A.H. Stouthamer (Nijmegen),
(b) gift from Prof. R.H. Pritchard (Leicester), see [7];
(c) gift from Prof. A.L. Taylor (Denver).

2.2. *Isolation of umg-mutants*

The uptake of fructose by *E. coli* is powerfully inhibited by glucose; in many strains, non-catabolizable analogues of glucose, such as αMG, 2-deoxyglucose (DG) and 3-deoxy 3-fluoroglucose (DFG), have the same effect. By incubating cultures of such strains at 37° for

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2 – 4 days on media, solidified with 1% (w/ v) 'Oxoid' Agar No. 1, containing salts [6], 5 mM fructose and either 5 mM DG or 0.2 mM DFG, mutants were readily obtained whose growth on fructose was unaffected by the presence of the glucose analogues. These mutants were purified by repeated single-colony isolation and were grown on 'Oxoid' nutrient agar supplemented with 10 μM methyl-α-[^{14}C] glucoside (3.8 Ci/mole; Radiochemical Centre, Amersham). After growth at 37° for 8 hr, Whatman No. 40 filter paper was pressed onto the colonies; the paper was air-dried and left in contact with Kodak 'Blue Brand' X-ray film overnight. Only clones that take up the labelled glucose analogue blacken the film; clones corresponding to those that do not were presumed to be UMG$^-$ and were studied further.

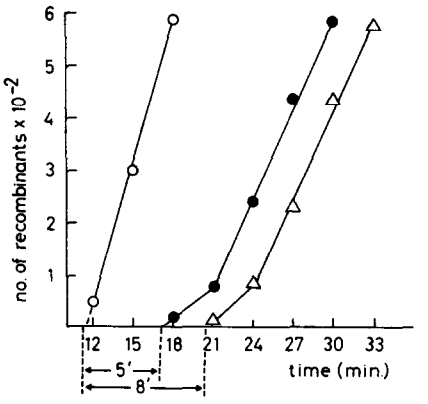

Fig. 1. Kinetics of transfer at 37° of the markers conferring ability to grow on galactose (o-o-o), and on glucose (•-•-•), and the loss of requirement for tryptophan (△-△-△), from the Hfr-strain B11 to the F$^-$-recipient K2.2w.

3. Results and discussion

A DFG-resistant mutant K2.2w was obtained from the F$^-$ strain K2; it differed from its parent particularly in its slow growth on glucose (doubling time approx. 4 hr), the reduced rate at which washed suspensions of cells grown on nutrient broth supplemented with glucose took up [^{14}C] glucose, and the failure of such washed suspensions to take up α[^{14}C] MG. On the other hand, the rates of growth of K2.2w on glucose 6-phosphate, gluconate, fructose or gluconeogenic substrates do not differ significantly from those of the parent organism, K2. This behaviour indicates that K2.2w is a mutant in an Enzyme II for glucose utilization via the PT-system.

The gene specifying this defective system for the uptake of glucose and, most noticeably, for the uptake of αMG (*umg*) was located by periodic interruption of conjugation between the Hfr strain B11 (which injects its genome in the order *o-gal-trp-his*, [7]) and K2.2w; as apparent from fig. 1, the UMG$^+$-character, specifying the ability of K2.2w to grow rapidly on glucose and to take up α[^{14}C] MG, entered approx. 5 min after *gal* and 3 min before *trp*. This location was confirmed by phage P1kc-mediated transduction of the UMG$^-$-character from K2.2w into a recipient strain (0144) that carried the *pur*B marker and that therefore requires adenine for growth. The transductants, isolated from plates devoid of adenine and containing fructose as sole source of carbon, were tested for their ability to take up methyl-α-[^{14}C] glucoside from agar plates (see sect. 2.2): approx. 22% of such transductants failed to take up the labelled glucose analogue and were thus UMG$^-$. In contrast, there was no linkage of *umg* to *pyr*C when, in a similar transduction, strain 1075 served as recipient.

Two classes of recombinants, obtained from the cross [B11 × K2.2w] illustrated in fig. 1, were tested for their ability to grow on glucose and to take up methyl-α-[^{14}C] glucoside from solution [8]. As expected, colonies identified as UMG$^-$ grew poorly on glucose and failed to take up the labelled glucose analogue; this applied to both GAL$^+$ and GAL$^-$ recombinants. In contrast, GAL$^+$ and GAL$^-$ recombinants identified as UMG$^+$ grew at wild-type rates on glucose and suspensions of cells harvested therefrom took up α[^{14}C] MG at rates similar to those observed with suspensions of the glucose-grown parent, K2 (fig. 2). However, although such UMG$^+$-recombinants also took up methyl-α-[^{14}C] glucoside after growth on fructose (fig. 2), on glucose 6-phosphate, and on a variety of other substrates, the rates and the extent of the uptake was much less than observed with suspensions of K2, similarly grown. Clearly, the "constitutive" UMG-system present in K2 had been replaced by the "inducible" UMG-system known [8] to be present in strain B11, in consequence of this genetic cross.

A number of observations indicate that this change, from a (potentially) "constitutive" UMG$^-$ to the "inducible" UMG$^+$ character is not an artefactual consequence of the selection of DFG- or DF-resistant mutants.

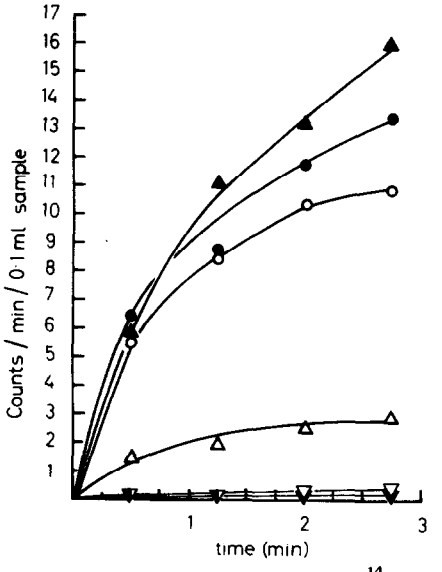

Fig. 2. Rates of uptake of 0.1 mM methyl-α-[^{14}C]glucoside by washed suspensions of strain K2 grown on fructose (○-○-○) or on glucose (●-●-●), of a UMG^{+}-recombinant from the cross [B11 × K2.2w] grown on fructose (△-△-△) or on glucose (▲-▲-▲), and a UMG^{-}-recombinant from the same cross grown on fructose (▽-▽-▽) or on glucose (▼-▼-▼).

In the first place, the "inducible" UMG-character is introduced by strain B11, and by other "inducible" strains such as K1 and AT2571 (see table 1), into the "constitutive" wild-type strain K2 at the same locus as it is introduced into K2.2w. Secondly, transduction of the UMG^{+}-character, via phage P1kc grown on the "constitutive" organism K2, into the DFG-resistant (UMG^{-}) recipient 0144.W (*pur*B) yields transductants that are constitutive for α[^{14}C]MG uptake, whereas the introduction of the UMG^{+}-character from the "inducible" strain AT2571 yields "inducible" recombinants for the uptake of α[^{14}C]MG. The evidence thus favours the view that the uptake system for methyl-α-glucoside is complex and may include both structural and regulatory components.

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