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Glucose Catabolite Repression in *Escherichia coli* K12 Mutants Defective in Methyl-α-D-Glucoside Transport

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1. Two spontaneous *Escherichia coli* K12 mutants resistant to glucose catabolite repression were isolated using minimal agar plates with methyl α-D-glucoside. Mutants grow well on glucose and mannitol.

2. Glucose does not inhibit the inducible enzyme synthesis in isolated mutants: mutant cell (in contrast to parent cells) produce high levels of β-galactosidase and L-tryptophanase under the conditions of glucose catabolite repression.

3. The isolated mutants are negative in methyl-α-D-glucoside transport; glucose uptake is not severely damaged. But the mutants (named *tgl*, transport of glucose) retained the ability to phosphorylate methyl α-D-glucoside *in vitro* at the expense of phospho*enol*pyruvate.

4. The *tgl* mutation is cotransduced with *purB* and *pyrC* markers, *i.e.* locates near 24 min of the *E. coli* chromosome map.

5. It is thought that *E. coli* cells possess two glucose transport systems. The first one is represented by the glucose-specific enzyme II of the phospho*enol*pyruvate-dependent phosphotransferase system. The second glucose transport system (coded for *tgl* gene) functions as permease and possesses high affinity to methyl α-D-glucoside. The integrity of glucose permease determine the sensitivity of the cell to glucose catabolite repression.

The transfer of glucose into *Escherichia coli* K 12 is mediated by the phospho*enol*pyruvate : carbohydrate phosphotransferase system [1]. The phosphotransferase reaction coupled with the glucose transport involves the necessary activity of some proteins. Enzyme I catalyses the transfer of the phosphate group from phospho*enol*pyruvate to a small histidine containing protein (Hpr) [2, 3]. The phosphorylated form of Hpr (phospho ~ Hpr) is the phosphate donor in the second step of the phosphotransferase reaction at which the membrane-linked enzyme II, specific for glucose, carries out phosphorylation of the carbohydrate [2, 4]. At present there is some evidence that in *E. coli* a cytoplasmic factor III may participate in the last step of the phosphotransferase reaction [4].

Recently it has been reported that there exist two phospho ~ Hpr : glucose phosphotransferase systems in crude extracts of *E. coli* cells. The two systems differ in their substrate specificity [5]. In the preliminary communication it was mentioned that system A phosphorylates both glucose and methyl α-D-glucoside (nonmetabolizable glucose analogue); in the wild type of *E. coli* 40% of the glucose phosphotransferase activity is due to system A. The remaining 60% of the activity is maintained by system B which phosphorylates only glucose and has little affinity for methyl α-D-glucoside. Curtis and Epstein consider that system B represents the membrane-linked glucose-specific enzyme II but not the glucokinase or some other soluble enzyme [5]. Damage of system A leads to glucose catabolite repression resistance. The two previously described mutants, LA-12 [6] and W1895D1

Genetic Nomenclature. *tgl* = lack of the glucose permease; *gptA* = lack of the phospho ~ Hpr : glucose phosphotransferase activity; *umg* = lack of the methyl-α-D-glucoside uptake system. Other symbols are standard, see [9].

Enzymes. β-Galactosidase (EC 3.2.1.23); L-tryptophanase (EC 4.2.1.20).

[7], do not have glucose phosphotransferase A activity; therefore it is possible that the *gptA* mutation is identical to the *cat* mutation, rendering enzyme synthesis resistant to catabolite repression and mapping at 24 min of the *E. coli* chromosome [7].

Kornberg *et al.* [8] described a mutant with an altered methyl-α-D-glucoside transport and phospho*enol*pyruvate-dependent phosphorylation of this carbohydrate *in vitro*. The K22w mutation (named *umg*, uptake of methyl glucoside) was located at the 24th min of the linkage map of *E. coli* K12 [9]. However, the resistance of this mutation to catabolite repression was not investigated.

With the information given above as a background, it may be supposed that at the region of 24 min there is (are) located gene(s) for one of the phospho ~ Hpr : glucose phosphotransferase activities. This system mediates the transport of methyl α-D-glucoside and it defines the sensitivity of *E. coli* to glucose catabolite repression.

It is the main purpose of this paper to report the isolation and properties of two *E. coli* mutants resistant to glucose catabolite repression. Though these mutants have an altered methyl-α-D-glucoside transport, they (in contrast to those mentioned above) effectively phosphorylate *in vitro* methyl α-D-glucoside in the presence of phospho*enol*pyruvate. The different degree of alteration in methyl α-D-glucoside uptake leads to the equal resistance of the mutants to glucose repression. In both strains the mutation (named *tgl*, transport of glucose) is cotransduced with *purB* and *pyrC* markers (*i.e.* locates at the 24th min of the *E. coli* chromosome map). A preliminary note about one of the mutants has been published [10].

MATERIALS AND METHODS

Bacterial strains used are listed in Table 1. Phage $P1_{vir}$ was received from Dr G.B.Smirnov (Laboratory of Genetics of Bacteria).

Media

Minimal liquid media were made on the basis of the synthetic salt medium M-9 [11] and contained 0.4% of the source of carbon and all necessary additions (20 μg/ml, thiamine 0.1 μg/ml).

In phage $P1_{vir}$-mediated transduction experiments we used media proposed by Willetts *et al.* [12].

Matings were carried out in broth according to the standard procedure [11].

The minimal agar was prepared as proposed by Hayes [11]; EMB glucose indicator medium was described previously [13]. Streptomycin, when used, was present at a concentration of 0.2 mg/ml.

Table 1. *List of* Escherichia coli *K12 strains*

Strain	Genotype	Notes
Hfr Cavalli	Hfr, *met*, str^s	
P5	HfrC, *met*, str^s, *tgl*	isolated from HfrC
ts19	HfrC, *met*, $ptsI^{ts}$, str^s	[13]
ts194	HfrC, *met*, $ptsI^{ts}$, *tgl*, str^s	isolated from ts19 [10]
W1895D1	Hfr, *met*, *gptA*, bgl^+, str^s	received from V. N. Kuznetsova
W1655	$F'lac^+/met$, str^s	received from V. N. Kuznetsova
0144	F^-, *thi*, *purB*, *lac*, *gal*, *mal*, *lam*, *tonA*, *tsx*, *xyl*, str^s	received from H. L. Kornberg
X5028	F^-, *pro*, *lac*, *tre*, *arg*, *gal*, *pyrC*, str^s	received from B.J. Bachman
A1	F^-, *thi*, *purB*, *gal*, *mal*, *lam*, *tonA*, *tsx*, *xyl*, str^r	W1655 × 0144 recombinant
U7	F^-, *tre*, *arg*, *gal*, *pyrC*, str^r	W1655 × X5028 recombinant

Growth of Bacteria

Routinely cultures were grown aerobically in salt liquid medium with casamino acids (0.4%, Serva) and glucose (0.4%). Since strains ts19 and ts194 possessed thermosensitive enzyme I of the phosphotransferase system, cells of these strains were grown at 27 °C, while strains HfrC and P5 were cultivated at 37 °C. Cells were collected on membrane filters (HUFS, Synpor, pore size 0.45 μm) in the middle phase of exponential growth, washed with fresh salt medium of room temperature and resuspended ($\approx 0.05-0.1$ mg of protein per ml) in M-9 medium without supplements.

Determination of Resistance of Cultures to Glucose Catabolite Repression

Resistance of cultures to glucose catabolite repression was determined using plates with minimal agar containing glucose (1%) and lactose (0.2%) as carbon sources [14]. Single colonies of cultures were suspended in 0.3 ml of sterile 0.85% NaCl and drops of suspension were placed on the agar. Plates were incubated for 18 h at 37 °C. The grown cultures were treated with vapour of toluene (30 min at 37 °C) and a small amount of 1 mM *o*-nitrophenyl β-D-galactopyranoside was layered on the surface of the agar. The reaction of hydrolysis of β-galactoside was stopped in 5 min by addition of 1 M Na_2CO_3. Cultures resistant to glucose catabolite repression produced β-galactosidase on this medium and they

were coloured yellow, but the sensitive ones remained colourless.

Measurement of Carbohydrate Accumulation

Cell suspension was incubated with 10 μM labelled sugar at the appropriate temperature in the water bath. Samples (2 ml) were withdrawn at intervals and filtered with suction through HUFS membrane filters. The filters were washed with 5–7 ml of salt medium at the temperature of incubation, dried and their radioactivity was determined. Cell filtration and washing routinely took 15–20 s. Methyl α-D-[U-^{14}C]-pyranoside (specific activity 3 mCi/mmol) and [U-^{14}C]-glucose (specific activity 3 mCi/mmol) were from Amersham.

Preparation of Bacterial Extracts

Cells grown for 4 h in medium with casamino acids and glucose were harvested, washed with 0.85% NaCl and resuspended in a buffer for disruption: 0.01 M Tris-HCl pH 7.8 (Serva), 0.1 mM $MgCl_2$, 0.1 mM EDTA (Serva), 1 mM dithiothreitol (BDH). The suspension was sonicated at 0 °C for 4 min (4 × 1 min with 1-min intervals) on an MSE oscillator. The sonicate was centrifuged (20 min, 12000 × *g* at 0 °C) and supernatant (4–6 mg of protein per ml) was used in experiments.

*Assay of Phospho*enol*pyruvate-Dependent Phosphotransferase*

Phospho*enol*pyruvate-dependent phosphotransferase activity was measured by the amount of methyl α-D-[^{14}C]glucoside phosphate formed during a 30-min incubation period at 27 °C in a mixture containing: 50 mM potassium salt of phospho*enol*pyruvate (Calbiochem), 0.5 mM $MgSO_4$, 2 mM dithiothreitol, 0.5 mM [^{14}C]glucose, 0.1 ml (or 0.05 ml) of cell extract (100–300 μg of protein) and 0.01 M Tris-HCl (pH 7.6) to a final volume of 0.5 ml. Phospho*enol*-pyruvate was omitted from the blank. The reaction was stopped by adding 0.5 ml of an ice-cold 0.2 M unlabelled sugar. The mixture was transferred onto a Dowex 1X8 formate column (0.7 × 1.5 cm). The columns were washed with 6 ml of water. Sugar phosphate was eluted with 2.5 ml of 0.2 M formic acid in 0.5 M ammonium formate solution and the radioactivity of 1 ml of eluate was determined.

Assays of β-Galactosidase and L-Tryptophanase

Cultures were grown aerobically to the middle of the log phase in the salt medium with glucose (0.4%) and methionine (strains ts19 and ts194 at 27 °C, strains P5 and HfrC at 37 °C). Then [U-^{14}C]leucine (specific activity 10 mCi/mmol, Amersham) was added to cultures together with inducer (0.1 mM isopropyl β-D-thiogalactopyranoside or 1.5 mM L-tryptophan).

Samples of the suspension were taken at the appropriate time intervals and activities of β-galactosidase or L-tryptophanase were determined by the methods described earlier [15, 16]. The rate of [^{14}C]-leucine incorporation into trichloroacetic-acid-insoluble cell fraction was the measure of protein synthesis. A correction corresponding to basal enzyme activity was subtracted from the estimated values.

Measurement of Radioactivities of Samples

The radioactivities of samples were counted in a liquid scintillation counter SL-20 (Intertechnique) by the method of Bray [17] or in a toluene scintillator liquid [13].

Transduction and Mating Experiments

Matings were performed at 37 °C by the method of Clowes and Hayes [11]. *lac*$^+$ recombinants of *pyrC* and *purB* strains have been isolated for mapping of the *tgl* mutation.

Genetic mapping by $P1_{vir}$ phage transduction was carried out according to procedure described by Willetts *et al.* [12]. (The multiplicity of infection was 0.1.)

Determination of Doubling Time of Cultures

Studies on growth rates of cultures were carried out using biophotometer B10-LOG II (Jouan, France). Growth was monitored by reading (every 2 min) the turbidity of cell suspension.

The cultures were grown aerobically in thermostated glass cuvettes (volume 10 ml, with 30-mm light path). Cells were adapted to the appropriate substrate overnight, and cultures were diluted 10-fold with the fresh medium before experiments.

Protein concentrations in cell suspensions and extracts were measured according to Lowry *et al.* [18].

RESULTS

Isolation of Mutants Resistant to Glucose Catabolite Repression

Methyl α-D-glucoside, as well as glucose, produces a severe repression of the inducible enzyme production. However methyl α-D-glucoside, in contrast to glucose, is a non-metabolizable substance for *E. coli*. So, by

Table 2. *Growth properties of selected mutants*

Strain	Growth temperature	Colour of colonies on EMB agar + glucose	Doubling time with	
			glucose	mannitol
	°C		min	
ts19	27	bronze	117.5	139.5
ts194	27	bronze	121	150
HfrC	37	bronze	70	115
P5	37	dark	78.5	128

our supposition, in the presence of this glucose analogue it is possible that only those cells in which induced enzyme synthesis is insensitive to methyl α-D-glucoside repression will grow.

The *E. coli* systems for utilization of mannitol and lactose are inducible [19,20]. Therefore on the minimal agar plates containing 1 mM methyl α-D-glucoside, lactose (0.4%) and mannitol (0.4%) will appear colonies of the cells with the systems of mannitol and lactose utilization insensitive to methyl-α-D-glucoside repression. The mutants were isolated by spreading cells on agar plates with methyl α-D-glucoside. Plates were incubated for 48 h at 27 °C when strain ts19 was used as the parent strain and at 37 °C in the case of strain HfrC. The grown colonies were tested for their resistance of β-galactosidase formation to catabolite repression by glucose.

Two spontaneous mutants resistant to glucose catabolite repression, ts194 and P5, were taken for the following investigations. Table 2 contains some growth properties of mutants and their parents.

The ability of mutant strains to grow well on glucose as the sole carbon source gave us the possibility to examine in detail the synthesis of inducible enzyme under the conditions of the glucose catabolite repression. It became clear that cells of strains ts194 and P5 (in contrast to their parent cells) manifested high rates of induction of β-galactosidase and L-tryptophanase (Fig. 1 and 2). According to Curtis and Epstein [5] the strain W1895D1, which is resistant to methyl-α-D-glucoside repression [7,21], has an altered phospho*enol*pyruvate-dependent phosphorylation of this carbohydrate *in vitro*. However, to our surprise, we found that in ts194 and P5 mutant strains the appearance of the resistance to glucose catabolite repression was not accompanied by a decrease in the rate of phospho*enol*pyruvate-dependent phosphorylation of methyl α-D-glucoside in crude cell extracts (Table 3). In fact, the phosphotransferase activity registered with methyl α-D-glucoside is somewhat higher in extracts of mutant than of parent cells.

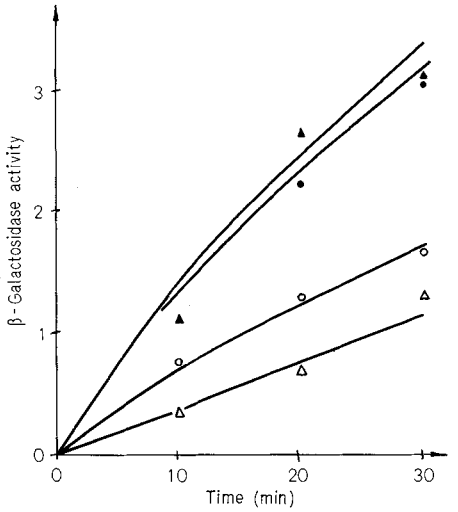

Fig. 1. *β-Galactosidase synthesis under the conditions of glucose catabolite repression in strains HfrC (○), P5 (●), ts19 (△), ts194 (▲).* β-Galactosidase activity was measured as nmol *o*-nitrophenol formed per min per 10^6 counts of [^{14}C]leucine

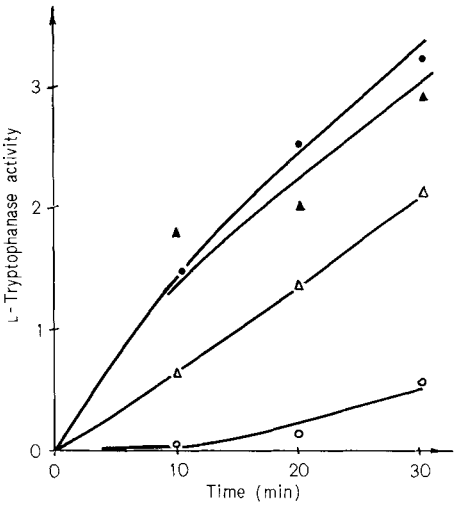

Fig. 2. *L-Tryptophanase synthesis under the conditions of glucose catabolite repression in strains HfrC (○), P5 (●), ts19 (△), ts194 (▲).* L-Tryptophanase activity was measured as nmol indole formed per min per 10^4 counts of [^{14}C]leucine

Table 3. *Phospho*enol*pyruvate-dependent phosphorylation of methyl α-D-glucoside*
The activity was measured as nmol of methyl α-D-glucoside phosphate formed per mg protein per 60 min at 27 °C

Strain	Activity
	nmol × mg^{-1} × h^{-1}
ts19	4.48
ts194	6.51
HfrC	20.48
P5	44.28

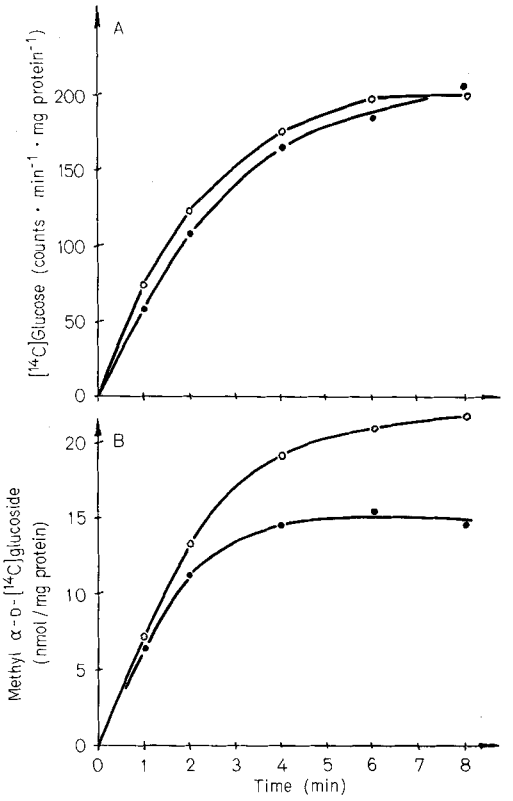

Fig. 3. *Accumulation of [^{14}C]glucose (A) and methyl α-D-[^{14}C]-glucoside (B) by cells of strains HfrC (○) and P5 (●)*

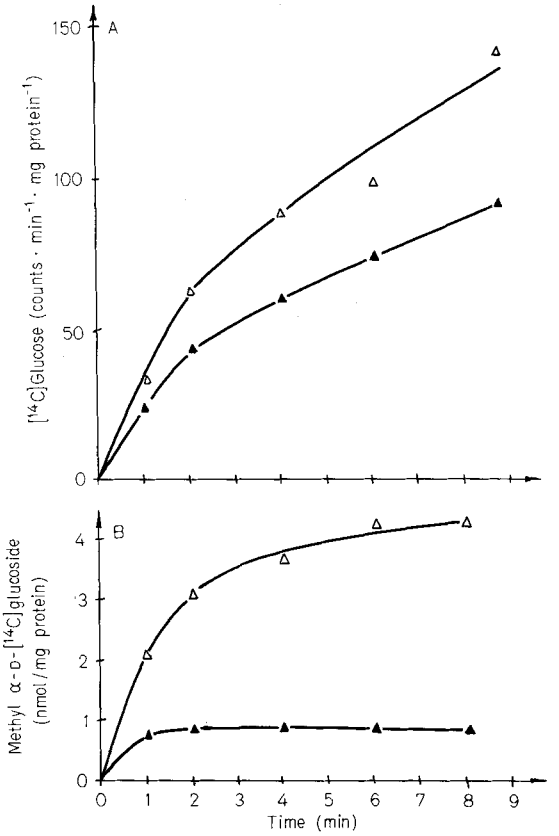

Fig. 4. *Accumulation of [^{14}C]glucose (A) and methyl α-D-[^{14}C]-glucoside (B) by cells of strains ts19 (△) and ts194 (▲)*

Decrease of Methyl-α-D-Glucoside Transport in ts194 and P5

The absence of the repressive effect of methyl α-D-glucoside on mutant cells may be due to their inability to accumulate this glucose analogue. Indeed, the experiments indicated the low rate of methyl-α-D-[^{14}C]glucoside accumulation in mutant cells: P5 accumulated the carbohydrate 40–50% less than the parent strain HfrC, and in ts194 the reduction of methyl-α-D-[^{14}C]glucoside accumulation was more than 90% in comparison with ts19 (Fig. 3B and 4B). It appeared also that strains P5 and HfrC were practically similar in their ability to take up [^{14}C]-glucose, while in mutant strain ts194 the glucose uptake was less than in ts19 (Fig. 3A and 4A).

However the reduction of methyl-α-D-glucoside transport in mutant cells might be due to increasing rate of carbohydrate efflux because of the activation of the acid phosphatase, since the steady-state level of cell saturation with methyl α-D-[^{14}C]glucoside depends on the relation between the rates of influx and efflux of free carbohydrate [22]. However, the latter are determined by rates of methyl-α-D-glucoside-phosphate dephosphorylation with acid phosphatase [23, 24].

Activity of acid phosphatase of methyl-α-D-glucoside phosphate and phospho*enol*pyruvate-dependent phosphorylation of methyl α-D-glucoside were found to be inhibited by NaF [24]. Since the addition of NaF at the steady-state step of methyl-α-D-glucoside accumulation will fix the amount of methyl-α-D-glucoside phosphate in the cell, we should be able to determine its level and the rate of efflux of free carbohydrate produced at the moment of addition of inhibitor [24].

It appears from the information of Fig. 5 and 6 that (a) the rates of efflux of free methyl α-D-glucoside from mutant and parent cells are practically equal; (b) the ratio of methyl α-D-glucoside and methyl α-D-glucoside phosphate in mutants is about the same as in the parents. These results do not support the assumption of increased activity of the acid phosphatase in mutant cells.

Therefore, the isolated mutants possess the following properties: (a) they are resistant to glucose catabolite repression; (b) they are unable to transport methyl α-D-glucoside and (c) the transport alterations

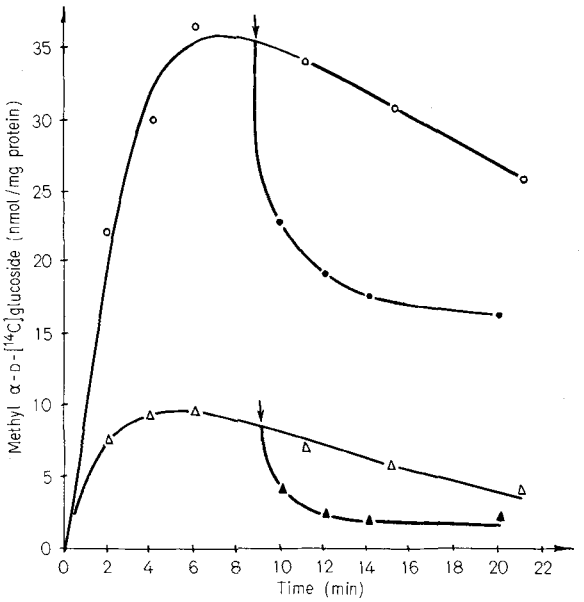

Fig. 5. *Effect of 50 mM NaF (pH 6.0; 30 °C) on methyl-α-D-[^{14}C]-glucoside accumulation in strains HfrC (○) and P5 (△).* The inhibitor was added to the bacterial suspension at the time indicated by the arrow. Dark symbols = accumulation in the presence of NaF.

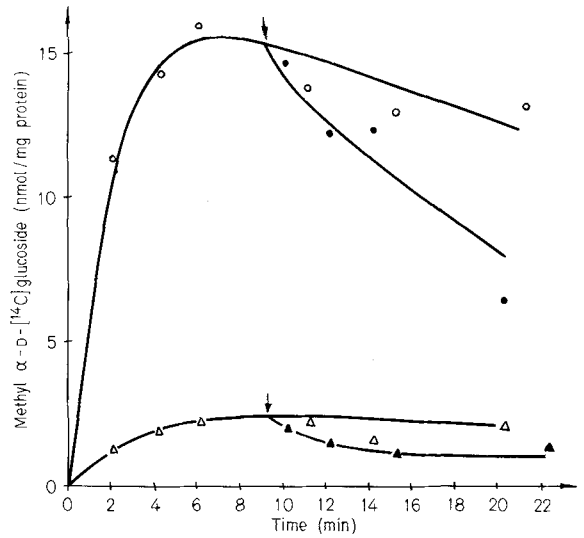

Fig. 6. *Effect of 50 mM NaF (pH 6.0; 27 °C) on methyl-α-D-[^{14}C]-glucoside accumulation in ts19 (○) and ts194 (△).* The inhibitor was added to bacterial suspension at the time indicated by the arrow. Dark symbols = accumulation in the presence of NaF

are not accompanied by a decrease of the phospho*enol*pyruvate-dependent phosphorylation of the carbohydrate *in vitro*.

However, the transport of sugars *via* the phospho*enol*pyruvate-dependent phosphotransferase system is always coupled with the phosphorylation of the substrate [1,3]. The analysis of our data leads us to a new suggestion that *E. coli* apparently has another transport system for glucose (with high afffnity to methyl α-D-glucoside) and the transport of the carbohydrates *via* this system is not coupled to modification of the substrate, *i.e.* this transport system functions as a permease. We named this transport system as glucose permease and the mutation altering the gene(s) coding for it was designated as *tgl* (transport of glucose). The carbohydrate transported *via* glucose permease is phosphorylated intracellularly at the expense of the protein phospho ~ Hpr.

Table 4. *Transport competition between glucose and methyl α-D-glucoside*
Results are given as a percentage of inhibition of the transport of the labelled sugar (10 μM) with the unlabelled one (100 μM); incubation time 1 min at 27 °C or 37 °C

Strain	Temperature	Methyl α-D-[^{14}C]glucoside + glucose	[^{14}C]Glucose + methyl α-D-glucoside
	°C	%	
ts19	27	85.5	69
ts194	27	53.5	10
HfrC	37	88.6	76.6
P5	37	70	50

Our suggestion about the existence in *E. coli* of two glucose transport systems is confirmed in experiments concerning competition between glucose and methyl α-D-glucoside for entry into the cells (Table 4).

It can be seen that glucose exerts inhibition of methyl-α-D-glucoside transport both in mutant and parent cells. However, methyl α-D-glucoside affects glucose transport only in the parent strain ts19. Probably, the two carbohydrates (because of their different affinities for enzyme II and glucose permease) compete in parent bacteria only for the protein phospho ~ Hpr. Therefore in mutant strain ts194, in the absence of a transport system for methyl α-D-glucoside, this carbohydrate is not able to compete with glucose for protein phospho ~ Hpr. The inhibition of glucose uptake in strain P5 is not so large since the damage of methyl-α-D-glucoside transport system (glucose permease) in this mutant is less expressed than in strain ts194 and the accumulated methyl α-D-glucoside can compete with glucose for protein phospho ~ Hpr.

The existence of two glucose transport systems with different affinities to glucose and its analogues confirms the fact that the glucose accumulation in *tgl* mutants decreases less than the uptake of methyl α-D-glucoside. Apparently the transport activity of en-

Table 5. *Mapping of ts194, P5, W1895D1 mutants*
The frequency of cotransduction is the percentage of recombinants containing both the unselected marker (A, resistance to catabolite repression) and the selected marker (B) given in the table

Donor	Recipient	Selected marker (B)	Ratio A/B	Frequency of cotransduction
				%
ts194	A1	*purB*$^+$	14/460	3.1
ts194	U7	*pyrC*$^+$	12/107	11.3
P5	A1	*purB*$^+$	15/111	13.6
P5	U7	*pyrC*$^+$	20/331	6.0
W1895D1	A1	*purB*$^+$	7/80	8.8
W1895D1	U7	*pyrC*$^+$	11/172	6.4

zyme II is quite enough for the maintenance of growth of mutants on glucose as sole carbon source: the doubling time on glucose is practically the same both in mutant and parent cultures (Table 2).

Mapping of the Mutants

The ts194 mutation was located in conjugation between the ts194 strain and the recipient with *purB* and *his* markers. These experiments showed that the *tgl* mutation mapped closer to the *purB* than to *his* markers, since the major part of the forms resistant to glucose catabolite repression appeared among *purB*$^+$ recombinants [10].

The more detailed genetic analysis was performed with phage $P1_{vir}$-mediated transduction of the *tgl* character from strains ts194 and P5 into recipient strains that carried *purB* or *pyrC* markers. The transductants isolated from plates devoid of adenine or uracil and containing glucose as sole carbon source, were tested for their resistance to glucose catabolite repression.

Since it has been proposed [5] that the *gptA* mutation is identical to the *cat* mutation we decided to map the *gptA* mutant W1895D1, which in our experiments showed resistance to glucose catabolite repression. The results of transduction are represented in Table 5 and they indicate that ts194 and P5 mutations are cotransduced with *pyrC* and *purB* markers with the same frequency as *gptA* mutation does, *i.e.* *tgl* (and *gptA*) mutation is located on 24 min of the *E. coli* chromosome map.

DISCUSSION

The transport of methyl α-D-glucoside and glucose in the *E. coli* cell until now has been attributed to the phospho*enol*pyruvate-dependent phosphotransferase system [1]. The transfer of carbohydrate *via* this system is carried out on the principle of group translocation, *i.e.* the molecule of substrate is modified during transmembrane passage, in this case it is phosphorylated at the expense of protein phospho ~ Hpr. This reaction is carried out by membrane-linked enzyme II [1 – 3]. Decrease of the phosphotransferase activity (as the result of blocking the phospho ~ Hpr generation system or in the case of mutational damage of the sugar specific for enzyme II) leads to alteration of the transport function of the phospho*enol*pyruvate-dependent system [1]. However, as reported in this paper, we succeeded in selecting mutants in which the alterations in methyl-α-D-glucoside transport are not accompanied with the decrease of the phospho*enol*-pyruvate-dependent phosphotransferase activity *in vitro*. It must be emphasized that these transport-negative mutants (*tgl* mutants) are resistant to glucose catabolite repression. The *tgl* mutation is cotransduced with *purB* and *pyrC* genes, *i.e.* locates near 24 min of the *E. coli* chromosome map.

On the basis of our studies we assumed that there are two transport systems for glucose in *E. coli*: the first constitutes the glucose-specific enzyme II and glucose transport *via* this system is coupled to phospho*enol*pyruvate-dependent phosphorylation of the sugar. Another system represents glucose permease which mediates the transfer of glucose by means of facilitated diffusion or active transport. Glucose (and its analogue) transported *via* glucose permease is phosphorylated intracellularly at the expense of protein phospho ~ Hpr. The *tgl* gene which codes for glucose permease maps near the *purB* and *pyrC* genes (the 24th min). The location of the gene for glucose-specific enzyme II has not yet been established. The two transport systems possess different substrate specificities: glucose is transported *via* both enzyme II and glucose permease whereas methyl α-D-glucoside permeates mainly *via* glucose permease.

Support for our assumption may be found in the literature. Kornberg and his coworkers also suppose the existence of two glucose transport systems in *E. coli* [25], but they connect them with the function of inducible and constitutive enzyme II for glucose. The inducible enzyme II besides glucose also transports methyl α-D-glucoside but the constitutive enzyme II only has affinity for glucose. The genetic marker specifying the inducible uptake system (*umg*) is located near 24 min on the linkage map and is cotransducible with *purB* marker [8, 25].

Strain LA12 is resistant to glucose catabolite repression (*cat* mutant) and in cell extracts of this mutant the glucose phosphotransferase activity was not detected either (*gptA* mutation) [5]. However, there is no information concerning the transport of

methyl α-D-glucoside and glucose in LA12 cells. It is known, however, that the *cat* mutation is cotransduced with *purB* and *pyrC* [9,14].

Gachelin mentions in his paper about the *E. coli* mutant 3300-GG-21 [26]. The author considers this mutant defective in the glucose-specific enzyme II of the phospho*enol*pyruvate-dependent phosphotransferase system. However, the mutant resembles the *umg* mutant K22w. It retains the ability to grow on glucose as the sole source of carbon, but the phospho*enol*pyruvate-dependent phosphorylation of methyl α-D-glucoside *in vivo* and the uptake of this glucose analogue are much less than those in the wild-type strain [26]. The 3300-GG-21 mutant was not mapped and its sensitivity to catabolite repression was not investigated.

The *tgl* mutants selected by us are negative in methyl-α-D-glucoside transport (the glucose uptake is not severely damaged). This mutation also leads to catabolite repression resistance and maps near *pyrC* and *purB* genes. In this connection it is of great interest to compare our data with the results obtained by Kornberg *et al.* concerning the observation about the existence in *E. coli* crude extracts of two phospho ~ Hpr: glucose phosphotransferase activities [8].

The *umg* mutation in K22w causes the alteration of methyl-α-D-glucoside uptake (partially reducing the glucose accumulation) and *in vitro* it leads to great decrease in the rate of the phospho*enol*pyruvate-dependent phosphorylation of methyl α-D-glucoside (and to a lesser extent of glucose) [8]. However, the resistance of this mutant to glucose catabolite repression was not verified.

Synthesis of β-galactosidase in mutant W1895D1 (considered earlier as defective in the glucose-specific enzyme II) is resistant to repression by glucose or methyl α-D-glucoside [7,21]. Also in this mutant the lack of methyl-α-D-glucoside transport is accompanied by absence of the phospho*enol*pyruvate-dependent phosphorylation of the carbohydrate *in vivo* (*gptA* mutation) [5]. Examination of W1895D1 in our laboratory confirmed the above results and we also found a decrease of phospho*enol*pyruvate-dependent phosphorylation of glucose in cell extracts of this mutant (data not shown). We also found that the mutation in W1895D1 was cotransduced with the *purB* and *pyrC* markers (Table 5).

All the mutations mentioned above (*umg*, *gptA*, *cat*, *tgl*) are cotransduced with *purB* markers, *i.e.* are mapped near 24 min of chromosome map. It may well be that in the region of *purB* and *pyrC* loci of the *E. coli* chromosome there are located the genes coded for (a) one of the glucose transport systems and (b) the system of the phospho ~ Hpr-dependent phosphorylation of glucose and methyl α-D-glucoside.

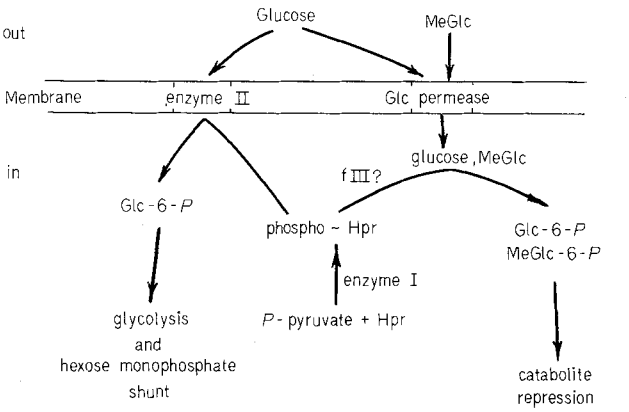

Fig. 7. *Hypothetical scheme of glucose transport in Escherichia coli K12.* MeGlc = methyl α-D-glucoside; Glc permease = glucose permease; Glc-6-*P* = glucose 6-phosphate; *P*-pyruvate = phospho*enol*pyruvate; MeGlc-6-*P* = methyl α-D-glucoside 6-phosphate; fIII = factor III

The mutational damage of these genes may produce alterations either in transport or both in transport and phosphorylation of glucose and its analogue. However, any disturbance of the genes integrity leads to the appearance of resistance to glucose catabolite repression in mutants. The proposed function of the two transport systems for glucose is schematically shown in Fig. 7.

It is probable that the *tgl* gene coding for glucose permease is linked to gene(s) for the protein(s) carrying out the intracellular phospho ~ Hpr-dependent phosphorylation of glucose and methyl α-D-glucoside. Therefore, in mutants W1895D1, K22w (and probably in 3300-GG-21) one mutation may lead to loss of the two functions (transport and phosphorylation) at once.

The nature of the protein(s) responsible for the intracellular phospho ~ Hpr-dependent phosphorylation of glucose and methyl α-D-glucoside is still an open question.

The role of the glucokinase must be excluded since in *E. coli* it does not have an affinity for methyl α-D-glucoside and besides this enzyme carries out the ATP-dependent phosphotransferase reaction.

As was mentioned in the Introduction, the *E. coli* factor III takes part in transfer of the phosphate group from phospho ~ Hpr on glucose. Factor III is a protein of molecular weight ≈ 20000 and is always found in the soluble fraction of the *E. coli* cell [4].

According to preliminary communications of Roseman and his coworkers, the *crr* gene either codes for or regulates the synthesis of factor III [4,27,28]. The *crr* mutants of *E. coli* were resistant to the repression mediated by the phospho*enol*pyruvate-dependent phosphotransferase system for synthesis of the enzymes required for catabolism of lactose and tryptophan [27,28]. It is possible that factor III is that

protein which phosphorylates (at the expense of phospho ~ Hpr) glucose and methyl-α-D-glucoside transported *via* glucose permease.

An effect of the *tgl* mutation can be interpreted in terms of an hypothesis about the existence of two sites in enzyme II of the phospho*enol*pyruvate-dependent phosphotransferase system. According to Tanaka *et al.* [29] and Gachelin [26] one site is responsible for the facilitated diffusion of methyl α-D-glucoside and glucose and the phospho*enol*pyruvate-dependent phosphorylation of the sugars takes place on another site of enzyme II. It may be also that the *tgl* mutation leads to a loss of the first site. In some cases (mutants K22w, W1895D1 and 3300-GG-21) the mutation affects the synthesis of the whole glucose-specific enzyme II, blocking the transport and phosphorylative function of the enzyme.

However, from the preceding discussion it is clear that our assumption is simpler and more attractive and the glucose transport system studied functions as represented in Fig. 7, *i.e.* there is the glucose permease (coded for by the *tgl* gene) and phosphorylation of the sugar (the limiting step in methyl-α-D-glucoside accumulation) is carried out by factor III intracellularly.

Analysis of our data leads to a new conclusion, that is that the function of one of the glucose transport systems and intracellular phospho*enol*-pyruvate-dependent phosphorylation of glucose determine the ability of glucose to repress the synthesis of catabolite enzymes in *E. coli*.

An intact glucose permease is necessary for the manifestation of catabolite repression, *i.e.* probably it is important to that pool of glucose 6-phosphate which is produced intracellularly during phospho*enol*pyruvate-dependent phosphorylation of sugar.

Resistance to glucose catabolite repression will be observed in the case of blocking of the intracellular phospho*enol*pyruvate-dependent phosphorylation. The same result will have a mutation affecting production of phospho ~ Hpr (*ptsI* or *ptsH* mutations), or mutation in gene(s) for factor III. In fact, it has been established in our laboratory that in a *ptsI*, *ptsH* mutant P34lac$^+$ an inducible synthesis of β-galactosidase, L-tryptophanase and D-serine deaminase (in different degrees) was resistant to glucose catabolite repression [30]. According to Tyler *et al.*, *ptsI* mutants GN2 and MM6 were also resistant to catabolite repression [31].

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