ISOLATION AND INVESTIGATION OF THE *ESCHERICHIA COLI* MUTANT WITH THE DELETION IN THE *ptsH* GENE

T. N. BOLSHAKOVA, O. Y. DOBRYNINA and V. N. GERSHANOVITCH
The Gamaleya Institute for Epidemiology and Microbiology, Academy of Medical Sciences, Moscow D98, USSR

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1. Introduction

The phosphoenolpyruvate (PEP): sugar phosphotransferase system (the PT-system) is known to regulate the synthesis of certain inducible enzymes [1,2]. The behaviour of *ptsI* and *ptsH* mutants (i.e., mutants with defective common components of the PT-system enzyme I and protein HPr) indicates that *pts* gene products are functional in inducible enzyme synthesis [2]. These mutations lead to the abrupt decrease in phospho~HPr – a donor of the phosphoryl group in the vector reactions of carbohydrate phosphorylation catalyzed by the specific components of the PT-system [3,4]. Besides alteration of the in vitro phosphotransferase reaction and transport function of the PT-system, the lack of phospho~HPr leads to changes in regulation of inducible enzyme synthesis, characteristic to *pts* mutants [1]:

(i) Enzyme synthesis is repressed and not restored with cyclic AMP to the wild-type level;
(ii) *pts* mutants are resistant to glucose catabolite repression.

According to [1] these effects are due to disruption of transcription. It has been supposed that phospho~HPr is necessary for the effective activation of the promotor of catabolite-sensitive operons with the cyclic AMP–receptor protein complex [1]. Thus it would be of interest to study the behaviour of a mutant with a deletion for the *ptsH* gene. We have described a mutant with a deletion covering the *ptsH* gene [5]. But more extensive investigations have demonstrated that this deletion also covers the *ptsI* gene. Therefore, we tried to select an *Escherichia coli* K12 mutant with a deletion which encompasses only the *ptsH* gene. Here we present the results of investigations of PT-system functions in the absence of the common component, HPr.

2. Experimental

Escherichia coli K12 strains used in this work are listed in table 1. Growth media, genetic procedure,

Table 1
Escherichia coli K12 strains used in this work

Strain	Genotype	Origin
KS5	*HfrH, thi Δ (bio nic attBB')*	[5,7,8]
j623ilv	*F⁻, trp purC ilv* str^I	[1]
ts19	*HfrC, met* $ptsI^{ts}$	[1]
ORF1/P648	*recA56 purC trp* (+Mu-1 cts61)/*F' trp⁺ purC⁺ ptsI ptsH⁺* (+Mu-1 cts61)	From Dr O. Y. Rusina
OD62	*HfrH, thi Δ (bio nic attBB')* (λ*C1857* in *ptsH*)	This work
OD628	*HfrH, thi Δ (bio nic attBB') ptsH mal lamB*	This work
jOD5	*F⁻, trp Δ ptsh ilv* str^I	This work

Table 2
PEP-dependent PT-activity in cell-free extracts (nmol sugar phosphate .mg protein^{-1} .30 min^{-1} at 37°C)

Strain	Methyl-α-glucoside 10^{-4} M	Glucose 10^{-4} M	Fructose (0.89×10^{-5} M)
j623ilv	13 16[a]	45	19[a]
jOD5	0.5 2[a]	37	17[a]
OD62[b]	0	6	n.d.

[a] Cells were grown in the presence of 0.5% fructose
[b] Cells were grown at 32°C

n.d., not determined

enzyme assays and materials were as in [5–8]. For the preparation of extracts, cells were grown to the middle log phase in 2 vol. mineral salt medium M9 with 1 vol. broth. All sugars (except L-arabinose) are of D-configuration.

3. Results and discussion

The desired deletion mutant was selected from cells cured of the thermosensitive prophage λCI857 transposed into the *ptsH* gene. Initially we isolated a lysogen strain OD62 with λCI857 prophage introduced into the *ptsH* gene attachment site. Phenotypical behaviour of strain OD62 indicated *ptsH* mutation: at 32°C this mutant fermented fructose, arabinose and gluconate, but formed colourless colonies on the EMB agar indicator plates with glucose, mannose, mannitol, sorbitol, lactose, maltose and glycerol. The isolated strain was identified as a *ptsH* mutant (tables 2,3). The attachment site of λCI857 prophage is unusual in OD62, so the titres of phage after thermoinduction are not very high (~10^5 p.f.u./ml) [7–9]. Curing of the lysogen culture leads to formation of two classes of cells (at 37°C):

(1) Cells with a completely restored wild-type Pts$^+$ phenotype;
(2) Bacteria which retain the parental PtsH phenotype.

The two classes of bacteria did not produce phage after thermoinduction, but we failed to isolate clones which could not ferment fructose. An isogenic form of a cured *pts* mutant was obtained using P1kc phage-mediated transduction.

The frequency of *ptsH* recombinants among the *purC*$^+$ transductants was ~2%, which pointed to the typical linkage of this mutation with the *purC* locus [10]. At 37°C mutant strain jOD5 did not ferment glucose, mannose, sorbitol, mannitol, lactose, maltose or glycerol, but formed coloured colonies on the EMB agar plates with fructose, gluconate, arabinose and galactose; i.e., it had the phenotype of a *ptsH* mutant. This was confirmed in genetic and biochemical studies.

In mating ts19 × jOD5 we found Pts$^+$ recombinants among Ilv$^+$ colonies, as well as among the Trp$^+$ colonies. The jOD5 mutation also complemented *ptsI* mutation from strain 1103 [10] which together with the *ptsH*$^+$ gene was transposed into the *F' trp*$^+$ episome using Mu-1 cts61 phage. These genetic data show that the mutation in strain jOD5 covers only the *ptsH* gene and that the *ptsI* locus is intact. Biochemical characterization of mutant jOD5 showed

Table 3
Activity of the common components of the PT-system (nmol sugar phosphate .mg protein^{-1} .30 min^{-1} at 37°C)

Strain	Methyl-α-glucoside (10^{-4} M)		Mannitol (4×10^{-4} M)	
	Enzyme I	HPr	Enzyme I	HPr
j623ilv	64	32	5	5
jOD5	55	3	5	0
OD62[a]	58	2	5	0.45

[a] Cells were grown at 32°C

that the decreased in vitro PEP-dependent phosphorylation of methyl-α-glucoside (table 2) was due to the lack of HPr (table 3).

The activity of enzyme I was not changed (table 3). However, grown in the presence of 0.5% fructose, the mutant possessed high PEP: fructose phosphotransferase activity (table 2). The measured alterations of phosphotransferase activity were well correlated with alterations of the transport properties of mutant cells. Transport of mannitol and of methyl-α-glucoside (at 10^{-5} M) was decreased, while uptake of fructose (0.89×10^{-6} M) was not.

Genetic and biochemical studies show that the mutation in jOD5 is *ptsH*. The following indicates the deletion character of this mutation:

(i) It was obtained from of a lysogenic strain which possessed the phenotype of a *ptsH* mutant;
(ii) Strain jOD5 does not produce Pts^+ revertants on the EMB agar plates with glucose after its treatment with mutagen (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine);
(iii) We did not detect HPr activity in vitro in assays with mannitol (table 3).

It is well known that only HPr (besides enzyme I and mannitol-specific enzyme II) is necessary for the in vitro PEP-dependent phosphorylation [2–4] of mannitol.

The absence of HPr in jOD5 causes the following changes in enzyme-inducible synthesis (table 4):

(1) In a medium without carbohydrate (0.4% casamino acids) the differential rate of β-galactosidase synthesis is repressed;
(2) Addition of cyclic AMP does not restore the enzyme production;
(3) Glucose does not produce its repressive effect on β-galactosidase synthesis.

The inability of jOD5 strain to ferment lactose, maltose and glycerol (compounds which are not substrates for the PT-system) also indicates the repressed level of enzymes and transport proteins necessary for their transport and utilization. These data confirm our previous suppositions [1] that phospho~HPr could serve as an activating factor of transcription of catabolite-sensitive operons. The lack of HPr in a mutant cell disturbs all functions of the PTS:

(i) PEP-dependent phosphorylation of sugar;
(ii) Transport of carbohydrates;
(iii) Regulation of enzyme synthesis.

We must emphasize some unusual observations in strain jOD5.

(1) OD62 (lysogenic strain) and jOD5 were resistant to phosphomycin (2.5 μg/ml) in contrast to *ptsH* mutants of *Salmonella typhimurium*.
(2) A significant level of PEP-dependent phosphorylation of methyl-α-glucoside was measured in cell-free extracts of mutant bacteria and the decrease of PEP:glucose phosphotransferase activity was ⩽ 20% (table 2).
(3) In assays of HPr with methyl-α-glucoside we also found a rather high level of sugar phosphorylation (table 3).

In our opinion the activities detected could be due to a protein (or proteins) which substitutes for HPr in the in vitro phosphotransferase reaction. Factor III (which is necessary for the phosphorylation of methyl-α-D-glucoside and glucose) [2–4] could accept the phosphoryl group from PEP in the absence of HPr.

Retained phosphorylation and transport of fructose in mutant cells is of interest. The above-mentioned abnormal behaviour of *ptsH* mutants was discussed [3,4,10], but there is no satisfactory explanation of these facts as yet. We hope that the isolated *ptsH* mutant with a deletion for the *ptsH* gene will help us to resolve the problem.

Table 4
Effect of Δ*ptsH* mutation on differential rate of β-galactosidase synthesis[a] (nmol *o*-nitrophenol .mg protein^{-1} .min^{-1} at 30°C)

	7623ilv	70D5
Without additions	1625	898
+10^{-3} M glucose	329	899
+10^{-3} M cyclic AMP	1457	1128

[a] Cells were induced with 10^{-3} M isopropyl-β-thiogalactopyranoside; glucose or cyclic AMP were added simultaneously with inducer

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References

[1] Bolshakova, T. N., Gabrielyan, T. R., Bourd, G. I. and Gershanovitch, V. N. (1978) Eur. J. Biochem. 89, 483–490.
[2] Postma, P. W. and Roseman, S. (1976) Biochim. Biophys. Acta 457, 213–257.
[3] Kundig, W. (1974) J. Supramol. Struct. 2, 695–714.
[4] Saier, M. H., Simoni, R. D. and Roseman, S. (1976) J. Biol. Chem. 251, 6584–6597.
[5] Gershanovitch, V. N., Ilyina, T. S., Rusina, O. Y., Yourovitskaya, N. V. and Bolshakova, T. N. (1977) Mol. Gen. Genet. 153, 185–190.
[6] Umyarov, A. M., Bolshakova, T. N., Haykinson, M. Y., Bourd, G. I. and Gershanovitch, V. N. (1977) Genetika 13, 300–308.
[7] Shimada, K., Weisberg, R. and Gottesman, M. M. (1972) J. Mol. Biol. 63, 483–503.
[8] Shimada, K., Weisberg, R., Gottesman, M. M. (1973) J. Mol. Biol. 80, 297–314.
[9] Gottesman, M. M. (1976) Virology 72, 33–45.
[10] Fox, C. F. and Wilson, G. (1968) Proc. Natl. Acad. Sci. USA 59, 988–992.
[11] Cordaro, J. C., Melton, T., Stratis, J. P., Atagun, M., Gladding, C., Hartman, P. E. and Roseman, S. (1976) J. Bacteriol. 128, 785–793.