EFFECT OF *ptsI* AND *ptsH* GENES DOSAGE ON MANIFESTATION OF GLUCOSE CATABOLITE REPRESSION OF β-GALACTOSIDASE SYNTHESIS IN *ESCHERICHIA COLI* K12

A. M. UMYAROV, A. G. VOLOSHIN, T. N. BOLSHAKOVA and V. N. GERSHANOVITCH
The Gamaleya Institute for Epidemiology and Microbiology, Academy of Medical Sciences, Moscow D98, USSR

Received 25 September 1978

1. Introduction

It is well established now that activity of catabolic operons depends to a large extent on function of the phosphoenolpyruvate-dependent phosphotransferase (PT)-system. It was shown that mutational damage of the general components of the PT-system (*ptsI* and *ptsH* mutations) leads to repression of enzyme-inducible synthesis (for instance, β-galactosidase) [1,2], but these mutants become insensitive to glucose catabolite repression [3,4].

Recently we found that introduction of pts^+ genes with plasmid into mutant cell completely restores β-galactosidase synthesis [5]. However, glucose catabolite repression of synthesis of this enzyme was not studied in constructed merodiploid strains [5]. Effect of pts^+ genes dosage on β-galactosidase induction in medium with and without glucose was thoroughly investigated using newly selected short F' pts^+ episomes. It was found that:

1. Introduction of pts^+ genes into mutant cells restores β-galactosidase synthesis and makes it sensitive to glucose;
2. Increase in pts^+ genes content leads to hypersensitivity to glucose of β-galactosidase synthesis in pts^+/pts^+ merodiploid strains, but this effect is not manifested in mutants with defective glucose-specific enzyme II of the PT-system.

2. Experimental

Escherichia coli K12 strains selected for the present study are listed in table 1. The following strains were used for their construction: P642 (*F⁻, recA56 purC trp* str^r *ptsI,H*) [5], P644 (*F⁻, recA56 purC trp nalA* str^r) [5], J6231 (*F⁻, purC trp* str^r) [2,4], J624 (*F⁻, tgl purC* str^r) [6,9], W4985 (*hfr, met gptA*) [6], MH99 (*his araD leu*::(+Mu-1 *cts* 61)) (from Dr H. Howe, USA), AT2092 (*his argH purF pheA*::(+Mul c^+) from

Table 1
Escherichia coli K12 strains studied in this work

Strain	Genotype
J62313	*F⁻, thyA recA56 purC trp* str^r (+Mu-1 *ctc* 61)
P648	*F⁻, recA56 purC trp* str^r *nalA* (+Mu-1 *cts*61)
P647	*F⁻, thyA recA56 purC ptsI,H* str^r (+Mu-1 *cts*61)
AUF2/P647	*thyA purC ptsI,H trp recA56* str^r (+Mu-1 *cts*61) /*F' his*$^+$ *purC*$^+$ (+Mu-1 *cts*61) pts^+
AUF2/P648	*recA56 purC trp nalA* str^r (+Mu-1 *cts*61) /*F' lac*$^+$ *purC*$^+$ pts^+ (+Mu-1 *cts*61) his^+
AUF3/P647	*thyA purC ptsI,H trp recA56* str^r (+Mu-1 *cts*61) /*F' lac*$^+$ *purC*$^+$ pts^+ (+Mu-1 *cts*61)
AUF3/P648	*purC recA trp nalA* str^r (+Mu-1 *cts*61) /*F' lac*$^+$ *purC*$^+$ pts^+ (+Mu-1 *cts*61)
AUF3/J62313	*thyA recA56 purC trp* str^r (+Mu-1 *cts*61) /*F' lac*$^+$ *purC*$^+$ (+Mu-1 *cts*61) pts^+
AUF3/J6263	*recA56 purC gptA* (+Mu-1 *cts*61) /*F' lac*$^+$ *purC*$^+$ pts^+ (+Mu-1 *cts*61)
AUF3/J6243	*thyA recA56 purC tgl* str^r /*F' lac*$^+$ *purC*$^+$ pts^+ (+Mu-1 *cts*61)

Dr B. Bachmann, USA), 200PS/*F' lac* (*lac str*^{s}/*F' lac*$^+$) (from Dr F. Jacob, France), KLF 131/JC1553 (*recA argG his metB leu*/*F' his*$^+$) (from Dr B. Bachmann, USA).

Growth media, genetical procedures, enzyme assay and materials were as in [2,3,5,8]. Mutation *tgl* (in gene coded for glucose-specific enzyme II) blocks transport of methyl-α-D-glucoside via PT-system, but retains phosphoenolpyruvate-dependent phosphorylation of glucose and its analogue in vitro. Mutation *gptA* completely excludes the function of glucose-specific enzyme II [6,9]. Other genetical symbols are given according to [7].

3. Results and discussion

Stable merodiploid strains carrying the double dose of *pts*$^+$ genes or *pts*$^+$ genes only in *trans* position were constructed using transposition of *pts*$^+$ region of chromosome into the preexisted *F'*-plasmids. This transposition was performed with the help of Mu-phage by the method in [8]. In these cases the length of chromosomal region linked to the phage genome and transposed into *F'*-plasmid does not exceed 3.5 min [8].

Effect of *pts*$^+$ genes dosage on β-galactosidase synthesis in media without glucose and under the conditions of glucose catabolite repression was studied using constructed merodiploid strains (table 2).

Mutational damage of the general components of the PT-system (enzyme I and protein HPr) leads to a decrease in β-galactosidase synthesis in medium without glucose, but *ptsI,H* mutation is resistant to glucose catabolite repression (table 2). These results are in good accord with our data in [1–3]. *pts*$^+$ genes in *trans* position completely correct the defect of *ptsI,H* mutation: induction of β-galactosidase is restored, but is sensitive to glucose repression (table 2). These data indicate on participation (in a direct or indirect manner) of *pts* gene products in regulation of expression of catabolite-sensitive operons.

The double dose of *pts*$^+$ genes does not increase the differential rate of β-galactosidase synthesis in glucose-less medium (table 2). However, the rate of the enzyme synthesis is abruptly reduced under the conditions of glucose catabolite repression, i.e., *pts*$^+$/*pts*$^+$ merodiploid strains are more sensitive in compar-

Table 2
Effects of *pts*$^+$ genes dosage on differential rate of β-galactosidase synthesis

Strain	Genotype studied	Enzyme synthesis[a] (nmol *o*-nitrophenol/min/mg protein at 30°C)	
		Medium without glucose	Medium with glucose (10^{-3} M)[b]
P648	*pts*$^+$	0.775	0.395 (49)
J62313	*pts*$^+$	1.78	0.781 (56)
		1.55	0.730 (53)
P647	*ptsI,H*	0.461	0.522 (0)
AUF2/P647	*pts*$^+$/*ptsI,H*	0.742	0.308 (58)
AUF3/P647	*pts*$^+$/*ptsI,H*	1.132	0.596 (47)
AUF2/P648	*pts*$^+$/*pts*$^+$	0.720	0.180 (75)
AUF3/P648	*pts*$^+$/*pts*$^+$	1.223	0.302 (75)
AUF3/J62313	*pts*$^+$/*pts*$^+$	1.994	0.540 (73)
		2.530	0.440 (86)
		–	0.480
AUF3/J6263	*pts*$^+$/*gptA pts*$^+$	0.590	0.490 (17)
		0.410	0.470 (0)
AUF3/J6243	*pts*$^+$/*tgl pts*$^+$	1.490	1.240 (16)
		1.970	1.50 (20)

[a] Cells were grown in Nutrient Broth (Difco) at 30°C; glucose was added with inducer – isopropyl-β-D-thiogalactopyranoside (10^{-3} M)

[b] % Glucose inhibition of enzyme synthesis is represented in parentheses

ison with haploid and *pts*$^+$/*ptsI,H* strains (table 2). This hypersensitivity was demonstrated on merodiploid strains with different *F'*-plasmids (table 1,2).

It is well established now that active glucose-specific enzyme II of the PT-system is necessary for the realization of the phenomenon of glucose catabolite repression [6,9–11]. Mutational damage of this component makes bacteria resistant to glucose action [6,9–11]. Merodiploid cells with increased content of *pts*$^+$ genes but inactive glucose-specific enzyme II (*tgl* and *gptA* mutations) are also resistant to glucose (table 2).

It is still difficult to envisage the mechanism whereby the products of the *ptsI* and *ptsH* genes participate in regulation of gene activity. However, it is obvious that the active state of glucose-specific enzyme II (i.e., intact genes coded for enzyme I, HPr and enzyme II) is necessary for the manifestation of glucose catabolite repression in bacteria.

References

[1] Gershanovitch, V. N., Bourd, G. I., Yourovitskaya, N. V., Skavronskaya, A. G., Klutcheva, V. V. and Shabolenko, V. P. (1967) Biochim. Biophys. Acta 134, 188–190.
[2] Bolshakova, T. N., Gabrielyan, T. R., Bourd, G. I. and Gershanovitch, V. N. (1978) Eur. J. Biochem. in press.
[3] Gershanovitch, V. N., Yourovitskaya, N. V., Saprykina, T. P. and Klutcheva, V. V. (1970) Dokl. Akad. Nauk SSR 190, 1232–1234.
[4] Gershanovitch, V. N., Yourovitskaya, N. V., Komissarova, T. V., Bolshakova, T. N., Erlagaeva, R. S. and Bourd, G. I. (1975) Molek. Gen. Genet. 140, 81–90.
[5] Umyarov, A. M., Bolshakova, T. N., Khaykinson, M. Ya., Bourd, G. I. and Gershanovitch, V. N. (1977) Genetika 13, 300–308.
[6] Bourd, G. I., Erlagaeva, R. S., Bolshakova, T. N. and Gershanovitch, V. N. (1975) Eur. J. Biochem. 53, 419–427.
[7] Bachmann, B. J., Low, K. B. and Taylor, A. L. (1976) Bacteriol. Rev. 4, 116–167.
[8] Failen, M. and Toussaint, A. (1976) J. Mol. Biol. 104, 525–531.
[9] Erlagaeva, R. S., Bolshakova, T. N., Shulgina, M. V., Bourd, G. I., and Gershanovitch, V. N. (1977). Eur. J. Biochem. 72, 127–132.
[10] Saier, M. H., Feucht, B. U. and Hofstadter, L. G. (1976) J. Biol. Chem. 251, 883–894.
[11] Harwood, G. P., Gazdar, C. and Peterkofsky, A. (1976) J. Biol. Chem. 251, 2462–2468.