tgs AND *crr*: GENES INVOLVED IN CATABOLITE INHIBITION AND INDUCER EXCLUSION IN *ESCHERICHIA COLI*

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1. Introduction

We recently described some properties of mutants of *Escherichia coli* that had been selected as being resistant to the growth inhibitory effects of 5-thio-D-glucose [1]. These mutants still grew on glucose, albeit at only half the rate at which the wild-type organisms grew, but glucose was no longer utilized in preference to other sugars taken up via the phosphoenolpyruvate-dependent phosphotransferase (PT) system; they were thus altered in 'catabolite inhibition' [2]. A further characteristic of these mutants was that they were strongly impaired in their ability to adapt to grow on media containing salts of C_4-dicarboxylic acids as sole carbon source, but that this impairment was overcome by the addition of adenosine 3′,5′-cyclic phosphate (cAMP); as expected from this finding, the adenylate cyclase activity [3] of toluenized suspensions of the mutants was much less than that of similarly treated wild-type organisms.

We had previously [4] described the isolation of mutants that had lost the property of 'inducer exclusion' [5]; such mutants readily adapted to grow on sugars not taken up through the PT-system even in the absence of the enzyme I (*ptsI*) or HPr (*ptsH*) components of the PT-system. Both the loss of 'catabolite inhibition' and the loss of 'inducer exclusion' were co-transducible with *ptsI*; however, even the evidence then available [1] indicated that more than one gene was involved.

It is the purpose of this paper to report that there are two genes concerned and to summarize some properties of organisms mutated in them. One gene, mapping between *cysA* and *ptsI* [6] and very close to if not identical with *ptsH*, specifies 'catabolite inhibition': since organisms altered in this property are selected for loss of thioglucose sensitivity, we propose to designate this phenotype Tgs$^-$ and this gene *tgs*. The other gene, specifying loss of 'inducer exclusion', is also located near *ptsI* but distal to *ptsH* and not co-transducible with *cysA*. Mutants carrying this gene manifest many of the characteristics of the *crr*-mutants of *Salmonella typhimurium* described by Roseman and his colleagues [7,8] and we adopt their nomenclature.

2. Experimental

tgs-Mutants were selected as thioglucose-resistant organisms on media containing fructose as main carbon source, and the *tgs*-marker was transferred by phage P1 mediated transduction to the genetic background of *E. coli* strain PA309, as previously described [1]. *crr*-Mutants were slected as *ptsI*^{ts}.-mutants that had regained the ability to grow on maltose or glycerol at 40°C despite the absence of enzyme I activity at the higher temperature [4]. As with the *tgs* marker, the *crr*-mutation, together with the temperature-sensitive enzyme I specified by *ptsI*^{ts}., was transferred by phage P1-mediated transduction to a *ptsI*-derivative of *E. coli*, strain PA309; transductants were selected that grew on a PT-sugar at 30°C but not at 40°C and such organisms were screened for their ability to grow on maltose, and on glycerol, at 40°C.

Measurements of growth, of the relative utilization of glucose in the presence of other sugars, of uptake of labelled sugars and of adenylate cyclase activity were performed as previously described [1,9].

3. Results and discussion

3.1. *Properties of* tgs-*mutants*

As reported previously [1], by transfer by phage P1-mediated transduction of the *ptsI*$^+$-allele from *tgs*-donor strains of *E. coli* to *ptsI*-derivatives of strain PA309 yielded *ptsI*$^+$*tgs*-transductants with very high frequency (>98%). Using the nearby marker *cysA* instead of *ptsI*, the frequency with which *cys*$^+$*tgs*-transductants were obtained was also high (>85%). All *tgs*-transductants showed some impairment of uptake of glucose and of its analogue methyl α-glucoside: the rates at which these substrates, at 0.1 mM, were taken up by suspensions of fructose-grown cells were only about 25% of those observed with wild-type *E. coli* strain PA309. Under these conditions of measurement, the impairment in glucose utilization is quantitatively more severe than when cultures of the mutants grow on glucose, which is usually supplied in growth media at concentrations greater than 2 mM, when both the enzymes II for glucose specified by *ptsG* and *ptsM* will function [10]. A further characteristic of *tgs*-mutants is that glucose is no longer effective in excluding other sugars when added to cultures growing on those other sugars, irrespective of whether they are taken up through the PT-system or not (table 1). The *tgs*-mutants have thus lost the property of 'catabolite inhibition' [2]. (It should be noted, however, that these other sugars are still largely excluded by glucose 6-phosphate.)

Since toluene-treated suspensions of *tgs*-mutants have much lower adenylate cyclase activities than do similarly treated suspensions of wild-type cells [1], it might be expected that the addition of cAMP would restore the wild-type phenotype. Such, however, is not the case. Inclusion of 2.5 mM cAMP markedly decreased the utilization of [^{14}C]glucose by cultures of *tgs*-mutants growing on a mixture of glucose and lactose; left unchanged its utilization when sorbitol was the other sugar; and increased it to nearly the levels observed with wild-type cells only when fructose or mannose were the other sugar growth substrates (fig.1). No such effects were observed with wild-type organisms, in which cAMP augmented or left virtually unchanged the utilization of glucose in preference to all other sugars. Moreover, these effects of cAMP were also not observed with *tgs ptsM* double mutants: this implies that the apparent

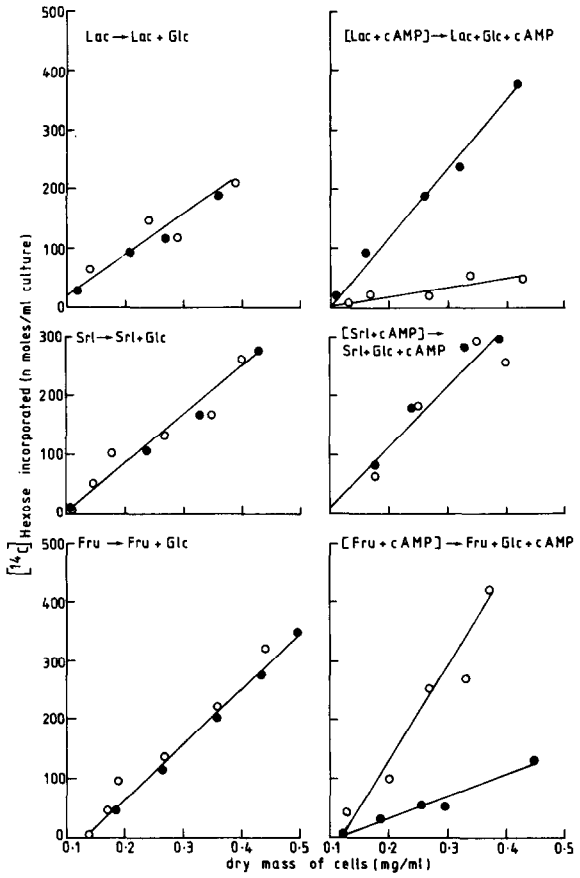

Fig.1. Effect of cAMP on the relative utilization of glucose and other sugars by *tgs*-mutants of *Escherichia coli*. 2.5 mM cAMP was included in all media on the right hand side of the diagram. In each case, the incorporation of [^{14}C]glucose is indicated by open circles and that of lactose (upper), sorbitol (middle) and fructose (lower) by closed circles.

restoration of the wild-type phenotype seen earlier with *tgs*-cells grown on fructose or mannose was the consequence of an increased uptake of [^{14}C]glucose through the PtsM system, which appears not to be induced in these mutants during their growth on sorbitol or lactose. In accordance with this interpretation, toluenized suspensions of *tgs*-mutants, which had been grown on 10 mM fructose in the presence of 2.5 mM cAMP, catalyzed the PEP-dependent phosphorylation [11,12] of glucose and of 2-deoxyglucose (which can be phosphorylated via the PtsM system) at nearly the rates observed with similar suspensions derived from wild-type cells, whereas the phosphoryla-

Table 1
The effect of mutations in *tgs* and *crr* on the preferential utilization of glucose by *E. coli* strain PA309

Genotype of organism	Fraction (%) of total cell C derived from [^{14}C]glucose in the presence of [a]				
	Fructose	Sorbitol	Maltose	Lactose	Glycerol
tgs$^+$ *crr*$^+$	88	94	92	93	92
tgs$^-$ *crr*$^+$	53	58	52	55	63
tgs$^+$ *crr*$^-$	67	68	56	35	76

[a] Cultures of organisms were grown overnight at 30°C on the appropriate carbohydrate, harvested by centrifugation at room temperature and resuspended (at approx. 0.1 mg dry mass/ml) in two flasks containing 10 ml of growth medium with that carbohydrate at 10 mM (glycerol), 5 mM (hexoses) or 2.5 mM (disaccharides). When growth at 30°C had recommenced, [^{14}C]-glucose (to 5 mM) was added to one flask and [^{12}C]glucose (to 5 mM) plus a small quantity of the other carbohydrate, labelled with ^{14}C, were added to the second flask. Samples were taken through the subsequent period of growth, and the utilization of the two substrates in the presence of each other measured as previously described [9]

tion of methyl α-glucoside (which is phosphorylated predominantly via the PtsG system) remained low.

3.2. *Properties of* crr-*mutants*

Although the Crr$^-$-phenotype was also found to be co-transducible with *ptsI*, it is so transferred with a frequency of only about 25%. Since the co-transduction frequency of *crr* with *cysA* is less than 5%, it is likely that the gene order is: ... *cysA tgs ptsI crr* ...

Suspensions of *crr* mutants of *E. coli* strain PA309 do not appear to differ from wild-type cells in their rates of uptake of glucose or of methyl α-glucoside, or in their adenylate cyclase activities. They also show only slight loss of catabolite inhibition when grown in the presence of sugars taken up through the PT-system, such as fructose or sorbitol (table 1). However, when non-PT sugars, such as lactose or maltose, are present in addition, they are not excluded by glucose. The presence of cAMP does not significantly affect the ratios of sugar utilization observed. This implies that the altered property in *crr*-mutants affects 'inducer exclusion' rather than interference with the function of the PT-system. But, since lactose and maltose are excluded as effectively by glucose 6-phosphate and by gluconate from *crr*-mutants as they are from wild-type cells (table 2), the 'inducer

Table 2
Specificity of inducer exclusion and the effect of cAMP on *crr*-mutants

Growth conditions[a]	Ratio of uptake [A : B]
Glucose(A) + lactose(B)	33 : 67
cAMP + glucose(A) + lactose(B)	15 : 85
Glucose(A) + fructose(B)	70 : 30
cAMP + glucose(A) + fructose(B)	72 : 28
Glucose 6-phosphate(A) + lactose(B)	95 : 5
Gluconate(A) + lactose(B)	92 : 8

[a] For conditions, see legend to table 1

exclusion' specified by *crr* is not a general loss of sensitivity to catabolites but is associated specifically with the effect of external glucose on the utilization of non-PT sugars.

The mechanisms involved in the function of the *tgs* and *crr* genes remain to be elucidated.

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