ROLES OF *crr*-GENE PRODUCTS IN REGULATING CARBOHYDRATE UPTAKE BY *ESCHERICHIA COLI*

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1. Introduction

The phosphoenolpyruvate-dependent phosphotransferase (PT-) system [1] plays an essential role in the uptake of a number of hexoses by enteric bacteria, such as *Salmonella typhimurium* and *Escherichia coli*. Its main features are:

(i) An initial transfer of phosphate from phosphoenolpyruvate (PEP) to a small histidine-containing protein HPr: this is catalysed by enzyme I;
(ii) Transfer of phosphate from the phosphorylated HPr to the appropriate hexose: this is catalysed by one or more relatively sugar-specific enzymes II [2,3].

As expected, mutants lacking either enzyme I (*pts*I) or HPr (*pts*H) are unable to utilize any sugar that is taken up via the PT-system (PT-sugars). However, they are also unable or reluctant to grow on a variety of carbohydrates (such as C_4-dicarboxylic acids, glycerol, lactose and maltose) the uptake of which does not directly involve the PT-system [4–7]. This appears to be due to an inability of *pts*I and *pts*H mutants to induce the proteins necessary for the rapid uptake of these substrates and can be overcome in some (but not all) such mutants in one of three ways:

(1) By adding non-catabolizable inducers, such as isopropyl-thiogalactoside to promote growth on lactose;
(2) By adding adenosine 3′,5′-cyclic phosphate (cAMP), to promote induction of catabolite-repressible genes [8];
(3) By mutation to a genotype designated *crr* [9,10], which is cotransducible with *pts*I.

However, *crr*-mutants which have been selected by these means may be impaired in more than one function, since the property of 'inducer exclusion' that permits growth on non PT-carbohydrates [11] is not co-transducible with *pts*I in the *E. coli* strains used in our laboratory (to be published).

We now describe the selection, by a different procedure, of mutants affected in the *ccr*-gene region but unimpaired in enzyme I or HPr activities. This procedure utilizes the finding [12] that *crr* mutants of *E. coli* are no longer inhibited in their growth on PT-sugars by glucose or glucose analogues. Since the lesion in mutants thus selected is co-transducible with *pts*I but they exhibit more than one phenotype, we conclude that there are several genes in the *crr*-locus and that the product(s) specified by them play different roles in the regulation of carbohydrate uptake.

2. Experimental

Cultures of the *E. coli* K12 strains K2.1t [13] or PW 7 [12] were grown overnight in defined media [14] on fructose as sole carbon source, either at 37°C or (since strain PW 7 carried the temperature-sensitive allele of *pts*I, *pts*I^{ts}, and thus does not grow on PT-sugars at elevated temperatures) at 30°C. To the cultures, diluted to approx. 0.1 mg dry mass/ml with fresh fructose growth medium, was added 5-thioglucose (TG) to final conc. 2 mM: growth was rapidly arrested. However, after 2–3 days further shaking, cells resistant to TG grew in the flasks.

In order to isolate from such cells mutants affected specifically in the *crr*-gene region, phage P1

was propagated [13] on them and was used to infect strain HK 488, a *pts*I-derivative of strain PA 309 [15]. Transductants were selected for their ability to grow on a PT-sugar, such as sorbitol, either at 37°C (for *pts*I$^+$) or at 30°C (for *pts*I^{ts}): the latter transductants were further screened for their inability to grow on PT sugars at the higher temperature.

The methods used for measurements of growth and of the incorporation of ^{14}C-labelled sugars have been described [16]. Adenylate cyclase activity was measured by the method in [17] after treatment of the cells with toluene [18]. As reported [19], this enzyme activity was found to be strongly inhibited by 1 mM glucose and thus differed from the soluble form of the enzyme.

3. Results and discussion

Two main types of *pts*I$^+$-transductants, designated HK 517 and HK 518, were obtained after infection of a *pts*I-derivative of *E. coli* strain PA 309 with phage P1 propagated on cultures of that PA 309 strain rendered resistant to TG.

1. HK 517 was slightly affected in its rate of growth on glucose but was strongly impaired in growth on fumarate, succinate or malate; this impairment was largely corrected by the inclusion of 2.5 mM cAMP in the C_4-dicarboxylate growth medium (table 1). When fructose-grown cultures of this mutant were placed on media containing either 5 mM [^{14}C]-fructose and 5 mM [^{12}C]glucose, or 5 mM [^{12}C]-fructose and 5 mM [^{14}C]glucose, 44–48% total hexose-carbon incorporated during subsequent growth [16] was derived from fructose and a roughly equal amount (52–56%) from glucose. This proportion, which had been previously noted with the *pts*I^{ts}.*crr*-strain PW 8 selected by a different procedure [12], was twice the 20–22% observed with prototrophic strains of *E. coli* such as the parental strain PA 309.

2. HK 518 was also slightly affected in its rate of growth on glucose and was also impaired in its ability to grow on salts of C_4-dicarboxylic acids; like strain HK 517, the inclusion of 2.5 mM cAMP largely overcame this impairment. However, unlike strain HK 517, glucose was barely used when added to cells growing on fructose: some 77% of the total hexose-carbon incorporated during growth on this mixture were derived from fructose and only 23% from glucose.

Although both these phenotypes are routinely observed in screening of TG-resistant mutants, it is not yet certain that they are affected in different genes. As expected from their response to cAMP, suspensions of both types of mutant treated with toluene manifested greatly reduced adenylate cyclase activities when compared to those found with the parent organism PA 309 (table 1).

In studies with strain PW 7, which contains an enzyme I active at 30°C but not at 37°C and above (*pts*I^{ts}), a third type of TG-resistant mutant came to light. Like the mutants HK 517 and HK 518, this type was affected in a gene highly co-transducible with *pts*I; when this lesion was transferred to the

Table 1
Some properties of *E. coli* strain PA 309 and *crr*-mutants thereof

Strain	Doubling time[a] (h)			Adenylate cyclase act. (pmol/mg protein/h)	Fructose utilized × 100 / Fructose + Glucose
	Fumarate	Fumarate + cAMP	Glucose		
PA 309	1.8	1.8	1.1	876	22
HK 517	>8	2.2	2.0	160	44
HK 518	4.5	2.4	2.3	26	77
HK 519	1.8	1.8	2.2	80	44
PW 7	2.8	2.8	1.8	800	20
PW 68	3	3	>10	880	83

[a] Strain PA 309 and those prefixed HK with grown at 37°C; PW strains at 30°C

*pts*I-recipient strain HK 488 by phage P1-mediated transduction, the resultant strain PW 68 contained the *pts*I^{ts}-gene of the donor. However, this transductant differed from other *pts*I^{ts}.*crr*-strains [12] by its virtual inability to grow (at 30°C) on glucose, although growth on other PT-sugars was normal. Furthermore, and unlike HK 517 and HK 518, strain PW 68 was unimpaired in its ability to grow on salts of C_4-dicarboxylic acids and (as expected from this property) was also unimpaired in its adenylate cyclase activity.

These findings suggest that the *crr*-gene, defined as a marker co-transducible with *pts*I that specifies resistance to repression by carbohydrate [9], is not a single entity. One type of *crr*-mutant in *Sal. typhimurium* has been reported [3] to lack the factor III component of the membrane-bound enzyme II (formerly designated *umg* [20] and now termed *pts*G [3]) specific for the uptake and phosphorylation of glucose and methyl-α-glucoside. It is conceivable that the *E. coli* mutant PW 68 (table 1) is of this type: absence of overall function of the *pts*G-system, which is known to be the predominant enzyme II of glucose utilization [21], would be expected to produce the phenotype observed. However, lack of the factor III component of this enzyme II would not be expected to lead to the loss of adenylate cyclase activity and would not explain the variations in the extent to which glucose is taken up in the presence of fructose, without any major impairment in utilization of glucose as sole carbon source. The existence of the '*crr*'-mutants of type HK 517 and HK 518 suggests that the PT-system includes proteins that link the phospho-HPr component to adenylate cyclase on the one hand, and to the factor III component of the *pts*G-system on the other, and that these proteins play a key role in the regulation of carbohydrate uptake. This view differs somewhat from the models of PT-mediated glucose uptake proposed [3,22].

Multiple forms of '*crr*'-mutants in *Sal. typhimurium* were also briefly reported [23]. Double mutants carrying *pts*I.*crr*A (which do not grow on salts of C_4-dicarboxylic acids) were used to select further mutants that grow on these compounds. Such further mutants (affected in a gene designated *crr*B, which mapped very close to *crr*A and *pts*I) were still impaired in cAMP synthesis. Mutants with this phenotype can also be readily derived from '*crr*'-mutants of *E. coli*, whether they be *pts*I$^+$ (such as HK 517) or *pts*I$^-$ (such as strain PW 8 [12] at elevated temperatures). One such derivative of strain HK 517, termed HK 519, is illustrated in table 1: it grows readily on salts of C_4-dicarboxylic acids, but has neither regained adenylate cyclase activity nor the utilization of glucose preferentially to fructose characteristic of prototrophic strains of *E. coli*; it is also still inducible for the uptake of C_4-dicarboxylic acids. Moreover, when phage P1 propagated on strain HK 519, was used to infect the usual *pts*I-recipient strain HK 488 and *pts*I$^+$-transductants were selected on sorbitol or fructose media, some of these transductants were (like the donor HK 519) able to grow on fumarate but others were not. This shows that the mutation of the *crr*-strain HK 517 to growth on fumarate had suppressed, but not reversed, the original lesion. Although both the *crr*-marker and the suppressor gene that permitted growth on fumarate were co-transducible with *pts*I, they clearly could be separated from each other.

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