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Role of III^{Glc} of the Phosphoenolpyruvate-Glucose Phosphotransferase System in Inducer Exclusion in *Escherichia coli*

STEPHEN O. NELSON,[1] JOSEPH LENGELER,[2] AND PIETER W. POSTMA[1]*

Laboratory of Biochemistry, B.C.P. Jansen Institute, University of Amsterdam, 1000 HD Amsterdam, The Netherlands[1]; and Fachbereich Biologie, Universität Regensburg, D-8400 Regensburg, Federal Republic of Germany[2]

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The phosphoenolpyruvate–D-glucose phosphotransferase system of *Enterobacteriaceae* is thought to regulate the synthesis and activity of a number of catabolite uptake systems, including those for maltose, lactose, and glycerol, via the phosphorylation state of one of its components, III^{Glc}. We have investigated the proposal by Kornberg and co-workers (FEBS Lett. 117(Suppl.):K28–K36, 1980) that not III^{Glc}, but an unknown protein, the product of the *iex* gene, is responsible for the exclusion of the above-mentioned compounds from the cell. The *iex* mutant HK738 of *Escherichia coli* contains normal amounts of III^{Glc} as measured by specific antibodies, in contrast to *crr* mutants that lack III^{Glc}. The III^{Glc} of the *iex* strain functions normally in glucose and methyl α-glucoside transport, and the specific activity in in vitro phosphorylation is approximately 60% of that of the parent. The III^{Glc} activity of the *iex* strain is, however, heat labile, in contrast to the parental III^{Glc}, suggesting that the mutant contains an altered III^{Glc}. This is supported by the observation that III^{Glc} from the *iex* strain cannot bind to the lactose carrier. Thus it cannot inhibit the carrier, and this explains why the uptake of non-phosphotransferase system compounds in an *iex* strain is resistant to phosphotransferase system sugars. The introduction of a plasmid containing a wild-type crr^+ allele into the *iex* strain restores the *iex* phenotype to that of the iex^+ parent. The III^{Glc} produced from the plasmid in the *iex* strain is heat stable and binds normally to the lactose carrier. These results lead to the conclusion that the *iex* mutation is most likely allelic with *crr* and results in an altered, temperature-sensitive III^{Glc} that is still able to function in D-glucose and methyl α-glucoside uptake and phosphorylation and in the activation of adenylate cyclase, but is unable to bind to and inhibit the lactose carrier.

The phosphoenolpyruvate-sugar phosphotransferase system (PTS) catalyzes the transport and concomitant phosphorylation of a number of carbohydrates in *Escherichia coli* and *Salmonella typhimurium* (for a review, see references 19 and 20). The PTS is composed of two general cytoplasmic proteins, enzyme I and HPr, and various sugar-specific, membrane-bound enzymes II. The glucose PTS contains an additional cytoplasmic phosphoprotein, III^{Glc}, which is required for glucose and methyl α-glucoside transport. In addition the phosphoprotein III^{Glc}, a product of the *crr* gene (12, 25), is thought to function in the regulation of the synthesis and activity of a number of non-PTS uptake systems, including those for lactose, maltose, melibiose, and glycerol. Phosphorylated III^{Glc} is supposed to activate adenylate cyclase. When III^{Glc} is dephosphorylated (by a PTS sugar) or absent (due to mutation), the adenylate cyclase activity is low, and the intracellular level of cyclic AMP is not sufficient for the expression of certain catabolic operons (1, 19, 20). Non-phosphorylated III^{Glc} inhibits the accumulation of carbohydrates via the non-PTS uptake systems mentioned above. This phenomenon is called inducer exclusion. A direct interaction between III^{Glc} and the lactose carrier has recently been demonstrated (14, 15). With purified III^{Glc} and purified lactose carrier reconstituted in liposomes, it could be shown that these two proteins are sufficient for the observed interaction; transport of β-galactosides is inhibited by non-phosphorylated III^{Glc} (14). In the case of glycerol uptake we have recently demonstrated that non-phosphorylated III^{Glc} inhibits glycerol kinase, but not the facilitator (18). As a consequence no glycerol phosphate, the inducer of the *glp* regulon, is produced in the cell. PTS sugars not only decrease the uptake of certain non-PTS compounds, as mentioned above, but can also inhibit the uptake of each other. This is the result of competition of the various enzymes II or factors III for the common phospho-HPr pool (4, 23). Thus glucose and methyl α-glucoside can inhibit the uptake of fructose via II^{Fru} (see below).

A different model for regulation has been advanced by Kornberg and co-workers (6–8, 16). In their view III^{Glc}, the product of the *crr* (*tgs*) gene, is only involved in the regulation of adenylate cyclase (19). The product of a second gene, *iex*, regulates the activity of the various non-PTS transport systems. The *crr* and *iex* genes are thought to be closely linked, but on opposite sides of the *ptsHI* genes that code for HPr and enzyme I (2, 7). *crr* and *iex* mutants have different phenotypes, a major difference being that methyl α-glucoside, a non-metabolizable analog of glucose, inhibits growth of an *iex* mutant, but not of a *crr* mutant, on fructose. According to Kornberg this is due to the fact that *iex* mutants contain an active III^{Glc}. Growth on non-PTS compounds is resistant to methyl α-glucoside in *crr* and *iex* mutants for different reasons. In the case of the *crr* mutation, methyl α-glucoside cannot enter the cell. In the case of the *iex* mutation, the inhibitory protein is absent or mutated.

Since these two models are not compatible, we have investigated the *iex* mutant in more biochemical detail. Our main conclusion is that the *iex* mutation results in an altered III^{Glc} that is still active in glucose and methyl α-glucoside phosphorylation and adenylate cyclase activation, but is unable to bind to and inhibit the lactose carrier.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* strains HK761 *ptsI*(Ts) iex^+ *umgC metB ilv* and HK738 *ptsI*(Ts) *iex umgC metB ilv* were obtained from H. Kornberg. HK738 was constructed by introducing the *iex* mutation from HK727

* Corresponding author.

(16) into ts19. HK761 is the isogenic *iex*$^+$ strain. *E. coli* strain ts19 *ptsI*(Ts) *ilv metB* was obtained from V. Gershanovitch. PPA3 *crr ptsI*(Ts) *ilv metB* was derived from ts19 as a strain being able to grow on maltose in the presence of 5 mM methyl α-glucoside. The plasmid pBCP20 consists of a 1,000-base-pair *S. typhimurium* chromosomal fragment inserted in pAT153 (26) and containing the *crr*$^+$ gene coding for III^{Glc} (13a).

Growth of cells and preparation of bacterial extracts. Cells were grown in minimal medium A (23) containing the required amino acids and 0.2% of a carbon source and were harvested at the midexponential phase. Cell extracts were prepared by passing the cells through a French pressure cell (24). Membranes were removed by centrifugation for 60 min at 150,000 × *g*.

Determination of PTS proteins. The activity of enzyme I, HPr, and III^{Glc} was determined in cell-free extracts by phosphorylation in vitro in the presence of an excess of the other purified components (24). Sugar phosphate formation was measured by the ion-exchange method (9). The amount of III^{Glc} was determined by rocket immunoelectrophoresis (24).

Transport assay. Transport of 0.1 mM methyl-α-D-[*U*-^{14}C]glucopyranoside (specific activity, 390 cpm/nmol; Amersham Corp.) was measured as described previously (17).

Binding of III^{Glc} to the lactose carrier. Binding of III^{Glc} to the lactose carrier of *E. coli* was determined as described previously (14). Membranes containing high, elevated levels of the lactose carrier were a gift of J. K. Wright. The membrane preparation contained 17.4 mg of protein per ml and 1.4 nmol of lactose carrier per mg of protein.

Purified enzymes. Purification of enzyme I, HPr, and III^{Glc} has been described previously (24).

Transformation. HK738 and HK761 were transformed with pBCP20 by the method of Mandel and Higa (11), selecting for ampicillin resistance.

RESULTS

Nomenclature. In *S. typhimurium* mutations have been isolated that restore growth of *pts* mutants on non-PTS compounds such as maltose, glycerol, and melibiose (21, 22). These *crr* mutants have lowered levels of III^{Glc} or lack it altogether. It has been shown that the *crr* gene is the structural gene for III^{Glc} (12, 25). In *E. coli* two types of suppressor mutations have been reported by Kornberg and co-workers. *tgs* mutations ([7], renamed *gsr* later [16]) render an *E. coli* strain resistant to methyl α-glucoside or 5-thio-D-glucose on both PTS and non-PTS compounds. Presumably they lack III^{Glc} since methyl α-glucoside transport is lowered or absent and they have a low level of adenylate cyclase activity (6, 8). *tgs* in *E. coli* has been equated with *crr* in *S. typhimurium*. In addition, an *E. coli crr* mutation has been described ([5, 8], renamed *iex* later [16]) that renders a strain resistant to methyl α-glucoside on non-PTS compounds, but leaves it sensitive on PTS sugars. It has been claimed that the *iex* gene is different from the *crr* gene [8, 16]. Not to confuse matters more than necessary, we will designate *tgs* (*gsr*) in *E. coli* and *crr* in *Salmonella* by *crr*; *iex* as described by Kornberg et al. will be used to designate the additional mutation found in *E. coli*.

TABLE 1. Phenotype of *iex* and *crr* strains

Strain	Relevant genotype	Growth[a]					
		Fru (30°C)	Fru + αMG (30°C)	Malt (30°C)	Malt + αMG (30°C)	Malt (42°C)	Suc (30°C)
HK761	*ptsI*(Ts)	+	−	+	−	−	+
HK761 (pBCP20)	*ptsI*(Ts)	+	−	+	−	−	+
HK738	*ptsI*(Ts) *iex*	+	−	+	+	+	+
HK738 (pBCP20)	*ptsI*(Ts) *iex*	+	−	+	−	−	+
PPA3	*ptsI*(Ts) *crr*	+	+	+	+	+	−

[a] Growth was monitored on chemically defined media containing 0.2% (wt/vol) of the carbon source. The final concentration of methyl α-glucoside, if present, was 5mM. Symbols and abbreviations: +, growth after 48 h of incubation at 30°C (or 42°C in the case of maltose); −, no growth under these conditions; Fru, fructose; Malt, maltose; αMG, methyl α-glucoside; Suc, succinate.

TABLE 2. Levels and activities of PTS proteins in *E. coli*

Strain	Enzyme activity				
	Enzyme I[a]	HPr[a]	III^{Glc-a}	III^{Glc}[b]	Methyl α-glucoside transport[c]
HK761	15	12.5	0.8	4.2	7.3
HK761(pBCP20)	ND[d]	ND	ND	7.2	ND
HK738	17	14	0.5	4.0	7.3
HK738(pBCP20)	ND	ND	1.0	8.1	ND
PPA3	15	12	0	0	0

[a] Enzyme activities were determined by phosphorylation in vitro of methyl α-glucoside and expressed as nanomoles of methyl α-glucoside phosphorylated per minute per milligram of protein.

[b] III^{Glc} was determined by rocket immunoelectrophoresis and expressed as micrograms of III^{Glc} per milligram of protein.

[c] Methyl α-glucoside transport was measured in glycerol-grown cells as described in the text and expressed as nanomoles of methyl-α-glucoside taken up per minute per milligram (dry weight).

[d] ND, Not determined.

Properties of *iex* and *crr* mutants. The growth characteristics of the *iex* mutant HK738 and its corresponding *iex*$^+$ parent HK761 are shown in Table 1. They confirm and extend the results reported by Kornberg and co-workers earlier on *iex* strains (8, 16), i.e., the *iex* mutant does not grow on fructose, but grows well on maltose in the presence of methyl α-glucoside (Table 1) or thioglucose (data not shown), as is the case with the parent strain. In contrast, *crr* mutants [isolated in the same *ptsI*(Ts) background, see below] are resistant to methyl α-glucoside on all carbon sources. A representative strain, PPA3, is shown in Table 1. Growth at 42°C on certain non-PTS compounds is impaired in the parent since it contains a temperature-sensitive enzyme I (3). The *iex* mutation in strain HK738 was isolated as a suppressor of *ptsI*(Ts) that allows the strain to grow on maltose at 42°C similar to the *iex* mutant HK727 described before [8]. Both regain growth on other non-PTS compounds such as glycerol and lactose as well, similar to *S. typhimurium crr* mutants previously described. However, in *S. typhimurium crr* mutants, III^{Glc} is absent, and methyl α-glucoside transport is impaired. The inhibition of growth on fructose by methyl α-glucoside suggests that III^{Glc} is still active in the *E. coli iex* strain. In addition, growth on non-PTS compounds such as succinate, which is strongly dependent on cyclic AMP, is not impaired. This also suggests that III^{Glc} is still functional. Indeed, Kornberg et al. (8) showed that adenylate cyclase activity in an *iex* strain is close to that in a wild-type strain, in contrast to a *crr* strain.

PTS proteins in *iex* and *crr* mutants. To resolve these conflicting results obtained with *E. coli iex* and *crr* mutants and *S. typhimurium crr* mutants, we determined the levels of the various PTS proteins in such strains. Table 2 shows that the *iex* strain HK738 contained the same amount of III^{Glc} (as

measured with an antibody against III^{Glc}) as the parent HK761. The *crr* mutant PPA3 lacked III^{Glc} completely. Most *pts crr* double mutants of *S. typhimurium*, isolated as being able to grow on non-PTS compounds, lack most or all III^{Glc} (21, 25). The same type of mutation has been isolated in *E. coli* strains resistant to methyl α-glucoside on glycerol (10). It should be remembered that the *iex* mutation in *E. coli* is isolated in the same way, i.e., suppressing the growth defect of a *ptsI*(Ts) mutant on maltose at 42°C.

Transport studies with methyl α-glucoside, a substrate of the III^{Glc}-II^{Glc} transport system, show that the rate of transport is almost the same in wild-type HK761 and *iex* mutant HK738 (Table 2). The *crr* strain PPA3 is unable to catalyze methyl α-glucoside transport. It should be pointed out that in intact cells (or, for that matter, in toluenized cells) III^{Glc} is most likely not the rate-limiting component for uptake or phosphorylation of methyl α-glucoside (4, 23).

A first indication that III^{Glc} might be altered in an *iex* mutant derives from phosphorylation studies in vitro. The specific activity of III^{Glc} was determined in the presence of an excess of enzyme I, HPr, and enzyme II^{Glc}. Table 2 shows that III^{Glc} from the *iex* strain has only about 60% of the activity of the parental strain. The specific activity of enzyme I and HPr is the same in the iex^+ and *iex* strains. A *crr* mutant is unable to phosphorylate methyl α-glucoside due to the absence of III^{Glc}. Since the *iex* strain contains the same amount of III^{Glc} protein as the iex^+ strain (Table 2), this result suggests that this III^{Glc} has altered properties. In yet another way one can show that III^{Glc} in HK738 is altered. Heating III^{Glc} of the parent and the *iex* strains for 6 or 30 min at various temperatures results in a negligible decrease of the activity in the parent and an almost complete loss at all but the lowest temperature in the *iex* strain (Table 3). Clearly, the stability of III^{Glc} has been altered by the *iex* mutation.

No difference could be detected in the apparent molecular weight of III^{Glc} in iex^+ and *iex* strains as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, electroblotting on nitrocellulose filters, and detection with antibody against III^{Glc} (data not shown). A slight difference is observed after crossed immunoelectrophoresis. III^{Glc} from the *iex* strain shows a somewhat broader peak compared with the iex^+ III^{Glc}, but lacks the small shoulder present in the iex^+ strain. Mixing both types of III^{Glc} shows that wild-type and mutant III^{Glc} are immunologically identical (Fig. 1).

TABLE 3. Heat stability of III^{Glc}

Source	Temp (°C)	Activity of III^{Glc}[a]		
		No heat treatment	6 min of heating	30 min of heating
HK761	4	0.78	ND	ND
HK761	70	0.82	0.62	ND
HK738	4	0.37	ND	ND
HK738	37	0.37	0.33	0.21
HK738	45	0.37	0.13	0.10
HK738	54	0.37	0.01	0
HK738	63	0.37	0	0
HK738	70	0.37	0	ND
HK738(pBCP20)	70	1.05	0.39	ND

[a] High-speed supernatant samples of the various strains were heated for the indicated times, cooled at 0°C for 1 min, and assayed for remaining activity of methyl α-glucoside phosphorylation at 30°C. Activity is expressed as nanomoles of methyl α-glucoside phosphorylated per minute per milligram of protein. Control samples, not heated, were kept on ice. ND, Not determined.

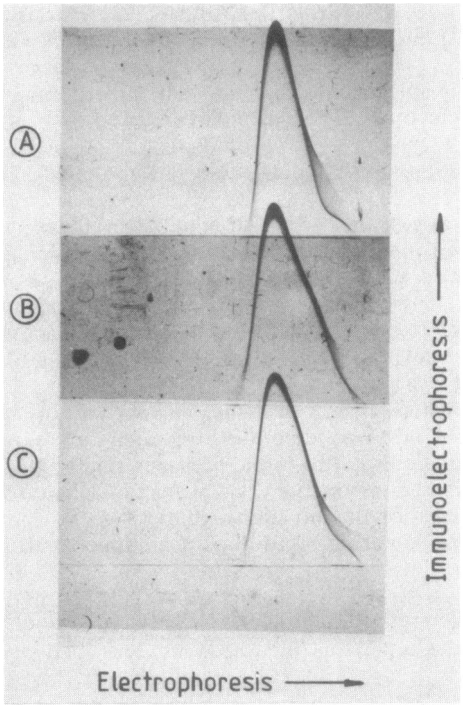

FIG. 1. Crossed immunoelectrophoresis of III^{Glc}. High-speed supernatants of strain HK761 (A), HK738 (B), and HK761 plus HK738 (C) were subjected to crossed immunoelectrophoresis as described in the text. Equal amounts of III^{Glc} were used in A and B. In C, half the amount of each was applied.

Binding of III^{Glc} to the lactose carrier. We have shown recently that non-phosphorylated III^{Glc} of *S. typhimurium* binds to and inactivates the lactose carrier of *E. coli* in the presence of β-galactosides (14). The properties of the *iex* mutant suggested to us that it might contain an altered III^{Glc} that is still active in phosphorylation, but unable to bind to the lactose carrier. Table 4 shows that the *E. coli* III^{Glc} from the parental strain (HK761) also binds to the lactose carrier and that this binding requires the presence of a β-galactoside. In contrast, III^{Glc} from the *iex* strain is unable to bind to the lactose carrier. To exclude the possibility that an inhibitory factor is present in the *iex* mutant, purified III^{Glc} was added to an *iex* supernatant, and the binding of this III^{Glc} to the lactose carrier was determined. The binding of purified III^{Glc} was equivalent to that in control under these conditions (Table 4).

Complementation by a crr^+ plasmid. We tested the possibility that the *iex* gene product is involved in modification of III^{Glc}. Conceivably, post-translational modification is required to allow the inhibitory action of III^{Glc}, although it does not influence its phosphorylating capacity. A plasmid containing the crr^+ gene was introduced in the *E. coli* strains. This plasmid produces III^{Glc} at about the same levels as the chromosomal gene (Table 2) (13). Table 1 shows that the *iex* strain HK738 acquires the parental phenotype upon transformation with the crr^+ plasmid pBCP20, i.e., it becomes sensitive again toward methyl α-glucoside on non-PTS compounds such as maltose, lactose, and glycerol. Thus the crr^+ gene is dominant.

Table 4 shows that under these conditions plasmid-produced III^{Glc} synthesized in the *iex* strain binds to the lactose carrier. The amount of III^{Glc} bound is lower, however, in the

TABLE 4. Binding of III^{Glc} to the lactose carrier[a]

Source of III^{Glc}[b]	TDG[c]	III^{Glc} concn[d]	III^{Glc} bound[e]
III^{Glc}	−	0.24	2.9
	+	0.21	9.6
HK761	−	0.18	3.3
	+	0.16	11.5
HK761(pBCP20)	−	0.17	2.9
	+	0.16	8.2
HK738	−	0.15	3.3
	+	0.15	3.3
HK738(pBCP20)	−	0.18	2.9
	+	0.16	5.3
HK738 + III^{Glc}	−	0.38	6.3
	+	0.31	14.2

[a] The binding of III^{Glc} from various sources to *E. coli* membrane vesicles containing 1.4 nmol of lactose carrier per mg of protein was determined as described in the text.

[b] Approximately 20 μg of III^{Glc} from each source was added to each binding assay. Pure III^{Glc} was purified from *S. typhimurium*.

[c] When present, the concentration of thiodigalactoside (TDG) was 4 mM.

[d] The free concentration of III^{Glc} was determined by rocket immunoelectrophoresis after centrifugation and is expressed as milligrams of III^{Glc} per milliliter.

[e] The bound III^{Glc} was determined by rocket immunoelectrophoresis and is expressed as micrograms of III^{Glc} bound per milligram of vesicle protein.

case of the HK738(pBCP20) strain compared with the HK761 strain lacking the plasmid, for the following reason. The final concentration of III^{Glc} was kept the same in both extracts. Since only about half of the III^{Glc} in the cell-free extract of HK738(pBCP20) originates from the plasmid *crr*$^+$ gene (Table 2) and the apparent K_d for III^{Glc} is 10 to 15 μM (14), one expects lowered binding at these III^{Glc} concentrations (approximately 5 μM each of mutant and wild-type III^{Glc}).

The heat inactivation of III^{Glc} produced by wild-type and mutant strains containing the plasmid also confirms the prediction. The III^{Glc} activity present in HK738(pBCP20) is only inactivated partly, whereas in HK738 the activity decreased to zero upon heating at 70°C (Table 3).

DISCUSSION

Two conflicting models have been advanced to explain the regulation of uptake and utilization of carbohydrates such as maltose, melibiose, glycerol, and lactose by the phosphoenolpyruvate-glucose PTS. According to one hypothesis, the protein III^{Glc} (product of *crr* gene) is the regulatory molecule (19–22), whereas according to Kornberg and co-workers (6–8, 16) an unidentified protein (product of the *iex* gene) is involved in the inhibition of non-PTS transport systems by PTS carbohydrates (inducer exclusion). Their main argument derives from the fact that two different mutations, *tgs* and *iex*, could be isolated in *E. coli* and that these mutations map at opposite sides of the *pts* operon. In their view III^{Glc}, the product of the *tgs* (*gsr*) gene, is only involved in glucose uptake and in the regulation of adenylate cyclase, but not in inducer exclusion of non-PTS carbohydrates. The data presented in this paper suggest, however, an alternative explanation for the *tgs* and *iex* mutations and the different phenotypes they cause, namely, that both are variable alleles of the *crr* gene.

E. coli tgs mutants as described by Kornberg are very similar to the *crr* mutants of *S. typhimurium* and of *E. coli* K-12 (10), whether selected as clones resistant to methyl α-glucoside or as *ptsI*(Ts) *crr* double mutants able to grow at 42°C on non-PTS carbohydrates. All are impaired in methyl α-glucoside transport and unable to grow on succinate. Growth of a *ptsI*(Ts) *tgs* double mutant on maltose at 42°C is partly restored compared with the *ptsI*(Ts) parent (8), similar again to *ptsI*(Ts) *crr* mutants (Table 1). The remaining difference in growth rate in the double mutant might be due to the fact that, as a result of the defective III^{Glc}, the cyclic AMP level is lowered. Expression of the maltose regulon is dependent on cyclic AMP. According to the *iex*-hypothesis, one would predict that *pts crr* double mutants of *E. coli* would not grow on non-PTS compounds like maltose, since the inhibitory *iex*-coded protein is still present. This, clearly, is not the case.

E. coli iex mutants and *S. typhimurium* and *E. coli crr* mutants apparently differ in one important phenotypic property. Whereas the latter lack III^{Glc}, the former are thought to possess normal, functional III^{Glc} as deduced from methyl α-glucoside transport and inhibition of growth on fructose by methyl α-glucoside. Thus, according to Kornberg, growth on non-PTS compounds is resistant to methyl α-glucoside in *crr* and *iex* mutants for different reasons. In the case of the *crr* mutation, the analog cannot enter the cell. In the case of the *iex* mutants, the unidentified *iex*-coded protein is mutated or absent.

The data presented in this paper show that in the *iex* mutant HK738 an altered III^{Glc} is found, active in phosphorylation in vitro, but unable to bind to the lactose carrier. Although strain HK738 seems different from the *S. typhimurium* or *E. coli crr* mutants, this is due to the fact that most *crr* mutants lack III^{Glc} altogether, whereas in *iex* mutants the various functions of III^{Glc} have been affected to different extents. The normal transport of methyl α-glucoside and the growth on succinate (which requires cyclic AMP) indicate that III^{Glc} can still be phosphorylated and can donate its phosphoryl group to II^{Glc}. It is also able to function in the activation of adenylate cyclase. Non-phosphorylated III^{Glc} is unable, however, to bind to the lactose carrier. A further strong indication for the presence of an altered III^{Glc} in the *iex* strain HK738 is the heat lability of the protein.

The allele specificity of *crr* (*tgs*) and *iex* mutations is sufficient to explain their phenotypic differences, assuming that only the inducer exclusion function with respect to non-PTS carbohydrates of III^{Glc} is altered in *iex* mutants. In such mutants, growth on non-PTS substrates is no longer inhibited by the altered III^{Glc}, whereas glucose and methyl α-glucoside can still inhibit the uptake of, for instance, fructose by competition for the common P~HPr pool (4, 23). Since a similar suppression by *iex* of PTS-mediated inhibition of growth on glycerol, maltose, or melibiose is obtained, it is likely that the interaction of III^{Glc} with those systems is also impaired.

The genetic evidence that *iex* and *crr* mutations lie at opposite sides of the *pts* operon seems an argument against our hypothesis that both are alleles of the same gene *crr*. Unfortunately, the published evidence is not sufficient to distinguish unequivocally between the two possibilities. The localization of *iex* has varied considerably, ranging from 25% cotransduction with *ptsI* (17) to possibly allelic with *ptsH* (2), whereas the apparent molecular weight of its gene product is given as 33,000 or 21,000. Furthermore, several of the *iex*-transducing lambda phages used had to be disregarded in the construction of the genetic map since either the gene products for which they code or the complementation pattern did not agree with the restriction enzyme site mapping. As a result the mapping was done with one lambda phage (e.g., lambda 206) lacking the *iex* proteins and having

an *iex* phenotype. Interestingly, this phage also produces a strongly decreased amount of III^{Glc} (3). In addition, the possibility has to be considered that different *iex* alleles produce III^{Glc} proteins having a residual activity at lower temperature (*iex* phenotype), but none at higher temperature (*crr* phenotype). This could explain several problems related to the complementation studies.

We think our studies have resolved the discrepancies between *crr* (*tgs* or *gsr*) mutations in *S. typhimurium* or *E. coli* and *iex* mutations with respect to their role in glucose-generated catabolite repression or inducer exclusion of non-PTS carbohydrates. Different mutations in the *crr* gene, coding for III^{Glc}, can affect the phenotype of *pts* mutants in different ways, *iex* and *tgs* (*crr*) being most likely alleles of the *crr* gene affecting different domains of III^{Glc} to variable extents.

It would be most interesting to sequence the mutated *iex* genes to determine what changes have occurred. This could possibly tell us something about the interaction between III^{Glc} and its many different target proteins.

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