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Regulation of Genes Coding for Enzyme Constituents of the Bacterial Phosphotransferase System

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Regulation of the synthesis of the proteins of the phosphoenolpyruvate:sugar phosphotransferase system was systematically studied in wild-type and mutant strains of *Salmonella typhimurium* and *Escherichia coli*. The results suggest that enzyme I and HPr as well as the glucose-specific and the mannose-specific enzymes II are synthesized by a mechanism which depends on (i) cyclic adenosine monophosphate and its receptor protein; (ii) extracellular inducer; (iii) the sugar-specific enzyme II complex which recognizes the inducing sugar; and (iv) the general energy-coupling proteins of the phosphotransferase system, enzyme I and HPr.

The bacterial phosphotransferase system (PTS) catalyzes the concomitant transport and phosphorylation of a variety of sugars (11-13, 17). The transport and phosphorylation of sugar at the expense of the high-energy phosphoryl bond of phosphoenolpyruvate require the participation of four enzymes. Enzyme I and HPr are the general energy-coupling proteins which are located in the cytoplasm, whereas enzymes II and III exhibit specificity for one or a few sugar substrates of the PTS (12, 17, 21). The enzymes II are integral membrane proteins which function as the sugar recognition constituents of the system. The genes which code for the general proteins HPr and enzyme I, *ptsH* and *ptsI*, respectively, comprise an operon, the *pts* operon (3-5, 7, 23-25). These genes are located at about 55 min on the new *Salmonella typhimurium* linkage map (5). The operon possesses an operator-promoter region (4), and the order of genes in the operon is *ptsOP*, *ptsH*, *ptsI*.

The genes which code for the sugar-specific proteins of the PTS are dispersed on the bacterial chromosome. Those which code for the enzymes II specific for mannitol, glucitol, galactitol, and fructose are included within operons comprised of genes which code for the catabolic enzymes involved in the metabolism of the particular sugar. The genes which code for enzymes II specific for glucose (enzyme II^{glc}), *glu*, and for mannose (enzyme II^{man}), *manA*, map separately from each other and from other genes involved in the catabolism of these sugars. Regulation of the expression of the *pts* genes and the sugar-specific *glu* and *manA* genes is the subject of the present communication. An account of some of the results has appeared in preliminary form (24).

MATERIALS AND METHODS

Materials. Phosphoenolpyruvate, sugar phosphates, and radioactive and nonradioactive sugars used in the present study were obtained as described previously (18).

Bacterial strains. The bacterial strains employed in the present study are listed in Table 1. *S. typhimurium* strain LJ144 with elevated enzyme I, HPr, and enzyme III^{glc} activities (22) was used as a source of the soluble PTS proteins.

Media and growth conditions. The growth medium was medium 63 without iron (20). The carbon sources were autoclaved separately and were added to the autoclaved medium at the concentrations indicated in the legends to the tables. Flasks were filled with the liquid medium to less than 50% of capacity. Cells were grown at 37°C with aeration at a rotation speed of 200 rpm.

Enzyme assays. Sugar uptake was conducted as previously described (25). The activities of HPr and enzyme I were measured as described elsewhere (5, 24). Enzyme II activities were measured with butanol-urea-extracted membranes (12). The in vitro phosphoenolpyruvate-dependent sugar phosphorylation reaction catalyzed by the enzymes II was conducted with saturating amounts of HPr and enzyme I obtained from strain LJ144. The reaction mixtures contained 50 mM potassium phosphate buffer (pH 7.5), 10 mM $MgCl_2$, 20 mM KF, 1 mM dithiothreitol, 10 mM phosphoenolpyruvate, and ^{14}C-sugar at 5 μM for methyl-α-glucoside and 10 μM for 2-deoxyglucose. The final volume of the reaction mixture was 200 μl. The reaction mixture for the transphosphorylation reactions catalyzed by enzyme II^{glc} contained, in a final volume of 200 μl, 50 mM potassium phosphate buffer (pH 6), 10 μM [^{14}C]methyl-α-glucoside, and 1 mM glucose-6-phosphate. The reaction mixture (final volume, 200 μl) for the transphosphorylation reaction catalyzed by enzyme II^{man} contained 50 mM potassium phosphate buffer (pH 6.0), 12 μM [^{14}C]2-deoxyglucose, and 15 mM mannose-6-phosphate (18). All assays were con-

TABLE 1. *Bacterial strains*[a]

Strain no.	Genotype	Reference or source
S. typhimurium		
LT-2	Wild type	(22)
SB1476	*ptsI17*	(22)
SB1477	*ptsI18*	(22)
SB1682	*ptsI19*	(22)
LJ163	*metE336 cpd-451*	(2)
LJ162	*metE336 cpd-451 cya-861*	From LJ163 with phosphomycin (22)
LJ173	*metE336 cpd-451 crp-177*	(16)
SB1795	cya-207	
SB1887	*manA12*	(22)
SB1744	*mtl-61*	(22)
SB783	*fru-2*	(22)
LJ62	*cpd-401*	(2)
LJ114	*cpd-401 ptsH15*	From SB1475
LJ135	*cpd-401 ptsI18*	From SB1477
LJ138	*cpd-401 crrA3*	(19)
LJ211	*cpd-401 Δ(cysK-ptsHI-crrA49)*	(5)
E. coli		
1100	*thi*	(12)
5333	*thi crp*	(12)
5336	*thi cya*	(13)

[a] The genetic nomenclature used is that recommended by Lin (14).

ducted under conditions where activity was linear with time and protein concentration. Incubations were at 37°C for 5 to 10 min.

RESULTS

Induction of HPr and enzyme I by extracellular sugar substrates of the PTS. Table 2 summarizes the relative specific activities of HPr and enzyme I after growth of wild-type *S. typhimurium* cells in the presence of a variety of carbon sources. Cells grown in the presence of lactate, pyruvate, or glycerol possessed basal (noninduced) activities of these proteins. However, when the cells were grown in the presence of any one of the natural sugar substrates of the PTS listed in the table, the specific activities of these two enzymes were coordinately induced to a maximal extent of threefold. No induction was observed when the cells were grown in the presence of maltose or melibiose which are taken into the cell by active transport and converted intracellularly to glucose and glucose phosphates. These observations suggest that the sugar must be present in the external medium for it to exert an inductive effect.

Induction of enzyme II^{glc} and enzyme II^{man} by extracellular sugars. The activities of enzyme II^{glc} and enzyme II^{man} were assayed in strain LJ62 after growth in the presence of the carbon sources indicated in Table 3. Both enzymes were induced when cells were grown in the presence of glucose, but not when maltose or melibiose was included in the growth medium as the carbon source. The transport activities of the enzymes were induced about threefold, whereas the in vitro phosphorylation activities of the enzymes were induced seven to elevenfold. The difference in the extent of induction between the in vivo and the in vitro activities suggests that the other components of the PTS were present in rate-limiting concentrations in the cell. The results suggest that enzyme II^{glc} and enzyme II^{man} are under similar genetic control and that extracellular glucose is the inducer.

Dependence of enzyme I and HPr synthesis on cyclic AMP and the cyclic AMP receptor protein. Studies were conducted with *Escherichia coli* and *S. typhimurium* strains. The wild-type strains 1100 (*E. coli*) and LT-2 (*S. typhimurium*) exhibited two- to threefold-higher activities of enzyme I and HPr after growth in the presence of fructose as compared with growth on pyruvate (Table 4). In an *S. typhimurium* mutant which lacked adenylate cyclase activity, fructose did not induce the activities of enzyme I and HPr unless cyclic AMP was added to the growth medium. Similarly, mutants of *E. coli* which were defective for either adenylate cyclase activity or functional cyclic AMP receptor protein were not fully in-

TABLE 2. *Induction of enzyme I and HPr by exogenous sugars*[a]

Carbon source in growth medium	Relative sp act	
	HPr	Enzyme I
Potassium lactate	1.0[b]	1.0[c]
Sodium pyruvate	1.0	1.1
Glycerol	1.1	1.1
Glucose	2.7	3.0
Lactate and methyl-α-glucoside	1.1	0.9
N-acetylglucosamine	2.5	2.6
Mannose	2.9	2.5
Glucosamine	1.7	1.9
Fructose	2.9	3.2
Mannitol	2.9	3.2
Maltose	1.2	1.4
Melibiose	1.4	1.0

[a] The experiments were conducted with *S. typhimurium* strain LT-2. Cells were grown and assayed for enzyme I and HPr activities as described in the text. The indicated carbon source was present in the growth medium at a concentration of 0.5%.

[b] The value of 1.0 corresponds to a specific activity of 600 nmol of methyl-α-glucoside phosphorylated per h per mg of protein at 37°C employing the assay conditions described (24, 25).

[c] The value of 1.0 corresponds to a specific activity of 5,400 nmol of methyl-α-glucoside phosphorylated per h per mg of protein when assayed as described (24, 25).

TABLE 3. *Induction of enzyme II^{glc} and enzyme II^{man} measured by in vivo transport and in vitro phosphorylation activities*[a]

Carbon source for growth	Relative sp act[b] for:					
	Sugar uptake		Phosphoenolpyruvate-dependent phosphorylation		Sugar-phosphate dependent phosphorylation	
	Enzyme II^{glc}	Enzyme II^{man}	Enzyme II^{glc}	Enzyme II^{man}	Enzyme II^{glc}	Enzyme II^{man}
Glycerol	1.0	1.0	1.0	1.0	1.0	1.0
Glucose	3.0	2.5	11	8	10	7
Maltose	0.8	1.2	2	1.2	1	1.3
Melibiose	0.9	1.0	3	1.5	2	1.5

[a] Cells (strain LJ62) were grown in medium 63 supplemented with the carbon sources indicated in the table. The cells were harvested during the mid-exponential phase of growth and were washed three times with medium 63. The cells were resuspended to a cell density of 0.16 mg (dry weight) per ml for measuring the transport activity of the enzyme II^{glc} and 0.08 mg (dry weight) per ml for measuring the transport activity of the enzyme II^{man}. The experiment was conducted as described previously (25). The concentrations of the carbon sources were 0.4% glycerol, 0.2% glucose, 0.4% maltose, and 0.4% melibiose. Phosphorylation assays were conducted as described in the text.

[b] The value of 1.0 for the rate of methyl-α-glucoside (40 μM) uptake corresponded to 0.6 μmol/min per g (dry weight) of cells, whereas that for mannose (40 μM) uptake corresponded to 1.8 μmol/min per g (dry weight) of cells. The value of 1.0 for the phosphoenolpyruvate-dependent phosphorylation reactions by enzyme II^{glc} and enzyme II^{man} corresponded to 7 and 10 μmol/min per g of protein, respectively, and to 0.3 μmol/min per g of protein for the two sugar phosphate-dependent phosphorylation reactions.

TABLE 4. *Induction of enzyme I and HPr in mutants lacking proteins involved in cyclic AMP action*[a]

Strain and carbon source	Relative sp act[b]	
	HPr	Enzyme I
S. typhimurium		
LT-2		
Pyruvate	1.0	1.0
Fructose	2.6	2.8
SB1795 (*cya-207*)		
Pyruvate	0.9	1.1
Fructose	0.9	0.9
Fructose + cyclic AMP	2.9	2.3
E. coli		
1100		
Pyruvate	1.0	1.0
Fructose	2.0	2.2
5333 (*crp*)		
Pyruvate	0.9	1.2
Fructose	1.2	1.6
5336 (*cya*)		
Pyruvate	1.0	1.1
Fructose	1.3	1.6

[a] The bacterial cells specified in the table were grown and assayed for HPr and enzyme I activities as described in the text. The carbon sources were present in the growth medium at a concentration of 0.5%.

[b] Absolute specific activities were as in Table 2.

ducible for the general proteins of the PTS.

Dependence of enzyme II^{glc} and enzyme II^{man} induction on cyclic AMP. The experiments were conducted with *S. typhimurium* strains which possessed wild-type activities of the proteins involved in cyclic AMP action and with isogenic *crp* and *cya* mutants. The activities of enzyme II^{glc} and of enzyme II^{man}, assayed in vivo, are shown in Table 5. The levels of the enzymes II were low in the mutant strains (*cya* and *crp*) as compared with the uninduced parent. The transport activities were induced threefold in the wild-type strain when glucose was present in the growth medium. The *cya* and *crp* mutants were not inducible. Inclusion of 10 mM cyclic AMP in the growth medium which contained glucose resulted in induction of both the enzyme II^{glc} and the enzyme II^{man} in the *cya* mutant, but not in the *crp* mutant. The in vitro activities catalyzed by enzyme II^{glc} and enzyme II^{man} gave similar results (Table 5), although the extent of induction was greater. These results show that induction of enzyme II^{glc} and enzyme II^{man} is under cyclic AMP control.

Induction of PTS constituents in strains defective for various proteins of the PTS. The induction of the general proteins of the PTS was examined in the wild type and enzyme II mutants of *S. typhimurium*. The results summarized in Table 6 suggest that the proteins which recognize the extracellular inducers are the sugar-specific enzymes II. HPr and enzyme I activities were induced in the wild-type strain, LT-2, by growth in the presence of either fructose or mannitol. In strain SB783 (*fru-2*), which lacks enzyme II^{fru} activity, fructose no longer induced the *pts* operon, although mannitol retained its inductive effect. Similarly, loss of mannitol or mannose enzyme II activity altered the inductive response to mannitol or mannose, respectively, but did not affect the inductive response to other PTS sugars present in the growth medium.

TABLE 5. *Dependence of enzyme II^{glc} and enzyme II^{man} induction on proteins involved in cyclic AMP action*[a]

S. typhimurium strains	Carbon source in the growth media	Relative sp act[b] for: Sugar uptake catalyzed by: Enzyme II^{glc}	Sugar uptake catalyzed by: Enzyme II^{man}	PEP-dependent phosphorylation: Enzyme II^{glc}	PEP-dependent phosphorylation: Enzyme II^{man}	Sugar-phosphate-dependent phosphorylation: Enzyme II^{glc}	Sugar-phosphate-dependent phosphorylation: Enzyme II^{man}
LJ163 (*metE336 cpd-451*)	Lactate	1	1	1	1	1	1
	Lactate+ glucose	3.5	3	7	10	10	6
LJ162 (*metE336 cpd-451 cya-861*)	Lactate	0.4	0.2	0.9	1.0	0.5	0.5
	Lactate + glucose	0.4	0.3	1.2	1.0	0.5	0.5
	Lactate + glucose + cAMP	3	2.7	8	10	7	8
LJ173 (*metE336 cpd-451 crp-177*)	Lactate	0.3	0.3	0.7	0.5	0.8	0.3
	Lactate + glucose	0.3	0.4	1.1	0.5	0.7	0.3
	Lactate + glucose + cAMP	0.5	0.4	1.2	0.7	0.7	0.3

[a] Cells were grown to the mid-exponential growth phase as described in the text, harvested by centrifugation, and washed three times with medium 63. The uptake studies with 50 μM [^{14}C]mannose or [^{14}C]methyl-α-glucoside were conducted with a cell density of 0.04 mg (dry weight) per ml or 0.16 mg (dry weight), respectively. Glucose and DL-lactate were added at concentrations of 0.2 and 0.5%, respectively. Cyclic AMP, when present, was added to a concentration of 10 mM. Phosphorylation assays were as described under "Materials and Methods".

[b] Specific activities were similar to those reported in Table 3. PEP, Phosphoenolpyruvate.

TABLE 6. *Induction of enzyme I and HPr in enzyme II mutants*[a]

S. typhimurium strain	Carbon source in the growth medium	Relative sp act[b]: HPr	Relative sp act[b]: Enzyme I
Expt 1			
LT-2	Lactate	1.0	1.0
	Lactate + fructose	2.9	2.7
	Lactate + mannitol	2.8	2.4
SB783 (*fru-2*)	Lactate	1.2	1.0
	Lactate + fructose	1.2	1.5
	Lactate + mannitol	2.9	2.1
SB1744 (*mtl-61*)	Lactate	1.2	1.2
	Lactate + fructose	2.9	2.6
	Lactate + mannitol	1.4	1.3
Expt 2			
LT-2	Lactate	1.0	1.0
	Lactate + mannose	2.1	2.2
	Lactate + mannitol	2.2	2.3
SB1887 (*manA12*)	Lactate	1.1	1.1
	Lactate + mannose	1.6	1.7
	Lactate + mannitol	2.4	2.7

[a] Assays were conducted as described in the text and in footnote *a* of Table 2.

[b] Absolute specific activities were as given in Table 2.

TABLE 7. *Induction of HPr activity in enzyme I mutants*[a]

Mutant strain	Carbon source in medium	Relative sp act[b]: Enzyme I	Relative sp act[b]: HPr
LT-2	Lactate	1.0	1.0
	Lactate + fructose	2.8	2.8
ptsI17	Lactate	0.006	1.3
	Lactate + fructose	0.017	2.8
ptsI18	Lactate	<0.001	2.5
	Lactate + fructose	<0.001	2.6
ptsI19	Lactate	<0.001	1.3
	Lactate + fructose	<0.001	1.2

[a] Assays were conducted as described in the text and in footnote *a* of Table 2.

[b] Absolute specific activities were as given in Table 2.

A regulatory function of enzyme I is suggested from the results summarized in Table 7. In wild-type cells, HPr and enzyme I were induced after growth in the presence of fructose. In the "leaky" enzyme I mutant, *ptsI17*, which possessed about 1% of wild-type enzyme I activity, both enzyme I and HPr activities were inducible. By contrast, in "tight" enzyme I mutants, *ptsI18* and *ptsI19*, HPr synthesis was constitutive. These results suggest that enzyme I, even when present in low amounts, regulates expression of the *pts* genes.

The effects of mutations in the genes which code for various constituents of the PTS on the induction of Enzyme II^{glc} and Enzyme II^{man} are shown in Table 8. The strain which possesses wild-type PTS activities showed 7- to 10-fold increases of both enzymes after growth in the presence of glucose. Mutants which lacked the activities of enzyme I, HPr, or both were constitutive for the synthesis of enzyme II^{glc} and enzyme II^{man}. The levels of enzyme activities in these mutants were elevated about twofold over the induced level of the wild type. Enzyme II activities in the tight enzyme I mutant strain (LJ135) and in a deletion mutant strain (LJ211)

TABLE 8. *Induction of enzyme II^{glc} and enzyme II^{man} in mutant strains of S. typhimurium defective for components of the PTS*[a]

S. typhimurium strain	Carbon source	Relative sp act[b] for:			
		Phosphoenolpyruvate-dependent phosphorylation		Sugar-Phosphate-dependent phosphorylation	
		Enzyme II^{glc}	Enzyme II^{man}	Enzyme II^{glc}	Enzyme II^{man}
LJ62 (*cpd-401*)	Lactate	1	1	1	1
	Lactate + glucose	9	7	10	10
LJ114 (*cpd-401 ptsH15*)	Lactate	23	19	18	17.5
	Lactate + glucose	18	20	17	15.0
LJ135 (*cpd-401 ptsI18*)	Lactate	17	22	24	21
	Lactate + glucose	12	10	9	11
LJ138 (*cpd-401 crrA3*)	Lactate	0.7	0.6	0.5	0.5
	Lactate + glucose	6.0	4.5	5.5	5.4
LJ211 [*cpd-401* Δ(*cysK-ptsHI-crrA49*)]	Lactate	18	22	20	16
	Lactate + glucose	10	12	8	15

[a] *S. typhimurium* strains were grown in the presence of 0.5% DL-lactate or 0.5% DL-lactate and 0.2% glucose. The growth conditions and the assays for enzyme II activities are described in the text.

[b] Absolute specific activities for the parental strain were similar to those reported in Table 3.

were slightly depressed after growth in the presence of glucose. The enzymes II in the *crr* mutant (LJ138) were inducible, but the levels of these enzymes were low. This could be due to low cytoplasmic concentrations of cyclic AMP in this strain (22). These results show that the expression of enzyme II^{glc} and enzyme II^{man} is regulated by the protein products of the *pts* operon.

DISCUSSION

Extracellular induction has been clearly demonstrated for the hexose-phosphate transport system of *E. coli* (6). It was shown that in mutants which lacked glucose-6-phosphate dehydrogenase and phosphoglucoisomerase and therefore could not metabolize intracellular glucose-6-phosphate, the sugar-phosphate accumulated to a cytoplasmic concentration in excess of 50 mM. Under these conditions, the synthesis of the hexose phosphate permease was not induced. A low extracellular concentration of inducer (0.1 mM) resulted in rapid induction of the permease (6). These results suggested that the inducer had to be present in the extracellular medium for induction to occur.

The phosphoglycerate transport system in *S. typhimurium* also appeared to be induced only when extracellular inducer is present (26), and preliminary observations suggested that the citrate permease in *S. typhimurium* is similarly controlled (21). Thus, expression of the genes which code for a variety of bacterial permeases appears to be dependent on extracellular inducer.

The present studies show that the PTS constituents are induced from without and, furthermore, that the inducer must interact with a functional sugar-specific enzyme II for induction to occur. Consequently, induction may result from interaction of an enzyme II with its extracellular sugar substrate, and this interaction may generate a cytoplasmic signal which is transmitted to the genes coding for the PTS constituents (21). The nature of the interaction between the sugar and the membrane-associated enzyme II is not clear. The binding of sugar to the enzyme, or the coupled translocation and phosphorylation of the sugar, or both might provide the inductive signal.

It has been shown previously that the transport, phosphorylation, and chemotactic activities of the enzyme II^{glc} are cyclic AMP dependent (1, 19). Inclusion of cyclic AMP in the growth medium restored the ability of *cya* mutants to induce the PTS protein, indicating that synthesis rather than the activity of the enzyme is under cyclic AMP control. Regulation of the expression of the *pts* genes by cyclic AMP renders virtually all of the proteins of the PTS subject to catabolite repression (8, 13; Tables 4 and 5). Whether regulation of the *pts* operon by cyclic AMP is a direct effect or an indirect effect resulting from prior induction of the enzymes II is not known. The slight induction in the *E. coli cya* and *crp* mutants may result from the fact that these mutants are phenotypically leaky.

Mutations in one of the *pts* genes rendered synthesis of enzyme I, HPr, enzyme II^{glc}, and enzyme II^{man} constitutive (27; Tables 7 and 8). The latter two enzymes exhibited higher levels of PTS enzyme activities than were found in the

induced wild-type strain. The elevated levels of PTS enzyme activities may reflect the nonrepressible maximal activities of the enzymes. The constitutive expression of the PTS enzymes in *ptsH* and *ptsI* mutants suggest that although the *pts*, *glu*, and *manA* genes map in distinct locations on the bacterial genome, they are subject to a common regulatory mechanism. It should be noted, however, that different strains of *E. coli* show different induction patterns. Thus, the extent of induction is strain dependent (10), and glucose repression of the glucose enzyme II has been reported (9). The studies reported here, in addition to defining the physiological conditions necessary for induction and repression of genes coding for specific carbohydrate catabolic enzymes in *S. typhimurium*, provide preliminary evidence for a novel mechanism responsible for the regulation of gene expression.

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