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Fine Control of Adenylate Cyclase by the Phosphoenolpyruvate:Sugar Phosphotransferase Systems in *Escherichia coli* and *Salmonella typhimurium*

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Inhibition of cellular adenylate cyclase activity by sugar substrates of the phosphoenolpyruvate-dependent phosphotransferase system was reliant on the activities of the protein components of this enzyme system and on a gene designated *crrA*. In bacterial strains containing very low enzyme I activity, inhibition could be elicited by nanomolar concentrations of sugar. An antagonistic effect between methyl α-glucoside and phosphoenolpyruvate was observed in permeabilized *Escherichia coli* cells containing normal activities of the phosphotransferase system enzymes. In contrast, phosphoenolpyruvate could not overcome the inhibitory effect of this sugar in strains deficient for enzyme I or HPr. Although the in vivo sensitivity of adenylate cyclase to inhibition correlated with sensitivity of carbohydrate permease function to inhibition in most strains studied, a few mutant strains were isolated in which sensitivity of carbohydrate uptake to inhibition was lost and sensitivity of adenylate cyclase to regulation was retained. These results are consistent with the conclusions that adenylate cyclase and the carbohydrate permeases are regulated by a common mechanism involving phosphorylation of a cellular constituent by the phosphotransferase system, but that bacterial cells possess mechanisms for selectively uncoupling carbohydrate transport from regulation.

Escherichia coli and *Salmonella typhimurium* mutant cells lacking either enzyme I or HPr of the phosphoenolpyruvate-dependent phosphotransferase system (PTS) cannot grow on a large number of sugars. Some of these sugars (PTS sugars) are transported into bacterial cells by a group translocation mechanism which requires phosphorylation of the sugar (15). Other sugars (non-PTS sugars) are transported by facilitated diffusion or by active transport processes which do not require the participation of PTS protein constituents (2, 8). Nevertheless, some of these non-PTS carbohydrates (glycerol, melibiose, maltose, and lactose) are not utilized by mutants lacking enzyme I or HPr (15, 22, 25).

Several reports have considered this problem. Gershanovitch et al. (6) and Fox and Wilson (5) first reported abnormal induction of β-galactosidase synthesis in cells lacking enzyme I. Similar results have been reported for the enzyme systems responsible for the catabolism of glycerol (1), melibiose (22), and maltose (20). Pastan and Perlman provided evidence that poor induction of β-galactosidase synthesis in certain *pts* mutants of *E. coli* resulted from hypersensitivity of the synthesis of the lactose-catabolic enzyme system to repression (13). They showed that repression interfered with *lac* mRNA synthesis and, in addition, that high extracellular concentrations of cyclic AMP overcame this repression (12, 26).

In previous communications, studies concerned with the regulation of non-PTS permease function by the phosphotransferase system were reported (3, 18, 21). It was shown that in *E. coli* and *S. typhimurium*, inducer exclusion, a manifestation of transport regulation, contributed substantially to the repression of carbohydrate catabolic enzyme synthesis (19, 24). Moreover, it was demonstrated that the activities of several non-PTS carbohydrate permeases and of adenylate cyclase were subject to coordinate regulation when the cellular components of the PTS (enzyme I, HPr, and the enzyme II complexes) were manipulated by mutation or induction (17, 18). By employing a mutant in which enzyme I was thermolabile, it was further shown that this protein (and therefore presumably HPr and enzyme II as well) functions as a catalytic component of the regulatory systems (3). These observations led to the suggestion that a common mechanism was responsible for the regulation of the activities of the proteins responsible for in-

ducer uptake and cyclic AMP synthesis.

In this paper the results of additional experiments which bear on the molecular mechanism of adenylate cyclase regulation are presented. It is shown that the in vivo inhibition of adenylate cyclase by sugar substrates of the PTS is dependent on the cellular levels of phosphoenolpyruvate, on the activities of the protein components of the phosphotransferase system, and on a gene designated *crrA* (17, 20). The effect of the mutant *crrA* gene depends on the genetic background of the strain into which it is introduced. In certain mutant strains, exceptionally low sugar concentrations (as low as 0.05 μM) can inhibit net cyclic AMP synthesis. In a few mutants the regulation of permease function is uncoupled from that of adenylate cyclase. A preliminary account of some of this work has appeared previously (17).

MATERIALS AND METHODS

Most materials were obtained commercially and were of the highest purity available. Lactose (Calbiochem) was recrystallized twice from aqueous ethanol and was free of detectable glucose. Methyl α-glucoside was purified free of glucose as described previously (16). Assays for phosphoenolpyruvate:sugar phosphotransferase activities (11), transport (22), β-galactosidase in toluene-treated cells (22), cyclic AMP (7, 18), and adenylate cyclase in toluene-treated cells (9, 10) have been described previously. Growth rates and fermentation responses were determined as described previously (22). The minimal medium used was medium 63 without iron (22). The complex medium was doubly concentrated nutrient broth. The *E. coli* strains used in this study and their pertinent genotypes are listed, in Table 1. The strains of *S. typhimurium* used are listed in Table 3. All bacterial strains were isolated or constructed by published procedures (3, 4, 18, 22). The *ptsH307* mutation was introduced into

TABLE 1. *Effect of methyl α-glucoside on the induction of β-galactosidase in E. coli strains*[a]

Expt	Strain	Genotype	o-Nitrophenyl-β-galactoside hydrolysis (optical density units/min per mg [dry wt])		% Methyl α-glucoside-mediated repression
			Without methyl α-glucoside	With methyl α-glucoside	
1	1100	*thi*	4.2	2.4	43
	1101	*thi ptsH315*	4.9	0.8	84
	LJ139	*thi ptsH315 crrA156*	4.6	5.3	0
	1103	*thi ptsI316*	2.5	1.6	36
	SB2273	*thi ptsI316 crrA13*	4.4	4.7	0
2	SB2252 (AB257^{pc-1})	*met cpd-454*	6.8	6.9	0
	SB2260	*met cpd-454 ptsI109*	6.7	5.0	25
	SB2876	*met cpd-454 ptsI109 crrA27*	7.0	8.1	0
3	SB2249 (Crook)	*cpd-453*	4.7	3.7	21
	LJ176	*cpd-453 ptsI313*	4.9	3.1	37
	LJ177	*cpd-453 ptsI313 crrA158*	1.8	1.9	0
4	3300 (SB2889)	*thi lacI*	26	24	8
	3300-1 (SB2890)	*thi lacI ptsI*	18	17	5
5	9036G (SB2891)	*pro his lacY*	5.6	4.3	23
	9036GB (SB2892)	*pro his lacY ptsI*	2.7	2.7	0

[a] Cells were grown overnight with aeration in medium 63 containing 1% DL-lactate (experiments 1 through 3) or 1% pyruvate (experiments 4 and 5) as the sole source of carbon. Thiamine (5 μg/ml) was included in experiment 1, methionine (20 μg/ml) was present in experiment 2, thiamine (5 μg/ml) was present in experiment 4, and proline and histidine (10 μg/ml) were included in experiment 5. The growth temperature was 35°C in experiments 1 and 2, 28°C in experiment 3, and 37°C in experiments 4 and 5. Fresh medium of the same composition was inoculated with the stationary-phase cultures, and cells were allowed to grow for 4 h with aeration before initiation of β-galatosidase induction in experiments 1 through 3 and 5 by the addition of isopropyl β-thiogalactoside (0.5 mM) when all cultures were in the exponential growth phase. Methyl α-glucoside (5 mM), when present, was added simultaneously with inducer. After a 20-min induction period, the cell suspensions were chilled to 0°C. Cell densities were determined, and β-galactosidase activity was measured in toluene-treated cells (22). The strains employed in experiments 1 (22), 2 (18), and 3 (3) have been described previously. All strains in each experiment are isogenic except for the indicated markers. *ptsH* and *ptsI* mutants are deficient for HPr and enzyme I, respectively (22). The mutants used in the experiments exhibit from 1 to 5% of wild-type activity. *crrA* mutations have been described previously (17, 19, 20). The *ptsH315* mutant is phenotypically leaky because of the presence of a fructose-inducible protein which can substitute for HPr (22).

strain SB2249 by the fosfomycin resistance selection procedure described previously (3).

RESULTS

Effects of *pts* and *crrA* mutations on induction of β-galactosidase synthesis in *E. coli*. The induction of β-galactosidase synthesis was studied in several series of *E. coli* strains under a variety of experimental conditions. Table 1 summarizes the effects of *pts* and *crrA* mutations on isopropyl β-thiogalactoside-induced synthesis of β-galactosidase in several genetic backgrounds. In a wild-type *E. coli* K-12 genetic background (Table 1, experiment 1) the phenotypically leaky *ptsH315* mutation exerted little effect on β-galactosidase synthesis in the absence of methyl α-glucoside, but the mutation enhanced sensitivity of β-galactosidase synthesis to repression in the presence of low concentrations of sugar. On the other hand, the "leaky" *ptsI316* mutation depressed the rate and extent of β-galactosidase synthesis in the absence of methyl α-glucoside, but the glucoside exerted no more of a repressive effect in the mutant than in the wild-type parent. The effects of leaky *ptsI* mutations on the repression of β-galactosidase synthesis by methyl α-glucoside were less pronounced when the cells lacked cyclic AMP phosphodiesterase (Table 1, experiments 2 and 3). In all cases, a *crrA* mutation abolished the repressive effects of methyl α-glucoside.

The consequences of *ptsI* mutations were also studied in *lacI* (lactose repressor-negative) and *lacY* (lactose permease-negative) strains (Table I, experiments 4 and 5). With cells growing exponentially in pyruvate minimal medium and with 1 mM isopropyl β-thiogalactoside as inducer for the permease-negative strains, rates of β-galactosidase synthesis were depressed 30 to 50% by "tight" *ptsI* mutations.

Effects of *pts* and *crr* mutations on net cyclic AMP synthesis in *E. coli* strains. By employing the bacterial strains listed in Table 1, the net production of cyclic AMP during growth in nutrient broth was determined (Table 2). A qualitative correlation was observed between net cyclic AMP production and sensitivity to repression of β-galactosidase synthesis in the presence of isopropyl β-thiogalactoside (Table 1). For example, the *ptsH315* mutation did not depress the rate of β-galactosidase synthesis or of cyclic AMP production in the absence of methyl α-glucoside, but this sugar depressed both rates more than in the parental strain. In contrast, the *ptsI316* mutation, which depressed the rate of β-galactosidase synthesis in the absence of methyl α-glucoside, also inhibited cyclic AMP production. The *crrA* mutations tested restored induction rates and enhanced the amounts of cyclic AMP synthesized in both strains. The effect of *crrA* mutations on cyclic AMP production in the genetic background of strain 1100 was different from that observed in the other strains of *E. coli* and *S. typhimurium* studied. For example, leaky *ptsI* mutations in cyclic AMP phosphodiesterase-negative strains of *E. coli* generally enhanced sensitivity of β-galactosidase synthesis to repression by methyl α-glucoside (Ta-

TABLE 2. *Net production of cyclic AMP by E. coli strains*[a]

Expt	Strain	Genotype	Total cyclic AMP[b]		% Methyl α-glucoside-mediated inhibition
			Without methyl α-glucoside	With methyl α-glucoside	
1	1100	*thi*	0.31	0.12	61
	1101	*thi ptsH315*	0.30	0.01	97
	LJ139	*thi ptsH315 crrA156*	0.37	0.34	8
	1103	*thi ptsI316*	0.05	0.04	20
	SB2273	*thi ptsI316 crrA13*	0.08	0.07	12
2	SB2252	*met cpd-454*	1.46	0.52	64
	Sb2260	*met cpd-454 ptsI109*	2.41	0.17	93
	SB2876	*met cpd-454 ptsI109 crrA27*	0.27	0.16	40
3	SB2249	*cpd-453*	0.82	0.67	17
	LJ176	*cpd-453 ptsI313*	0.95	0.17	82
	LJ177	*cpd-453 ptsI313 crrA158*	0.15	0.12	20

[a] Cells were grown with constant rotation (250 rpm) in twofold-concentrated nutrient broth. Methyl α-glucoside, when present, was added to a final concentration of 5 mM before initiation of growth. The growth temperature was 35°C in experiments 1 and 2 and 28°C in experiment 3. After 24 h, with cells in the stationary phase of growth, cyclic AMP was measured in the culture fluids as described previously (7, 18). Reported values represent averages of triplicate determinations. Bacterial strains were the same as in Table 1. Results can be compared with those reported in Table 1 because none of the PTS enzymes was induced by the growth conditions described in either table.

[b] Micromoles per gram (dry weight).

ble 1, experiments 2 and 3). A *crrA* mutation abolished the PTS-mediated repression of β-galactosidase synthesis but depressed net production of cyclic AMP. Residual adenylate cyclase activity in *crrA* mutants was relatively insensitive to inhibition by methyl α-glucoside. It should be noted that the *crrA* mutations in *E. coli* have not been mapped genetically.

In contrast with results obtained with the parental strain, all sugar substrates of the PTS were potent inhibitors of adenylate cyclase in the leaky enzyme I mutant SB2876. Sugars which were not transported and phosphorylated by the PTS were less effective in depressing rates of cyclic AMP production under the conditions of the experiment, and the *ptsI* mutation did not influence this inhibition (data not shown).

Regulation of cyclic AMP production and glycerol uptake in *Salmonella* strains. Both leaky and tight *pts* mutations were introduced into a cyclic AMP phosphodiesterase-negative strain of *S. typhimurium*, and the effects of these mutations and of *crrA* mutations on the regulation of glycerol uptake and cyclic AMP production were studied (Table 3). As reported previously (17), the presence of a leaky *ptsH* mutation (strain LJ114) or a leaky *ptsI* mutation (strain LJ101) resulted in enhanced sensitivity of glycerol uptake and cyclic AMP synthesis to inhibition by methyl α-glucoside. Tight *ptsI* mutations (strains LJ135 and LJ94) depressed cyclic AMP synthetic rates to low values, and strains which were deleted for the *ptsH* and *ptsI* genes did not synthesize measurable cyclic AMP under the conditions employed. Introduction of a *crrA* mutation into one of the leaky mutants which was capable of cyclic AMP synthesis generally depressed cyclic AMP synthetic rates to low values (Table 3). These results were similar to those obtained with phosphodiesterase-negative strains of *E. coli* (Table 2; unpublished data).

In most wild-type and *pts* mutant strains studied, coordinate regulation of glycerol uptake and adenylate cyclase activities was observed (17). However, exceptions were noted. In strain LJ135, which contained less than 0.05% of wild-type enzyme I activity, glycerol uptake occurred at a maximal rate and was not sensitive to inhibition by sugar substrates of the PTS. In contrast, cyclic AMP production in this strain was appreciable in the absence of sugar, but was sensitive to inhibition by exceptionally low concentrations of methyl α-glucoside; 70 nM methyl α-glucoside was sufficient for half-maximal inhibition (Fig. 1). This concentration is more than 1,000-fold below the apparent K_m for methyl α-glucoside uptake measured with the wild-type strain.

TABLE 3. *Cyclic AMP production by mutant strains of S. typhimurium*[a]

Strain	Genotype	Methyl α-glucoside	Glycerol uptake (μmol/g [dry wt] per min)	Cyclic AMP production (nmol/g [dry wt] per min)
LJ62	*cpd-401*	−	0.89	25
		+	0.75	7
LJ137	*cpd-401 crrA6*	−	0.60	<1
		+	0.60	<1
LJ114	*cpd-401 ptsH15*	−	0.66	30
		+	0.21	<1
LJ134	*cpd-401 ptsH15 crrA6*	−	0.62	<1
		+	0.65	<1
LJ101	*cpd-401 ptsI17*	−	1.1	54
		+	0.23	<1
LJ132	*cpd-401 ptsI17 crrA154*	−	0.60	<1
		+	0.71	2
LJ102	*cpd-401 ptsI17 crrA1*	−	0.76	6
		+	0.84	<1
LJ135	*cpd-401 ptsI18*	−	0.79	11
		+	0.76	<1
LJ136	*cpd-401 ptsI18 crrA3*	−	0.45	<1
		+	0.45	<1
LJ94	*cpd-401 ptsI305*	−	0.18	2
		+	0.11	<1
LJ210	*cpd-401 ΔcysK ptsHI41*	−	<0.05	<1
		+	<0.05	<1

[a] Bacterial strains were grown in medium 63 containing 0.4% galactose as the sole source of carbon and were harvested during exponential growth. The cells were washed three times by centrifugation at 4°C and resuspended in medium 63 to a cell density of 0.4 mg [dry weight] of cells per ml for measurement of cyclic AMP production or to a cell density of 0.16 mg [dry weight] of cells per ml for measurement of [^{14}C]glycerol (5 μCi/μmol) uptake. Concentrations of methyl α-glucoside (0.5 mM for the glycerol uptake experiments and 5 mM for the cyclic AMP production experiments) were sufficient to saturate glucose enzyme II. The incubation temperature was 37°C.

The *crrA* mutations generally abolished PTS-mediated transport regulation and depressed cyclic AMP synthetic rates to low values. However, an exception was noted with strain LJ102. Although glycerol uptake by this strain was completely insensitive to inhibition by methyl α-glucoside, adenylate cyclase activity was appreciable and was depressed at the same sugar concentrations which were inhibitory in the parental *ptsI* mutant (Fig. 2). The *crrA1* mutation apparently uncoupled glycerol uptake from adenylate cyclase regulation.

Regulation of adenylate cyclase in toluene-treated *E. coli* cells. Harwood and Peterkofsky have shown that the regulation of adenylate cyclase can be demonstrated by using

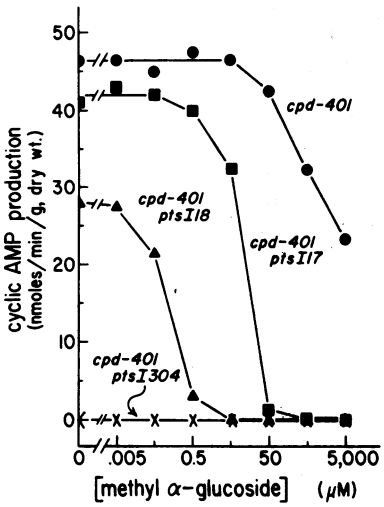

FIG. 1. *Cyclic AMP production by mutant strains of S. typhimurium containing differing amounts of enzyme I activity. Cells were grown in medium 63 containing 1% Casamino Acids and 0.4% galactose, harvested during logarithmic growth, washed three times, and resuspended in medium 63 to a cell density of 0.50 mg [dry weight] of cells per ml for measurement of cyclic AMP production during a 20-min incubation period at 37°C in the presence of the concentrations of methyl α-glucoside indicated on the abscissa. Symbols: ●, strain LJ62 (cpd-401); ■, strain LJ101 (cpd-401 ptsI17); ▲, strain LJ135 (cpd-401 ptsI18); ×, strain LJ93 (cpd-401 ptsI304).*

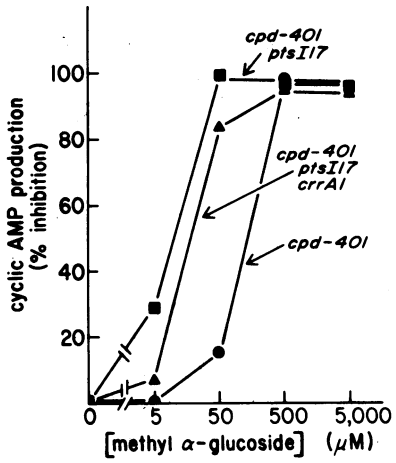

FIG. 2. *Effect of the crrA1 mutation on inhibition of cyclic AMP production by methyl α-glucoside. Cells were grown in medium 63 containing 0.4% galactose as the sole source of carbon and prepared for measurement of total cyclic AMP production as described in the legend to Fig. 1. Strain LJ102 was used in this study.*

[^{32}P]ATP in an assay with cells permeabilized with toluene (10). Figure 3 shows the effects of methyl α-glucoside and phosphoenolpyruvate on adenylate cyclase activity when this assay was used. Data are included for isogenic cyclic AMP phosphodiesterase-negative strains which differed only with respect to their contents of enzyme I and HPr. In the parental strain, which contained normal amounts of the enzymes of the phosphotransferase system, methyl α-glucoside was inhibitory at a concentration of 50 μM. In contrast, when phosphoenolpyruvate was present in the incubation mixture, a 100-fold-higher concentration of the sugar was required to exert a comparable inhibitory effect (Fig. 3). Thus, methyl α-glucoside and phosphoenolpyruvate exerted antagonistic effects on the regulation of adenylate cyclase. This behavior contrasts with that observed for enzyme I- and HPr-deficient strains. In the latter strains, 5 and 50 μM concentrations of methyl α-glucoside were inhibitory, and inhibition was not reversed by inclusion of phosphoenolpyruvate in the incubation mixture (Fig. 3B and C). When strain LJ176, which contained a heat-labile enzyme I, was grown at the permissive temperature (28°C) and then incubated at 42°C in order to partially inactivate enzyme I, adenylate cyclase showed greatly enhanced sensitivity to inhibition by low concentrations of methyl α-glucoside in toluene-treated cells (Fig. 3D).

Further experiments revealed that the effects of *ptsI*, *ptsH*, and *crrA* mutations on the regulation of adenylate cyclase were similar when intact cells and toluene-treated cells were studied (data not shown). For example, the *crrA158* mutation reduced the rate of cyclic AMP synthesis to a low value, whereas the *crrA159* mutation permitted substantial cyclic AMP synthesis but largely abolished sensitivity to inhibition. The effects of methyl α-glucoside on wild-type and mutant cells shown in Fig. 3 were similar to the effects observed with intact cells (3, 17, 18).

DISCUSSION

Several early reports provided evidence for an involvement of the PTS in the regulation of carbohydrate-catabolic enzyme induction, the activities of several carbohydrate permeases, and the activity of adenylate cyclase (3, 10, 13–15, 17, 21). More recent experiments have confirmed this suggestion and have permitted speculation regarding the mechanism by which these activities are modulated (10, 15, 17, 23). Regulation appears to involve a phosphorylation mechanism, with enzyme I functioning as a catalytic component of the regulatory system (3, 23). In this report, additional evidence for a phosphorylation mechanism is presented.

As noted previously, an inverse relationship

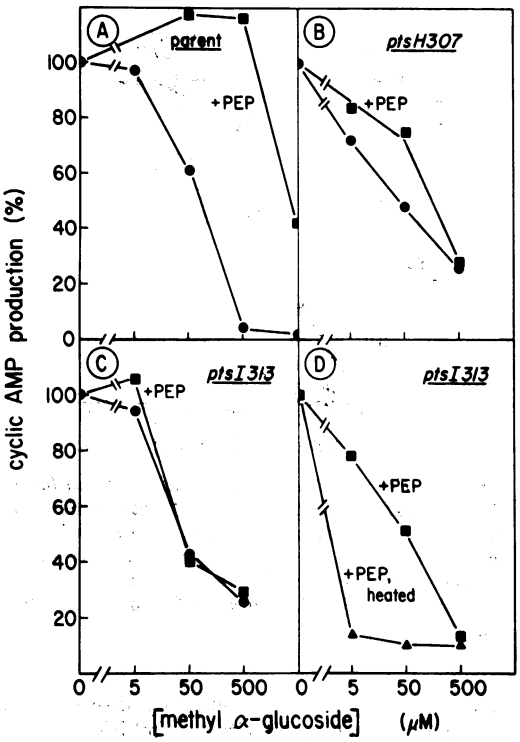

FIG. 3. *Inhibition of adenylate cyclase activity in toluene-treated cells as a function of methyl α-glucoside concentration. Strains and growth conditions were as follows. (A) LJ50 (Crook's strain; cpd-453) was grown at 37°C in medium 63 containing 1% DL-lactate and 0.4% glucose. (B) LJ156 (cpd-453 ptsH307) was grown at 37°C in medium 63 containing 0.4% galactose and 0.4% fructose. (C) LJ176 (cpd-453 ptsI313) was grown at 30°C in medium 63 containing 1% lactate and 0.4% glucose. (D) LJ176 (cpd-453 ptsI313) was grown at 28°C in medium 63 containing 0.4% galactose. Cells were harvested during exponential growth and washed three times with 10 mM Tris-hydrochloride buffer (pH 8.5) before suspension to a cell density of 2,000 Klett units (8 mg [dry weight] per ml) in the adenylate cyclase assay mixture for treatment with toluene. The assay mixture contained 25 mM Tris-hydrochloride (pH 8.5), 20 mM $MgCl_2$, 15 mM K_2HPO_4, 1 mM dithiothreitol, 20 mM creatine phosphate, excess creatine phosphokinase, and 1 mM [α-^{32}P]ATP (2 μCi/tube); the mixture was with (■) or without (●) phosphoenolpyruvate (PEP) at a final concentration of 2 mM. Toluene (1%) was added, and the tubes were blended in a Vortex mixer three times for 10 s. Incubations were for 20 min at 34°C. In the two pts mutants, phosphoenolpyruvate increased the total adenylate cyclase activity in the absence of methyl α-glucoside. In (D) the solid triangles indicate data for LJ214 cells in which enzyme I was heat inactivated during a 1-h incubation at 42°C before initiation of the adenylate cyclase assay (3). The control rates of cyclic AMP synthesis for strains LJ50, LJ156, and LJ176 in the absence of phosphoenolpyruvate and methyl α-glucoside were about 14 nmol/g (dry weight) per min.*

appears to exist between cellular enzyme I or HPr activity and the sensitivity of adenylate cyclase and several carbohydrate permeases to inhibition by PTS sugars. In this report we show that an *S. typhimurium* strain with a very low enzyme I content (strain LJ135; <0.1% of wild-type activity due to an ochre mutation in the structural gene for enzyme I [22]) could synthesize appreciable cyclic AMP in the absence of a sugar substrate of the PTS. In this strain nanomolar concentrations of methyl α-glucoside were strongly inhibitory (Fig. 1). This observation was worthy of note for two reasons. First, the apparent K_m for methyl α-glucoside uptake in the parental strain is more than three orders of magnitude higher than the concentration of sugar required for half-maximal inhibition of adenylate cyclase (A. W. Rephaeli and M. H. Saier, Jr., unpublished data). This fact renders any regulatory mechanism which involves ligand binding and direct interaction between an enzyme II of the PTS and adenylate cyclase extremely unlikely. Second, in the same strain, inhibition of glycerol uptake by methyl α-glucoside could not be demonstrated (Table 3), suggesting that the mutation uncoupled regulation of the glycerol permease from regulation of adenylate cyclase.

crrA mutations in *S. typhimurium* and *E. coli* strains have the effect of rendering the carbohydrate permeases resistent to regulation by sugar substrates of the PTS. The same mutations greatly reduced the rates of cyclic AMP synthesis in most strains tested (Tables 2 and 3). However, two exceptions were noted. First, in the genetic background of *E. coli* strain 1100, the *crrA* mutation enhanced cyclic AMP synthetic rates relative to the parental *pts* mutants and rendered adenylate cyclase resistant to PTS-mediated control (Table 2). Second, a rare *crrA* mutation in *S. typhimurium* (the *crrA1* mutation in strain LJ102) abolished PTS-mediated control of carbohydrate uptake (21) without appreciably altering the sensitivity of adenylate cyclase to inhibition (Fig. 2). These results show that, by genetic manipulation, the regulation of inducer uptake can be uncoupled from the regulation of adenylate cyclase. Recent experiments in our laboratory have shown that the uncoupling of these two regulatory functions can also be accomplished physiologically by a process termed transport desensitization (unpublished data). The desensitization mechanism has yet to be elucidated.

Three distinct mechanisms have been pro-

posed to account for the regulation of adenylate cyclase by the phosphotransferase system. Peterkofsky and his co-workers have suggested that phospho-enzyme I either phosphorylates or directly interacts with adenylate cyclase, converting it to a more active configuration (9, 10). In contrast, Saier and Feucht proposed that the enzyme is subject to allosteric activation by a regulatory protein, RPr, which can be phosphorylated at the expense of phospho-HPr (17). All three proposed mechanisms are consistent with the observation that a deficiency in either phosphoenolpyruvate or enzyme I results in hypersensitivity of adenylate cyclase to regulation by sugar substrates of the PTS (Fig. 3A, C, and D) (9, 10, 17). However, the different models can be experimentally distinguished by studying the effect of an HPr deficiency on adenylate cyclase regulation. Partial loss of HPr function would be expected to decrease sensitivity of adenylate cyclase to PTS-mediated regulation if either of the mechanisms proposed by Peterkofsky were operative, but the opposite effect should result if the model of Saier and Feucht is correct. Figure 3B clearly shows that an HPr deficiency renders adenylate cyclase hypersensitive to inhibition by methyl α-glucoside in toluene-treated *E. coli* cells, as was previously demonstrated for intact *S. typhimurium* cells (17). Moreover, inhibition in the *E. coli ptsH* mutant was not reversed by the addition of phosphoenolpyruvate to the toluene-treated cells (Fig. 3B). These results, together with the profound effects of *crrA* mutations on adenylate cyclase activity (Tables 2 and 3), lend support to the allosteric model of Saier and Feucht. It should be noted, however, that a mechanism of greater complexity is fully consistent with the available data. Thus, additional proteins and cofactors might be required for adenylate cyclase activation, and it is possible that the free form of the proposed allosteric regulatory protein (RPr) might inhibit adenylate cyclase, whereas the phosphorylated form of the protein may activate the enzyme (15). Detailed biochemical analyses will be required to define the regulatory mechanism in precise molecular terms.

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