Coordinate Regulation of Adenylate Cyclase and Carbohydrate Permeases by the Phosphoenolpyruvate:Sugar Phosphotransferase System in *Salmonella typhimurium*

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**SUMMARY**

Adenylate cyclase (EC 4.6.1.1) and several carbohydrate permeases are inhibited by D-glucose and other substrates of the phosphoenolpyruvate:sugar phosphotransferase system. These activities are coordinate ly altered by sugar substrates of the phosphotransferase system in a variety of bacterial strains which contain differing cellular levels of the protein components of the phosphotransferase system: Enzyme I, a small heat-stable protein, and Enzyme II. It is suggested that the activities of adenylate cyclase and the permease proteins are subject to allosteric regulation and that the allosteric effector is a regulatory protein which can be phosphorylated by the phosphotransferase system.

Transcription of bacterial operons coding for carbohydrate catabolic enzymes is subject to dual regulation: inducer and adenine 3':5'-monophosphate must be present simultaneously for transcription to occur at an appreciable rate (1). That the intracellular levels of inducer and cyclic AMP are subject to regulation has been established, and the phenomena of inducer exclusion and catabolite repression have been attributed to these regulatory processes (2-4). D-Glucose, when added to a bacterial cell suspension, inhibits the activities of several carbohydrate permeases (2, 5-9) and lowers intracellular cyclic AMP levels (1, 4, 10-14). Depressed cyclic AMP levels are due both to inhibition of adenylate cyclase (EC 4.6.1.1) activity and to stimulation of cyclic AMP efflux (4, 10-14).

D-Glucose and several other sugars enter the bacterial cell and are phosphorylated via a phosphotransferase system in a process termed group translocation (10-19). Phosphotransferase is transferred first from phosphoenolpyruvate to Enzyme I of the phosphotransferase system, then to HPr (a small heat-stable protein), third to one of several sugar-specific Enzymes II, and finally to sugar in a reaction which requires the participation of the membrane-associated sugar-specific Enzyme II. Thus, phosphorylation of a sugar by this mechanism requires the participation of four proteins which catalyze four phosphate transfer reactions.

The complexity of the phosphoenolpyruvate:sugar phosphotransferase system is bewildering, and one wonders what evolutionary advantages an organism might derive from the presence of so intricate a system. Our studies suggest that this complexity permits regulatory interactions which allow control over carbon and energy metabolism. Genetic investigations indicate that the phosphotransferase system is involved in the regulation of the activities of adenylate cyclase and several non-phosphotransferase system permeases. For example, sensitivity of the melibiose, maltose, and glycerol transport systems to inhibition by D-glucose or the nonmetabolizable n-glucose analogue, methyl α-D-glucoside, was enhanced by mutations which resulted in reduction in the cellular level of either Enzyme I (ptsI mutants) or HPr (ptsH mutants) (8). Moreover, inhibition by methyl α-D-glucoside was abolished by genetic loss of the Enzyme II specific for this sugar or by mutations in a gene (the crr gene) which mapped adjacent to the pts operon. Finally, a third class of mutations abolished sensitivity of each of several transport systems to inhibition and each of these mutations mapped with the gene(s) which coded for the respective transport system (22).

In this communication we describe studies on cyclic AMP synthesis and glycerol uptake in isogenic *Salmonella* strains which lack cyclic AMP phosphodiesterase (EC 3.1.4.17), the enzyme responsible for cyclic AMP degradation. These studies suggest that the phosphotransferase system functions as a protein kinase and that the degree of phosphorylation of a specific regulatory protein determines the rates of glycerol uptake and cyclic AMP synthesis.

The parental organism employed in our studies was *Salmonella typhimurium* strain TA3311, a cyclic AMP phosphodiesterase negative strain (less than 1% of wild type activity), generously provided by Drs. M. D. Alper and B. N. Ames (23). Other

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1. The abbreviations used are: cyclic AMP, cyclic adenosine 3':5'-monophosphate; HPr, a small heat-stable protein; RPr, a central regulatory protein.


3. Referred to as Factor III or Enzyme IIA by Roseman et al. (15, 17, 18).

4. Referred to as Enzyme II or Enzyme IIB by Roseman et al. (15, 17, 18).

5. The physiological and biochemical properties of *crr* (carbohydrate repression resistant) mutants (8, 9, 20) will be described in a forthcoming manuscript (M. H. Saier, Jr. and S. Roseman, in preparation). Briefly, *crr* mutants were isolated from *ptsI* and *ptsH* mutants as follows. A *pts* mutant (10^6 cells) was spread on solid minimal medium containing 0.2% glycerol as the sole source of carbon together with 0.1% methyl α-D-glucoside which inhibits glycerol utilization. *pts* mutants could not grow on this medium, but spontaneous or mutagen-induced clones appeared after 2 to 3 days. Those clones which simultaneously gained the ability to utilize maltose and melibiose in the presence of methyl α-D-glucoside and which utilized sugar substrates of the phosphotransferase system at the same rates as did the parental *pts* mutant were selected. *crr* mutants from *Salmonella typhimurium* and *Escherichia coli* were insensitive to phosphotransferase system-mediated repression for synthesis of the enzyme systems required for the catabolism of melibiose, maltose, glycerol, and (in *E. coli*) lactose and tryptophan. Additionally, the permease systems responsible for the uptake of melibiose, maltose, glycerol, and lactose were rendered insensitive to phosphotransferase system-mediated inhibition by the *crr* mutations (8). A biochemical deficiency associated with the *crr* mutation is the lack of the constitutive Enzyme II of the phosphotransferase system (10, 17). It has not yet been established that this protein is the product of the *crr* gene and the hypothetical regulatory protein, RPr (21).
strains were isolated from TA311 or were constructed by genetic transfer (21), and all were isogenic except for the pts and err defects. LJ144 (cpd-401 cyaA1150/F’198) was constructed by transfer of the Escherichia coli episome F’198, obtained from Dr. W. Epstein, to S. typhimurium strain LJ98 (cpd-401 cyaA1150). This episome carries the pts and err genes, but not the cya gene which codes for adenylate cyclase. In vitro analyses revealed that strain LJ144 possessed 4- to 6-fold increased activities of Enzyme I, HPr, and the soluble Enzyme IIIεε (6, 20). Cyclic AMP was measured by the Gilman procedure with the beef muscle cyclic AMP-binding protein purified through the DEAE-cellulose step (25). Total cyclic AMP in the cell suspension (cells + medium) was measured after extraction of cellular material by incubation at 100° for 5 min. Uptake of radioactive glycerol was followed as described previously (8). [3H]Glycerol and cyclic [3H]AMP were purchased from New England Nuclear. Other materials were obtained commercially and were of the highest purity available.

FIG. 1. Time courses for the production of cyclic AMP by Salmonella typhimurium strains in the presence ( ● ) and absence ( ○ ) of methyl α-D-glucoside (5 mM). Cells were grown in medium 63 containing 1% Casamino acids and 0.4% n-galactose, harvested during logarithmic growth, washed three times with medium 63, and resuspended in medium 63 to a cell density of 0.32 mg dry weight of cells per ml for measurement of cyclic AMP production at 37°. A, strain TA311 (cpd-401); B, strain LJ101 (cpd-401 pts117); C, strain LJ132 (cpd-401 pts117 err-54); D, strain LJ94 (cpd-401 pts1305).

Ten pts and 20 err mutations in strain TA311 were studied, and although the properties of the mutants described above were representative of the majority classes, exceptions were noted. For example, strain LJ135 (cpd-401 pts118) could be partially induced for uptake of glycerol, and it synthesized appreciable cyclic AMP in the absence of methyl α-D-glucoside even though it contained less than 0.1% of wild type Enzyme I activity. While cyclic AMP production by this strain was inhibited completely by 0.5 μM methyl α-D-glucoside, glycerol uptake was only weakly inhibited. Possibly the pts118 mutation, which has been characterized as an ochre nonsense mutation (26), is slightly leaky. err mutants isolated in strain LJ101 (cpd-401 pts117) had two phenotypes. The majority class, represented by LJ132 (cpd-401 pts117 err-1), fermented maltose and melibiose more efficiently and synthesized cyclic AMP in the absence of methyl α-D-glucoside. Although glycerol uptake was not inhibited by methyl α-D-glucoside in this strain, the sugar did inhibit cyclic AMP production at the same low concentrations observed for the parental strain (cpd-401 pts117, see Fig. 2). All pts and err mutations were shown by transductional analyses with phage P22 int-4 to map very near one another and to be co-transducible with cyaA1150 (8, 20).

FIG. 2. Inhibition of glycerol uptake (A) and cyclic AMP production (B) as a function of methyl α-D-glucoside concentration in isogenic Salmonella typhimurium strains which lack cyclic AMP phosphodiesterase and possess altered levels of either Enzyme I or HPr. Cells were grown in medium 63 containing 1% Casamino acids and 0.4% n-galactose, harvested during logarithmic growth, washed three times with medium 63, and resuspended in medium 63 to a cell density of 0.19 mg dry weight of cells per ml for measurement of glycerol uptake, or to a cell density of 0.32 mg dry weight of cells per ml for measurement of cyclic AMP production at 37°. A, strain LJ132 (cpd-401), 100% Enzyme I and HPr; B, strain LJ150, cpd-401 pts117, 5% of wild type Enzyme I activity; C, strain LJ101, cpd-401 pts117, 1% of wild type Enzyme I; X, strain LJ114, cpd-401 ptsH15, 67% of wild type HPr activity.

Unpublished observations.
of each of the components of the phosphotransferase system: Enzyme I, HPr, and Enzyme II. In contrast, several err mutations which abolished sensitivity to regulation, resulted in high Enzyme I, HPr, and Enzyme II. Additionally, we found that enhanced dosage of the err gene,4 and (b) that RPr (or phospho-RPr) allosterically regulate the activities of adenylate cyclase and the glycerol transport system to inhibition by methyl α-glucoside parallel each other under a wide variety of growth conditions while the rate of cyclic AMP synthesis was increased.

The results summarized above show that sensitivities of adenylate cyclase and the glycerol transport system to inhibition by methyl α-glucoside parallel each other under a wide variety of conditions. In the absence of a sugar substrate of the phosphotransferase system and in a cell with sufficient phosphoenolpyruvate, Enzyme I, and HPr, HPr would be fully phosphorylated. Under these conditions, glycerol transport and cyclic AMP synthesis were found to occur at maximal rates. Addition of a sugar substrate of the phosphotransferase system to the cell suspension should drain phosphate off of RPr. These substrates were found to inhibit glycerol uptake and net cyclic AMP synthesis. Consequently, allosteric regulatory interactions can account for the physiological properties of pts and err mutants. They rationalize the observations that energy depletion and the temperature-dependent loss of Enzyme I render glycerol uptake hypersensitive to inhibition by methyl α-glucoside (22, 27). This concept does not take into account regulatory interactions due to sugar metabolism.4 It is consistent with most available evidence concerning phosphotransferase system-mediated regulation and serves as a working hypothesis for future experiments.

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REFERENCES
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