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The Mechanism of Sugar-Dependent Repression of Synthesis of Catabolic Enzymes in Escherichia coli

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Previous studies have indicated that the Escherichia coli adenylate cyclase (AC) activity is controlled by an interaction with the phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS). A model for the regulation of AC involving the phosphorylation state of the PTS is described. Kinetic studies support the concept that the velocity of AC is determined by the opposing contributions of PEP-dependent phosphorylation (V_1) and sugar-dependent dephosphorylation (V_2) of the PTS proteins according to the expression $\% V_{AC} = 100/[1 + (\text{Max } V_2/\text{Max } V_1)]$. Physiological parameters influencing the rate of the PTS are discussed in the framework of their effects on cAMP metabolism. Factors that increase cellular concentration of PEP (and stimulate V_1) appear to enhance AC activity while increases in extracellular sugar concentration (which stimulate V_2) or internal levels of pyruvate (which inhibit V_1) inhibit the activity of this enzyme.

Key words: **adenylate cyclase, catabolite repression, sugar transport**

In E. coli, cAMP is required for the synthesis of the mRNA for many catabolic enzymes (1). E. coli growing exponentially on glycerol or succinate have elevated cAMP levels (2) and when exposed to the appropriate inducer rapidly synthesize enzymes for the transport and degradation of that inducer (3). Immediately following the addition of glucose to such cultures, cAMP levels decrease substantially (4, 5); this results in a period of transient repression during which induced enzyme synthesis is inhibited (3). If the level of cAMP is replenished by exogenous addition of this nucleotide, such cells recover from this transient repression (1). In this report, we examine a model for transient repression based on the regulation of cAMP levels by glucose, and other sugar substrates of the phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS).

The intracellular pool of cAMP is determined by the rates of synthesis, degradation and excretion of this nucleotide. The glucose-dependent decrease in cAMP levels cannot

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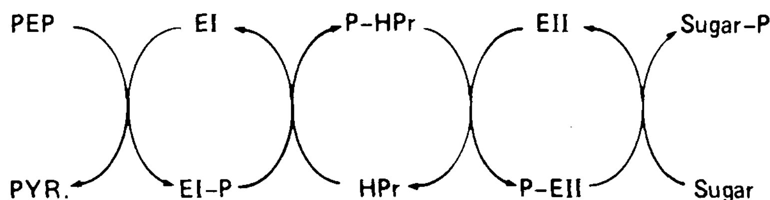

Fig. 1. Mechanism of the PEP:glucose phosphotransferase system in E. coli. The flow of the phosphate group, derived from PEP, to the sugar is thermodynamically favored. PEP can saturate the system with phosphate groups if the concentrations of pyruvate and glucose are low; in the absence of PEP, glucose or pyruvate can convert the proteins to the dephospho forms. EII is composed of 2 proteins, $F^{glc}III$ and $E^{glc}IIB$, specific for glucose. Each different PTS sugar has specific EIIs involved in its transport. The EIIB is a membrane-associated protein. Enzyme I (EI) and HPr are soluble proteins that are required for transport of all PTS sugars.

be explained on the basis of effects on the rates of degradation (6) or excretion (6–8). However, the rate of synthesis of cAMP can be inhibited by glucose (6). The regulatory model outlined below involves an interaction of the PTS with AC.

RESULTS

Figure 1 outlines the PTS, composed of 4 proteins, by which glucose and some other sugars are transported in E. coli. There is a sequential, covalent transfer of the phosphate group derived from PEP across this protein chain to a sugar acceptor (10). This paper is concerned with a model for the regulation of AC activity which is based on the idea that AC interacts with PTS proteins and that when PTS proteins are phosphorylated, AC is active. The model proposes that PEP, which phosphorylates PTS proteins, activates AC, while sugar substrates of the PTS, as well as pyruvate, dephosphorylate PTS proteins and inhibit AC. Since there are opposing effects of PEP compared to pyruvate and sugar in determining the phosphorylation state of the PTS proteins, intermediate levels of AC activity would be expected when both PEP and either pyruvate or sugar are present.

Several lines of evidence indicate that the glucose-dependent inhibition of adenylate cyclase (AC) is coupled to the glucose transport activity. Harwood and Peterkofsky (9) showed that AC activity in intact or permeabilized cells is inhibited by glucose, but not by glucose-6-phosphate, the immediate product of the sugar transport process (10). Magasanik has indicated that transient repression is caused not only by glucose but is also observed with other sugars if their transport systems are present (3). The induction of sugar-specific membrane-associated proteins permit the transport of other hexoses besides glucose. Once sugar transport activity is induced, the sugar is capable of inhibiting AC (11). Figure 2 shows that the sensitivity of AC to inhibition by mannitol parallels the induction of mannitol transport activity. When the mannitol-dependent PTS activity is low, AC activity is relatively insensitive to inhibition by mannitol. Under this condition, the capability of mannitol to dephosphorylate PTS proteins is relatively poor compared to the endogenous PEP-dependent reaction leading to the phosphorylation of PTS proteins. The observation that one PTS substrate (PEP) antagonizes the inhibition of AC by another PTS substrate (sugar) (14) provides further support for the model that AC activity depends on the state of phosphorylation of the PTS proteins.

Figure 3 describes a simplified model for the PTS system. An analysis of this model suggests that, at steady-state, the fraction of the PTS proteins in the phospho form (EP) is

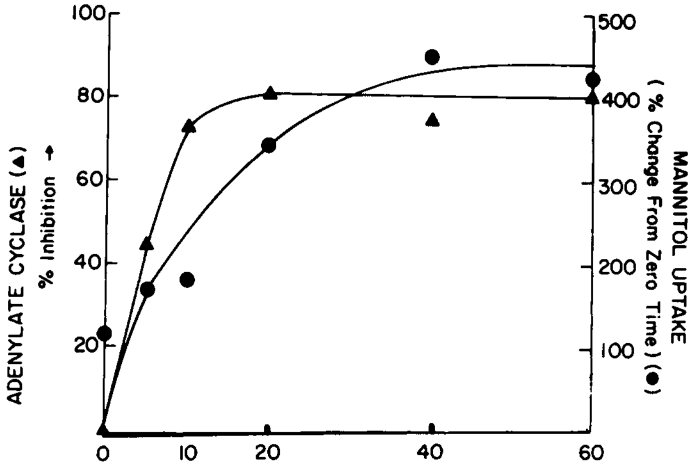

Fig. 2. Kinetics of induction of mannitol inhibition of adenylate cyclase compared to mannitol uptake during exposure of E. coli B to mannitol. E. coli B were grown in minimal medium (12) supplemented with 1% glucose to an A_{650} of 1. At that point, the culture was centrifuged, washed in minimal medium, and resuspended in minimal medium (original volume), adjusted to a mannitol concentration of 15 mM. The cell suspension was shaken at 37°C. At the indicated times, aliquots of the cells were collected and washed on large Millipore membranes (9 cm) then resuspended in their original volume. Aliquots (10 ml) of the washed cells were tested for adenylate cyclase activity in the absence or presence of mannitol (1 mM). The period of pulse-labeling with [^{3}H]adenosine was 1 min. The concentrations of [^{3}H]cAMP were determined as previously described (6). The data are presented as percentage inhibition of the adenylate cyclase activity by mannitol. Measurements of [^{14}C]mannitol uptake were as described by Solomon and Lin (13) using 0.025 ml aliquots of the cell suspension. Assays were done for 0.5, 1.0, 1.5, and 2.0 min. The data are expressed as the percentage change in mannitol uptake rate of the samples compared to the zero time samples. Legend: Effect of mannitol on adenylate cyclase (▲—▲); Mannitol uptake (●—●). Adapted from (11).

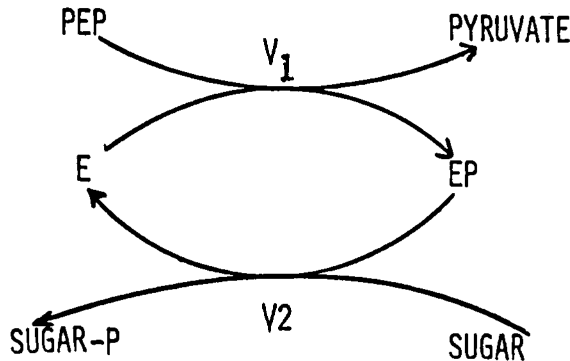

Fig. 3. The interconversion of the PTS proteins between phospho and dephospho forms. E represents the PTS proteins in the dephospho form; EP represents the PTS proteins in the phospho form. V_1 is the PEP-dependent rate of phosphorylation of E; V_2 represents the sugar-dependent rate of dephosphorylation of EP.

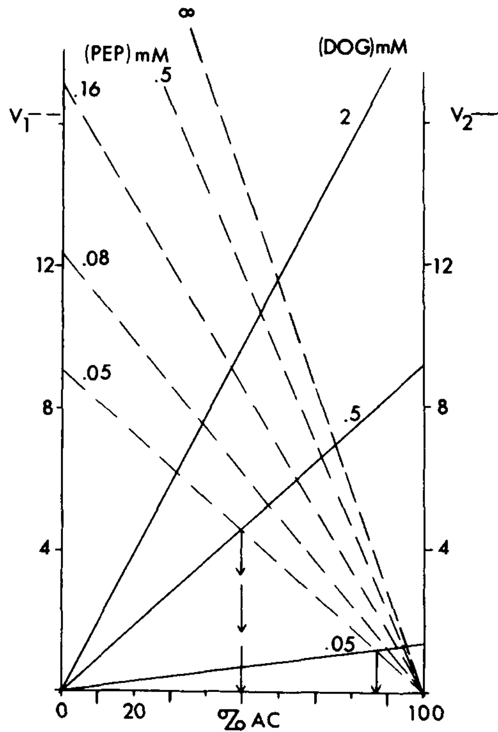

Fig. 4. Graphical determination of adenylate cyclase activity based on the PTS regulatory model. The abscissa labeled % AC also represents the percentage of the PTS proteins in the phospho form. The method used for calculation of maximum V_1 values (Max V_1, where PTS proteins are completely in the dephospho form) and maximum V_2 values (Max V_2, where PTS proteins are completely in the phospho form) at various concentrations of PEP and sugar, respectively, is described in Appendix B. The ordinates labeled V_1 and V_2 represent Max V_1 and Max V_2 values at the intercepts on the ordinates. At any specified concentration of PEP, V_1 is directly proportional to the concentration of the dephospho PTS, thus justifying the extrapolation (dashed lines) of Max V_1 to zero velocity when all of the PTS is in the phospho form. A similar analysis applies to the construction of the V_2 lines (solid lines). The intersection of any pair of V_1 and V_2 lines corresponds to the steady-state velocity of the PTS reaction at designated concentrations of PEP and DOG (2-deoxyglucose). Since, as indicated in Appendix A, the fractional velocity of AC is equal to the fraction of the PTS in the phospho form, the intersection of the lines also determines the % activity of adenylate cyclase (designated on the abscissa). The arrows indicate the fraction of maximum AC activity at 0.05 mM PEP and either 0.05 mM or 0.5 mM DOG. Values for fractional AC activity determined from this plot are compared with experimental data in Table I.

determined by the maximum V_1 (Max V_1, the velocity of the reaction at any specified concentration of PEP when all of the enzyme is in the dephospho form, E) and by the maximum V_2 (Max V_2, the velocity of the reaction at any specified sugar concentration when all of the enzyme is in the phospho form, EP). We propose that the fractional velocity of AC is equal to the fraction of the PTS in the phospho form, EP. The fractional velocity of AC can be expressed mathematically by the following equation (derived in Appendix A): Fractional Velocity of AC (in %) = $100/ [1 + (\text{Max } V_2/\text{Max } V_1)]$. A graphical solution to this equation is presented in Fig. 4. Steady-state PTS velocities can be read from this graph

TABLE I. Correlation of Experimentally Determined Fractional Adenylate Cyclase Activities With Values Generated by Kinetic Modeling*

PEP (mM)	V_1	DOG 0: Experimental V_{AC}	DOG 0: Experimental FV_{AC}	DOG 0.05 (V_2 = 1.4): Experimental V_{AC}	DOG 0.05 (V_2 = 1.4): Experimental FV_{AC}	DOG 0.05 (V_2 = 1.4): Modeling V_{AC}	DOG 0.05 (V_2 = 1.4): Modeling FV_{AC}	DOG 0.5 (V_2 = 9.2): Experimental V_{AC}	DOG 0.5 (V_2 = 9.2): Experimental FV_{AC}	DOG 0.5 (V_2 = 9.2): Modeling V_{AC}	DOG 0.5 (V_2 = 9.2): Modeling FV_{AC}
0.05	9.1	1.14	100	1.00	88	0.99	87	0.57	50	0.57	50
0.08	12.4	1.20	100	1.06	88	1.08	90	0.62	52	0.68	57
0.16	17.2	1.28	100	1.16	91	1.18	92	0.83	65	0.83	65
0.5	23.8	1.32	100	1.21	92	1.24	94	0.95	72	0.95	72

*Adenylate cyclase activity in permeabilized cells of E. coli 1,100 was determined as described (15). Velocity (V_{AC}) is expressed as nmoles cAMP formed per hour per mg of protein. Experimental values for FV_{AC} were calculated as the percentage of the activity obtained at the indicated concentrations of PEP in the absence of DOG. Modeling values for FV_{AC} were calculated from Equation 5 (Appendix B) using the indicated values for V_1 and V_2 obtained as described in Appendix B.

as the ordinate values at the intersection of pairs of V_1 and V_2 lines. These steady-state PTS velocities generate parallel lines in a Lineweaver-Burk plot. Such kinetics are consistent with the expected formation of the stable phosphorylated intermediates previously demonstrated for the PTS and required for the suggested model of PTS-dependent regulation of AC. The intersection point of a particular pair of V_1 and V_2 lines (extrapolated to the abscissa) determines the fractional velocity of AC. Table I summarizes the values of the fractional AC activity obtained graphically and experimentally. The good agreement of the values obtained by the 2 methods supports the notion that the proposed model describes the mechanism of AC regulation.

Other predictions generated by the model equation and fulfilled by preliminary experimental data are as follows:

1) The K_{PEP} and K_{DOG} values in the PTS reaction are the same as the K_{PEP} (for activation) and K_{DOG} (for inhibition) with respect to the AC activity.

2) Inhibition by saturating [DOG] decreases with increasing [PEP]; PEP-dependent activation cannot completely reverse DOG-dependent inhibition.

3) The apparent K_{aPEP} and K_{iDOG} increase as the concentration of the other ligand increases.

4) Subsaturating concentrations of either PEP or DOG are sufficient to completely activate or inhibit AC, respectively, in the absence of the other PTS substrate.

DISCUSSION

The phosphorylation model indicates that extensive inhibition of AC requires both an appropriate concentration in the medium of a sugar transportable by the PTS, as well as a relatively low concentration of PEP in the cell. Addition of glucose to an E. coli culture growing exponentially on glycerol- or succinate-salts medium causes a rapid decrease of the PEP pool (16) thereby enhancing the sensitivity of AC to sugar inhibition. This reduction of intracellular PEP may be the result of sugar phosphate formation via the PTS (16, 17). The high cAMP levels characteristic of growth on glycerol together with the decrease in cAMP after the addition of glucose may be a reflection of the differences in intracellular PEP levels under these conditions. The observed cAMP levels vary in the

direction predicted by the model presented here.

The model also predicts that mutants deficient in the production of PEP will be hypersensitive to glucose repression of induced enzyme synthesis. This is the case in PEP synthetase (18) and phosphofructokinase mutants (19).

Mutants leaky in the EI or HPr proteins of the PTS (Fig. 1) are also hypersensitive to glucose repression (20). Such mutants may be unable to effectively phosphorylate PTS proteins (i.e., have low V_1, normal V_2). In contrast, mutants in the membrane-associated sugar-specific proteins of the PTS are unable to dephosphorylate HPr and EI proteins of the PTS (i.e., have low V_2, normal V_1) and thereby, become resistant to sugar inhibition of AC (15). The properties of these mutants are consistent with the model outlined in Fig. 3.

Pyruvate inhibits AC activity (14). Lowry et al. (16) report that the internal concentrations of pyruvate range from 0.04 mM to 0.63 mM according to growth conditions. Since the K_i value for pyruvate inhibition of AC in permeabilized cells is 0.1 mM (data not shown), physiological changes in pyruvate levels may contribute significantly to the regulation of AC activity. The mechanism of AC inhibition by pyruvate may be mediated by the state of phosphorylation of the PTS since, pyruvate, like a sugar substrate, can dephosphorylate the PTS proteins (10) (see Fig. 1).

Pyruvate, like glucose, elicits catabolite repression. Addition of pyruvate to E. coli growing exponentially on glycerol leads to a reduction in the rate of tryptophanase (1, 21, 22, 23) and D-serine deaminase (22) synthesis. Cyclic AMP prevents the pyruvate-dependent repression of enzyme synthesis (21, 23). These observations are consistent with the suggestion (23) that pyruvate decreases cellular cAMP levels.

Cyclic AMP levels are a key factor in regulating induced enzyme synthesis. Various observations in the literature link the expression of induced enzymes to PTS sugars, pyruvate, and PEP. The discussion presented here provides a unifying model compatible with much of these apparently unrelated data. It proposes that phosphorylated PTS substrates increase the steady-state concentration of phospho-PTS proteins thereby activating AC while nonphosphorylated PTS substrates decrease the steady-state concentration of phospho-PTS proteins leading to a deactivation of AC.

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APPENDIX A

Derivation of the Fractional Velocity Equation for Adenylate Cyclase Activity

From the model in Fig. 3, at steady-state,

$$\frac{\text{Max } V_1}{\text{Max } V_2} = \frac{(EP)}{(E)} \tag{1}$$

Since the amount of PTS proteins is constant,

$$E + EP = 1 \tag{2}$$

From equations 1 and 2,

$$EP = \frac{1}{1 + \frac{\text{Max } V_2}{\text{Max } V_1}} \tag{3}$$

We define the fractional velocity of AC (FV_{AC}) as equivalent to the fraction of the PTS that is phosphorylated (EP). Therefore,

$$FV_{AC} = \frac{1}{1 + \frac{\text{Max } V_2}{\text{Max } V_1}} \tag{4}$$

APPENDIX B

Method for Calculation of Fractional V_{AC}

As shown in Appendix A the fractional velocity of AC in the presence of the PTS substrates, PEP and DOG is

$$FV_{AC} = \frac{1}{1 + \frac{\text{Max } V_2}{\text{Max } V_1}}, \text{ where} \tag{5}$$

$$\text{Max } V_1 = \frac{k_1 \text{ [E] [PEP]}}{K_{PEP} + \text{[PEP]}}, \text{ when all of the enzyme is in the E form, and} \tag{6}$$

$$\text{Max } V_2 = \frac{k_2 \text{ [EP] [DOG]}}{K_{DOG} + \text{DOG}}, \text{ when all of the enzyme is in the EP form.} \tag{7}$$

K_{PEP} and K_{DOG} were determined to be 0.11 mM and 0.8 mM, respectively by assays for PTS activity at various concentrations of PEP or DOG.* An experimentally determined value for FV_{AC} at 0.5 mM PEP and 0.5 mM DOG was 0.72. By arbitrarily assigning a value of Max V_2 as 9.2, equation 5 was solved for Max V_1 to give a value of 23.8. Using the values of K_{PEP} and Max V_1 at saturating PEP other values of Max V_1 at different [PEP] may be obtained graphically from the Lineweaver-Burk form of equation (6).

*The assay for phosphotransferase activity used in this study depended on the DOG-dependent conversion of [^{14}C] PEP to [^{14}C] pyruvate. Incubations for PTS activity were carried out as previously described (15), except that [^{14}C] PEP was used as the labeled compound. After a suitable incubation period, an aliquot (50 μl) of the incubation mixture together with 100 μl of pyruvate (1 N) was deposited on a column (0.5 × 2 cm) of Dowex 3-X4 200–400 mesh. [^{14}C] pyruvate was separated from [^{14}C] PEP by elution with 0.2 N HCl. The recovery of pyruvate in the fraction counted was determined by a spectrophotometric measurement at 340 nm. For other details, see the text.