The Escherichia coli Adenylate Cyclase Complex

STIMULATION BY POTASSIUM AND PHOSPHATE*

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In Escherichia coli, adenylate cyclase activity in toluene-treated cells can be inhibited by glucose while the activity in a broken cell preparation cannot. Adenylate cyclase activity in the permeabilized but not in broken cells is stimulated somewhat specifically and additively by potassium and phosphate. Kinetic studies show sigmoid substrate-velocity curves for the toluene-treated cells but hyperbolic curves for the broken cells. The stimulatory effects of potassium and phosphate on adenylate cyclase activity in toluene-treated cells are associated with increases in the V_max and K_m for ATP. While the enzyme activity in toluene-treated cells shows a preference for magnesium over manganese, the reverse is observed in broken cells. Stimulation of adenylate cyclase activity in toluene-treated cells requires the presence of the proteins of the phosphoenolpyruvate:sugar phosphotransferase system (PTS). The PTS proteins can be phosphorylated in a P-enolpyruvate-dependent reaction. The stimulatory effects of ions will not occur if the PTS proteins are not phosphorylated. Since potassium phosphate stimulates both adenylate cyclase and PTS activities in toluene-treated cells, it is proposed that the effect of potassium phosphate on adenylate cyclase activity is mediated through an effect on the PTS. A model for dual regulation by glucose of adenylate cyclase activity is presented. This model involves regulation of both the condition of the PTS proteins as well as the cellular concentration of phosphate.

In Escherichia coli, cAMP plays a crucial role in gene expression (1). The major mechanism by which cellular cAMP levels are regulated appears to involve a sugar-dependent inhibition of adenylate cyclase activity (2). While the details remain to be elucidated, it has been established that the sugar transport pathway called the phosphoenolpyruvate:sugar phosphotransferase system (PTS) is, in some way, involved in this sugar-dependent inhibition of adenylate cyclase activity. The PTS affects a P-enolpyruvate-dependent phosphorylation of a family of sugars coupled to their transport across the cell membrane (3). The system is composed of some general soluble proteins (Enzyme I, HP) as well as sugar specific proteins (for example, III^E which is soluble and glucose-specific and II^E which is membrane-bound and glucose-specific). The PTS mechanism entails a sequential passage of phosphate, originally derived from P-enolpyruvate, from protein to protein and ultimately to the sugar to form sugar phosphate.

The regulatory mechanism of adenylate cyclase has been partially defined in studies of permeable cells (2) but has not been reproduced in a cell-free system. In continuing studies of the regulation of adenylate cyclase activity, we have examined the effects of anions and cations on the system; it was found that, in addition to the expected requirement for magnesium, the regulated form of the enzyme also requires potassium and phosphate for full activity. The nature of the effects of these ions and of their interplay with the phosphoenolpyruvate:sugar phosphotransferase system, is described in this report.

EXPERIMENTAL PROCEDURES

E. coli Strains—The E. coli strains used in this study were wild-type 1100, a derivative of 1100 which synthesizes a mutant form of the Enzyme I of the PTS (4), a mutant of K12 deleted in the attachment site and a derivative of K12 in which the gene for the Enzyme I of the PTS has been deleted (kindly supplied by Dr. Michael Gottesman, National Cancer Institute (5)). Strain 1103, described previously (4, 6, 7), has an Enzyme I of the PTS with a lowered affinity for P-enolpyruvate such that P-enolpyruvate must be supplied exogenously to stimulate adenylate cyclase activity. All strains were made recA by transduction with a P1 lysate of strain JC19240 (kindly supplied by Dr. Barbara Bachmann, Yale University; CGSC 6074) as described (8). Tetracycline-resistant transductants were selected and tested for ultraviolet sensitivity. One of the UV-sensitive clones from each strain was transformed with pDIA100 (9), a pBR322 derivative carrying the cya gene, according to the CaCl_2 procedure described by Maniatis et al. (10). Ampicillin-resistant transformants were selected for high levels of adenylate cyclase activity. The resulting strains, 433 (1100), 471 (producing the mutant form of Enzyme I), 464 (K12), and 457 (deleted in the gene for Enzyme I) had differing levels of adenylate cyclase activity which reflected their state of regulation; however, they all had limited levels of adenylate cyclase activity which were approximately 10-fold higher than their respective parents.

The plasmid, pDIA100, was prepared from strain TP2010/pDIA100 (kindly supplied by Dr. Antoine Danchin, Pasteur Institute, Paris, France (9)). Cells were amplified in rich medium and lysed by the sodium dodecyl sulfate procedure (11). The plasmid DNA was purified by centrifugation to equilibrium in a cesium chloride/ethidium bromide gradient.

Growth of Cells—Adenylate cyclase and PTS assays were performed on cells grown at 37°C with shaking in salts medium (12) supplemented with Difco Nutrient Broth (1%). In the case of strain 433, 0.2% glucose was added to the medium. After addition, 0.4-0.5, 40 ml of cell suspension was harvested by centrifugation at 3,000 x g for 10 min, washed in 20 mM Bicine (sodium), pH 8.5 and then suspended in 1 ml of 20 mM Bicine. The washed cells were either treated with 1% toluene for 10 min (2) or passed through an Aminco French pressure cell (1 pass at 1,000 psi).

Adenylate Cyclase Assays—Adenylate cyclase assays were carried out at 30°C. Assay mixtures contained Bicine (sodium), pH 8.5, 25 mM; ATP (sodium or potassium salt as specified), 1 mM unless

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The abbreviations used are: PTS, phosphoenolpyruvate:sugar phosphotransferase system; Bicine, N,N-bis-(2-hydroxyethyl)glycine.

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Specified otherwise in the figure legends, MgCl₂, 10 mM unless otherwise specified; dithiothreitol, 1 mM; creatine phosphate (sodium salt), 20 mM and creatine phosphokinase, 100 units/ml. [α-32P]ATP was added to give a specific activity of 20–40 cpm/pmol. Where indicated in the figure legends, 5 mM cAMP was included in the incubation mixture. Master incubations were prepared in a volume of 0.5 ml at four time intervals (ranging from 1 to 20 min), aliquots (0.1 ml) were removed into tubes containing 0.2 ml of 1 N HClO₄. After addition of cyclic [3H]AMP (10,000–15,000 cpm), the [α-32P] cAMP was purified by a method involving Dowex 50 and alumina columns (13). Velocities were calculated from the best line that could be plotted through the four time points. In all the experiments reported here, the assays were linear. For studies involving the effect of varying ATP concentration on adenylate cyclase activity (Fig. 4), the data were fit to the Hill equation (14) by means of an interactive curve-fitting program, MLAB, developed at the National Institutes of Health and running on a PDP-10 digital computer (15). This program calculates values for Vmax, Km, and N (Hill coefficient).

Activity is expressed as nanomoles of cAMP formed per hour per milligram of protein. The constants for permeable cells assayed in the presence of phosphate (see Fig. 4B) were calculated from data that was not saturating because addition of ATP concentrations in excess of 3.5 mM resulted in inhibition.

PTS Assays—Assays for PTS activity were carried out with permeable cells at 30 °C containing the same additions as the adenylate cyclase assays except that the [γ-32P]ATP was replaced by methyl[(ß-D-UDP]-pyranoside (0.1 mM) at a specific activity of 5 cpn/pmol. Incubations were carried out in the presence of 1 mM P-enolpyruvate (potassium salt); controls without P-enolpyruvate were run in parallel to ensure that the reaction was P-enolpyruvate-dependent. The assays were performed as previously described (16).

Activity is expressed as nanomoles of α-methylglucoside-6-phosphate formed per hour per milligram of protein.

Reproducibility and Accuracy—Results shown are of representative experiments, each performed at least twice. Permeable cells and cell-free extracts were prepared freshly for each experiment. There was as much as a 2-fold variation from experiment to experiment in the level of both control and potassium- or phosphate-stimulated activities. The nature of the effects reported here were highly reproducible. There was also as much as a 2-fold variability from experiment to experiment in the level of activity determined in cell-free extracts.

Materials—[8-3H]cAMP (20 Ci/mmol) was obtained from Schwarz/Mann. [α-32P]ATP (31 Ci/mmol) was from New England Nuclear. Methyl-[(ß-D-UCP]-pyranoside (279 mCi/mmol) was purchased from The Radiochemical Centre, Amersham, England. All other chemicals were from standard sources.

RESULTS

Anion and Cation Effects on Adenylate Cyclase Activity in Permeable Cells and Extracts—It has previously been shown that E. coli cells permeabilized with toluene have adenylate cyclase activity which is inhibited by glucose or α-methylglucoside while the adenylate cyclase activity in crude French press extract is not glucose-inhibited (2). The major purpose of this study was to characterize the responses to anions and cations of the adenylate cyclase in these two types of preparations. The data in Panel A of Fig. 1 show that, in permeable cells of wild-type E. coli, adenylate cyclase activity was stimulated 3–4-fold by KCl and that the effect was essentially maximal at 20 mM. The activity was also stimulated about 3-fold by 20 mM Na₂HPO₄; higher phosphate concentrations did not stimulate further (data not shown). Addition to permeable cells of a combination of KCl (40 mM) and Na₂HPO₄ (20 mM) resulted in an approximately 7-fold stimulation. These data indicate that, in permeable cells, adenylate cyclase activity is stimulated independently and additively by KCl and Na₂HPO₄. It is noteworthy that the adenylate cyclase activity stimulated by all the agents mentioned was inhibited by α-methylglucoside.

The properties of adenylate cyclase in the broken cell suspension are quite different (Panel B of Fig. 1). The activity assayed in the absence of any added anions or cations was as high as the maximally stimulated adenylate cyclase activity in permeable cells (approximately 25 nmol of cAMP formed per hour per milligram of protein). Assay in the presence of 20–40 mM KCl produced 20% inhibition. Na₂HPO₄ at 20 mM resulted in 40% inhibition. Under no set of incubation conditions did the French press extract exhibit an inhibition of adenylate cyclase activity by α-methylglucoside.

Specificity of Cation Effects on Adenylate Cyclase Activity in Permeable Cells—The data in Fig. 2 demonstrate the specificity of the cation effect on adenylate cyclase activity in tolouene-treated cells. KCl and RbCl stimulated the activity while LiCl did not. The activity with RbCl was about 60% as great as with KCl when assayed in the absence of Na₂HPO₄. When assays were carried out in the presence of Na₂HPO₄, the
activity with RbCl was about 80% as high as with KCl.

Specificity of Anion Effects on Adenylate Cyclase Activity in Permeable Cells—A study of the effects of a variety of anions on adenylate cyclase activity in the absence or presence of potassium ions was carried out (Fig. 3). As shown in Fig. 1, the enzyme activity was stimulated by either KCl or Na₂HPO₄ alone. In this experiment, these stimulations were approximately 2-fold. When the activity was tested in the presence of a mixture of KCl and Na₂HPO₄ or K₂HPO₄, the stimulation of activity was approximately 5-fold. Sodium arsenate or sodium sulfate did not replace sodium phosphate. However, in the presence of KCl, sodium arsenate stimulated the activity, suggesting that arsenate could replace phosphate but only in the presence of potassium. This was verified by demonstrating a substantial stimulation (approximately 5-fold) by potassium arsenate. There was no stimulation by sulfate even in the presence of potassium ion. Therefore, it appears that the anion-dependent stimulation of adenylate cyclase activity in permeable cells shows some degree of specificity.

Effects of Anions and Cations on the Kinetic Properties of Adenylate Cyclase in Permeable Cells and Extracts—The curves shown in Fig. 4 demonstrate the effects of added ions on adenylate cyclase activity in the two types of preparations. A comparison of the curves in Panels A and B (permeable cells) compared to C and D (extract) indicates that permeable cells produce sigmoid substrate-velocity curves particularly in the presence of phosphate while extracts show hyperbolic curves. The calculated Hill coefficients (N values) are in agreement with this (see Table I); permeable cells show N values from 1.4 to 1.7, while extracts show N values from 1.1 to 1.3. Another major difference in the behavior of permeable cells compared to extracts is that the activity is saturated at approximately 3 times higher than in permeable cells.

The data shown in Table I summarizes the findings from the kinetic studies. In permeable cells, KCl increased the Vₘₐₓ about 5-fold and Na₂HPO₄ increased the Vₘₐₓ about 12-fold. The effects of potassium and phosphate on the Vₘₐₓ appear to be additive. In the absence of added potassium or phosphate, the Vₘₐₓ for adenylate cyclase in the extract was approximately three times higher than in permeable cells.

![Figure 3](image)

**Figure 3.** Specificity of anion effects on adenylate cyclase activity in permeable cells of E. coli. Toluene-treated cells (0.001 mg of protein/0.1-ml assay point) were assayed for adenylate cyclase activity as described under “Experimental Procedures.” Where indicated, assay mixtures were supplemented with 40 mM KCl and/or 20 mM Na₂HPO₄, Na₂AsO₄, K₂AsO₄, Na₂SO₄, or K₂SO₄. ATP was used as the sodium salt. Adenylate cyclase activity is expressed as nanomoles of cAMP formed per hour per milligram of protein.

![Figure 4](image)

**Figure 4.** Effect of potassium and phosphate on adenylate cyclase kinetics in permeable cells and extracts of E. coli. A, toluene-treated cells of strain 433 were assayed for adenylate cyclase activity as described under “Experimental Procedures.” Individual incubation mixtures contained the indicated concentration of ATP. Δ, no additions (each 0.1-ml assay point contained 0.043 mg of protein); ○, assays were supplemented with 40 mM KCl (each 0.1-ml assay point contained 0.054 mg of protein); ●, toluene-treated cells of strain 433 were assayed for adenylate cyclase activity as in Panel A, Δ, assays were supplemented with 20 mM Na₂HPO₄ (each 0.1-ml assay point contained 0.037 mg of protein); ○, assays were supplemented with 20 mM K₂HPO₄ (each 0.1-ml assay point contained 0.035 mg of protein). C, extracts of strain 433 were assayed for adenylate cyclase as in Panel A, Δ, no additions (each 0.1-ml assay point contained 0.03 mg of protein); ○, assays were supplemented with 40 mM KCl (each 0.1-ml assay point contained 0.042 mg of protein). D, extracts of strain 433 were assayed for adenylate cyclase activity as in Panel A, Δ, assays were supplemented with 20 mM Na₂HPO₄ (each 0.1-ml assay point contained 0.035 mg of protein). ATP was used throughout these studies as the sodium salt. Adenylate cyclase activity is expressed as nanomoles of cAMP formed per hour per milligram protein.

**Table I**

<table>
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<tr>
<th>Cell preparation</th>
<th>Additions</th>
<th>Vₘₐₓ</th>
<th>N°</th>
<th>K°</th>
</tr>
</thead>
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<tr>
<td>Permeable cells</td>
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<td>1.4 ± 0.07</td>
<td>0.73 ± 0.1</td>
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<td>Permeable cells</td>
<td>40 mM KCl</td>
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<td>1.2 ± 0.1</td>
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<td>20 mM Na₂HPO₄</td>
<td>58 ± 3</td>
<td>1.7 ± 0.2</td>
<td>3.0 ± 0.6</td>
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<tr>
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<td>80 ± 1</td>
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<td>1.7 ± 0.1</td>
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<tr>
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<td>1.3 ± 0.2</td>
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</tr>
<tr>
<td>Extract</td>
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<td>0.24 ± 0.04</td>
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<tr>
<td>Extract</td>
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<td>1.0 ± 0.2</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>Extract</td>
<td>20 mM K₂HPO₄</td>
<td>13 ± 4</td>
<td>1.2 ± 0.1</td>
<td>0.24 ± 0.01</td>
</tr>
</tbody>
</table>

*Expressed as nanomoles of cAMP formed per hour per milligram of protein.

*Calculated from the equation:

\[ v = \frac{V_{\text{max}}S^N}{K + S^N} \]

*Calculated as K°/N from the equation shown above and expressed as concentration of ATP (mM).
Inclusion of potassium or phosphate in the assays had no significant effect on the $V_{\text{max}}$ in the extract.

The $K_m$ for ATP in permeable cells (0.75 mM) was about six times higher than that in the extract (0.12 mM). In the extract, the $K_m$ for ATP was increased approximately 2-fold when the assays were carried out in the presence of added KCl or phosphate. In the case of permeable cells, KCl had a similar effect on $K_m$ as in the extract. However, the addition of sodium phosphate to permeable cells increased the $K_m$ approximately 4-fold ($K_m = 3.0$). The $K_m$ was only increased 2-fold when both potassium and phosphate were added to permeable cells ($K_m = 1.7$). These data suggest that interaction of potassium and phosphate with the adenylyl cyclase complex in permeable cells can modify its behavior such that both $K_m$ and $V_{\text{max}}$ are affected. Since changes of the magnitude observed in permeable cells were not observed in the extract, it appears that a functional complex is important to observe the ionic interactions.

Effects of Magnesium and Manganese on Adenylyl Cyclase Activity in Permeable Cells and Extracts—Fig. 5 shows the results of a study of the dependency on magnesium and manganese of adenylyl cyclase activity in permeable cells and extracts. As shown in Panels A (permeable cells, phosphate omitted) and B (permeable cells, phosphate added), magnesium was utilized in preference to manganese by permeable cells. The behavior of the extract was different as shown in Panels C (extract, phosphate omitted) and D (extract, phosphate added). In the extract, manganese promoted higher activity than magnesium, when tested at low concentrations.

The data of Figs. 1 and 4 show that phosphate inhibits adenylyl cyclase activity in the extract. The possibility was considered that this inhibition was due to the formation of a phosphate complex with Mg$^{2+}$ leading to a reduced concentration of free Mg$^{2+}$. If this were the case, higher Mg$^{2+}$ concentrations would, in the presence of phosphate, bring the adenylyl cyclase activity up to the level observed in the absence of phosphate. Inspection of the Mg$^{2+}$ concentration dependence of adenylyl cyclase activity in the presence or absence of phosphate (Fig. 5, Panels C and D) make this idea unlikely.

Effects of Potassium Phosphate on Adenylyl Cyclase and P-enolpyruvate: Sugar Phosphotransferase Activities in Mutant Strains—There is considerable evidence that, in permeable cells, the PTS system plays some role in regulating adenylyl cyclase activity (17). It was therefore of interest to test the effect of potassium phosphate on adenylyl cyclase activity in strains carrying mutations in this system. Fig. 6 presents the results of a study of the potassium phosphate concentration dependence of adenylyl cyclase activity in a wild-type strain (464) compared to an isogenic derivative (457) in which the gene for Enzyme I of the PTS has been deleted. While the wild-type strain showed a potassium phosphate-dependent increase in adenylyl cyclase activity, the PTS deletion strain showed the opposite effect; in that case, potassium phosphate inhibited the enzyme activity as it did.

**Fig. 5.** Effect of magnesium and manganese on adenylyl cyclase activity in permeable cells and extracts of E. coli. Toluene-treated cells (0.044 mg of protein/0.1 ml assay point in Panel A or 0.046 mg of protein/0.1 ml assay point in Panel B) and French press extracts (0.038 mg of protein/0.1 ml assay point in Panel C or 0.0425 mg of protein/0.1 ml assay point in Panel D) were assayed for adenylyl cyclase activity as described under "Experimental Procedures." Assay mixtures contained the indicated concentrations of MgCl$_2$ or MnCl$_2$, Δ, MnCl$_2$, □, MgCl$_2$. In Panels B and D, incubation mixtures were supplemented with 20 mM potassium phosphate, pH 8.5. In those cases, precipitates formed during the course of the reaction in mixtures containing 20 mM MgCl$_2$ or concentrations of MnCl$_2$ in excess of 2.5 mM. ATP was used as the potassium salt. Adenylyl cyclase activity is expressed as nanomoles of cAMP formed per hour per milligram of protein.

**Fig. 6.** Effect of potassium phosphate on adenylyl cyclase activity in permeable cells of wild-type and PTS-deletion strains. Toluene-treated cells (0.05-0.054 mg of protein/0.1 ml assay point) were assayed for adenylyl cyclase activity in reaction mixtures supplemented with 5 mM cAMP (see "Experimental Procedures"). Incubation mixtures contained the designated concentrations of K$_2$HPO$_4$. ATP was used as the potassium salt. Adenylyl cyclase activity is expressed as nanomoles of cAMP formed per hour per milligram of protein.
in the extract. These data indicate that potassium phosphate-dependent stimulation of adenylate cyclase activity depends on the presence of PTS components.

The properties of a PTS mutant characterized by a mutant form of Enzyme I were also examined. We previously showed that, in this strain, P-enolpyruvate increased adenylate cyclase activity severalfold in permeable cells (6). Fig. 7 documents the results of a study of the phosphate concentration dependence of adenylate cyclase activity in the absence or presence of added P-enolpyruvate. In the absence of added P-enolpyruvate, potassium phosphate inhibited the activity as it did in the PTS deletion strain (Fig. 6). However, in the presence of P-enolpyruvate, potassium phosphate (in the range of 5-15 mM) stimulated the adenylate cyclase activity. These results suggest that the stimulation of adenylate cyclase activity by potassium phosphate not only requires the presence of the PTS proteins (see Fig. 6) but also depends on those proteins being phosphorylated in a P-enolpyruvate-dependent reaction (see Fig. 7 and "Discussion").

The requirement for PTS proteins for the stimulation of adenylate cyclase activity by potassium phosphate prompted the consideration that potassium phosphate might also affect PTS activity. Fig. 8 shows the results of an experiment in which the effect of potassium phosphate on both adenylate cyclase and PTS activities was tested in strain 471 (which synthesizes an altered form of Enzyme I). The studies were performed in the presence of P-enolpyruvate which is required for PTS activity and stimulates the adenylate cyclase activity in this strain (see Fig. 7). The data indicate that, in the range of 5-15 mM potassium phosphate, both the activities of adenylate cyclase and PTS were stimulated. A likely interpretation of these findings is that the stimulation of adenylate cyclase by potassium phosphate is mediated by an interaction of potassium phosphate with one or more of the PTS proteins.

**DISCUSSION**

In this study, we show that *E. coli* cells, permeabilized with toluene, contain an adenylate cyclase activity that is stimulated by both potassium and phosphate. These stimulatory effects are additive and therefore, independent (Fig. 2) and both are abolished by α-methylglucoside. In the original description of a stimulatory effect of ions on adenylate cyclase activity in toluene-treated cells, it was reported that potassium had no effect on the activity (3). The data in the present report, showing a stimulation by potassium, is more reliable in two ways. First, in the older studies, ATP was used as the potassium salt. Adenylate cyclase and PTS activities are expressed as nanomoles of cAMP or α-methylglucoside-6-phosphate formed per hour per milligram of protein.

FIG. 7. Effect of potassium phosphate on adenylate cyclase activity in permeable cells of strain 471 in the absence or presence of P-enolpyruvate. Toluene-treated cells (0.046 mg of protein/0.1-ml assay point) were assayed for adenylate cyclase activity as described under "Experimental Procedures." Assay mixtures were supplemented with 5 mM cAMP and the designated concentrations of K₂HPO₄. Δ, incubation mixtures not supplemented with P-enolpyruvate; ○, incubation mixtures supplemented with 1 mM P-enolpyruvate. ATP was used as the potassium salt. Adenylate cyclase activity is expressed as nanomoles of cAMP formed per hour per milligram of protein.
The stimulation of adenylate cyclase activity by phosphate probably involves an interaction of phosphate with some component of the adenylate cyclase complex but not mediated by a high energy phosphorylated intermediate. Since arsenate effectively substitutes for phosphate in stimulation of adenylate cyclase activity (Fig. 3), that type of intermediate is unlikely.

Previous studies on the divalent cation specificity of adenylate cyclase of membranes derived from lymphoma cells (18) revealed that the enzyme coupled to receptors utilized magnesium in preference to manganese and that the uncoupled form of the enzyme was manganese-dependent. A study of the dependence on these two cations for adenylate cyclase activity in *E. coli* (Fig. 5) permeable cells and extracts showed that magnesium was utilized in preference to manganese by permeable cells; in contrast, at low cation concentrations, manganese promoted higher activity than magnesium in the extract. These data are consistent with the idea that permeable cells show properties of a coupled enzyme and the extract behaves like an uncoupled enzyme.

A further indication of the complex character of adenylate cyclase in permeable cells was derived from a study of the kinetic properties of the enzyme (Fig. 4). The substrate-velocity curves characteristic of permeable cells are sigmoid, suggesting a complex interaction while the curves from the extract are more hyperbolic. The *Km* for ATP for the enzyme in the extract is much lower than in permeable cells. Addition of potassium or phosphate to the extract increases *Km* by a factor of 2 with no effect on *Vmax* (Table I); these additions increase *Km* 2-4-fold and increase the *Vmax* as much as 16-fold in permeable cells.

In the absence of added ions, adenylate cyclase activity is five to six times higher in the extract than in permeable cells (Fig. 1). The nature of the processes regulating the activity of adenylate cyclase may therefore involve elements that prevent the enzyme from expressing its full potential activity. The stimulatory effects of potassium and phosphate on adenylate cyclase activity in permeable cells may reflect a relief of these inhibitory effects. An essential requirement for response of the permeable cell adenylate cyclase to potassium and phosphate appears to be a functional PTS (Fig. 6). Permeable cells of a strain in which the gene for Enzyme I of the PTS is deleted behave like an uncompensated enzyme. A study of the dependence on these two cations for adenylate cyclase activity in *E. coli* (Fig. 5) permeable cells and extracts showed that magnesium was utilized in preference to manganese by permeable cells; in contrast, at low cation concentrations, manganese promoted higher activity than magnesium in the extract. These data are consistent with the idea that permeable cells show properties of a coupled enzyme and the extract behaves like an uncoupled enzyme.

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An essential requirement for response of the permeable cell adenylate cyclase to potassium and phosphate appears to be a functional PTS (Fig. 6). Permeable cells of a strain in which the gene for Enzyme I of the PTS is deleted behave like an extract. This observation suggests that the unique loss of regulatory properties of adenylate cyclase in the extract is due to a breakdown of an association with PTS proteins.

Previous studies demonstrated a stimulation by P-enolpyruvate in permeable cells of adenylate cyclase activity in a mutant that synthesizes an altered form of Enzyme I of the PTS (6). This led to the proposal (6) that the high activity form of adenylate cyclase required that the PTS proteins be in the phosphorylated form. The data shown in Fig. 7 further suggest that the phosphorylated state of PTS proteins is necessary in order to demonstrate stimulation of adenylate cyclase activity by potassium phosphate.

The involvement of the PTS proteins in regulating adenylate cyclase activity at the level of the effects of potassium and phosphate is further implicated by the data of Fig. 8. It was shown that, in the presence of P-enolpyruvate, potassium phosphate stimulated the activity of both adenylate cyclase and the PTS. It seems likely, on the basis of these data, that the effects of potassium phosphate on adenylate cyclase are mediated via an interaction of these ions with PTS proteins.

The range of phosphate concentrations required for maximal stimulation of adenylate cyclase activity in permeable cells is 20–40 mM. This is a physiologically significant effect of phosphate since the normal phosphate concentration in *E. coli* has been reported to be 20–30 mM (19). Therefore, it may be assumed that the regulatory adenylate cyclase complex in intact cells contains phosphate derived from inorganic phosphate which is noncovalently bound as well as phosphate derived from P-enolpyruvate which is covalently bound to the PTS proteins. The data presented in this paper are consistent with a model of the regulatory complex as shown in Fig. 9. There is reason to believe that all the soluble PTS proteins interact with the specific membrane-bound transporters to form a functional transport complex (20). The stimulation by potassium phosphate of both PTS and adenylate cyclase activities provides a basis for suggesting an interaction of potassium phosphate with a PTS protein such as Enzyme I. The interaction of adenylate cyclase with the PTS complex is a logical prediction from the data presented here.

It is noteworthy that addition of glucose to intact cells results in an approximately 80% reduction of the internal phosphate concentration. When the added glucose is depleted, the internal phosphate level rises (21). While the studies reported here show that potassium stimulates adenylate cyclase activity, there is no evidence that exposure of cells to glucose results in a change in cellular potassium levels. This information, together with the phosphate-dependent stimulation of adenylate cyclase reported here, suggests that, in intact cells, glucose regulates the activity of adenylate cyclase in two ways. A working model describing such a multifunctional regulation of adenylate cyclase is described in Fig. 10. According to this model, the high activity form of adenylate cyclase in intact cells requires both the phosphorylated state of the PTS proteins derived from P-enolpyruvate (3) and in addition, bound inorganic phosphate. Exposure to glucose converts the coupled enzyme to a low activity form characterized by PTS proteins in the dephospho-form and a deficiency of bound phosphate. This regulation of adenylate cyclase by addition of glucose to intact cells results in an approximately 80% reduction of the internal phosphate concentration. When the added glucose is depleted, the internal phosphate level rises (21). While the studies reported here show that potassium stimulates adenylate cyclase activity, there is no evidence that exposure of cells to glucose results in a change in cellular potassium levels. This information, together with the phosphate-dependent stimulation of adenylate cyclase reported here, suggests that, in intact cells, glucose regulates the activity of adenylate cyclase in two ways. A working model describing such a multifunctional regulation of adenylate cyclase is described in Fig. 10. According to this model, the high activity form of adenylate cyclase in intact cells requires both the phosphorylated state of the PTS proteins derived from P-enolpyruvate (3) and in addition, bound inorganic phosphate. Exposure to glucose converts the coupled enzyme to a low activity form characterized by PTS proteins in the dephospho-form and a deficiency of bound phosphate. This regulation of adenylate cyclase by

![Fig. 9. A model of the regulatory complex of adenylate cyclase with PTS proteins and potassium phosphate.](image-url)
E. coli Adenylate Cyclase

**Fig. 10. The PTS-adenylate cyclase regulatory cycle.** In this scheme, it is proposed that the high activity state of adenylate cyclase is a form in which the PTS proteins are in the phospho-form (PTS-P), the PTS is coupled to adenylate cyclase (A.C.-PTS) and P is bound noncovalently in some undefined way to the complex. Exposure to glucose leads to a dephosphorylation of PTS phosphoproteins (PTS-P) as a result of the transfer of the phosphate to sugar to form sugar phosphate. In addition, exposure to glucose lowers the level of P in cells such that there is less available to interact noncovalently with the complex. The depletion of the complex of both covalently and noncovalently bound phosphate produces a low activity state of the enzyme. Reexposure of the low activity state (A.C.-PTS) to P-enolpyruvate (PEP) results in the conversion of dephospho-PTS proteins to phospho-proteins (PTS-P) concomitant with the conversion of P-enolpyruvate to pyruvate. When cellular levels of P increase, P becomes noncovalently bound to the complex. Conversion of the complex to a condition in which it contains both covalently and noncovalently bound phosphate regenerates the high activity state of the enzyme complex.

Simultaneously controlling two stimulatory factors provides a potent mechanism for rapidly controlling the activity of adenylate cyclase. The analogy to the cycle of phosphate release from GTP characteristic of hormone-dependent regulation of eucaryotic adenylate cyclase (22) is obvious.

Acknowledgment—We are grateful to Dr. Antoine Danchin for providing the plasmid encoding the gene for adenylate cyclase.

**REFERENCES**
