Differential Binding of Cyclic Adenosine 3',5'-Monophosphate to the Cyclic Adenosine 3',5'-Monophosphate Receptor Protein in Escherichia coli

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Binding of cyclic adenosine 3',5'-monophosphate (cAMP) by the cAMP receptor protein in crude cell-free extracts of Escherichia coli was characterized. When cells were grown in glucose, binding was inhibited 50% relative to extracts from cells grown with succinate as carbon source. This inhibition could be relieved by dialysis.

The induction of catabolic enzymes in Escherichia coli requires both cyclic 3',5'-adenosine monophosphate (cAMP) and a cAMP receptor protein referred to as the CRP (6) or the CAP (specific assay monophosphate (cAMP)). The experiments reported here show that growth of cells in glucose reduces the affinity of the CRP for cAMP.

cAMP binding in crude cell-free extracts was measured by the ammonium sulfate precipitation assay described by Anderson et al. (1, 11) with only minor modifications. The reaction mixture contained: 10^{-8} M cAMP with cyclic [5-^3H]AMP (specific activity, 38.5 Ci/mmol) diluted to give 10^6 cpm in the final reaction mixture; 0.01 M 5'-AMP (to minimize nonspecific binding of cAMP); 0.01 M potassium phosphate buffer (pH 7.5); and a variable amount of crude cell extract in a final volume of 100 µl. Each experimental value was corrected for nonspecific background binding by subtracting the counts found in an identical sample containing a 2,000-fold excess (2 mM) of nonradioactive cAMP in addition to the cyclic [5-^3H]AMP. By varying the protein concentration to provide at least four values for each determination, reproducible data could be obtained (Fig. 1). Binding activity was comparable when cells were harvested in mid-exponential, early stationary, or late stationary growth phase (18 h after inoculation). Occasionally extracts were obtained with very high binding activity.

Cells were routinely grown in a minimal salts medium (SBG) supplemented with 0.05% Casamino Acids and the appropriate carbon source at a concentration of 0.04 M as described previously (4, 5). L-Tryptphan (75 µg/ml) was present when required as a nutritional supplement. Ten-milliliter inocula were used to inoculate 1 liter of medium. Cells were grown aerobically at 37°C.

Cells were harvested by centrifugation at 4°C. Pellets were washed once, weighed, and suspended in cold buffer A as described by Anderson and Pastan (1). Washed cells were broken by means of the Eaton pressure cell. The disrupted cell suspensions were centrifuged at 30,000 × g for 1 h. The supernatant was utilized as the crude cell-free extract for cAMP binding determinations.

Binding activity in extracts from strains with various Crp phenotypes was determined to establish the validity of the assay (Table 1). Binding activity was determined from plots of cAMP bound versus protein as shown in Fig. 1. Extracts from strains lacking a functional crp gene (strains 5333 and X7901) bound less than 10% as much cAMP as wild-type strains. A strain diploid for the crp region (KLF41/JC1553) bound approximately twice that of the wild type.

Extracts of the wild-type strain were stable with respect to binding activity after 6 days of storage at 4°C. The half-life at 50°C was 6 min. Thus the stability of the binding activity in crude extracts is comparable to that of the purified CRP (1, 12). The dissociation constant, K_d, for the binding of cAMP by cell-free extracts was found to be approximately 10^{-8} M (Table 2), comparable to that observed with purified CRP assayed by the same ammonium sulfate precipitation assay (1, 10, 12).

The carbon source affording growth was
found to influence binding activity (Table 2). cAMP binding was consistently 50% lower in extracts of cells grown with glucose as a carbon source relative to cells grown with succinate. This was found to be independent of the growth phase of the culture at harvest. Even when grown in continuous culture with unlimiting nutrients but with a dilution rate of 0.25 h⁻¹, extracts of cells grown with glucose bound half as much cAMP as did extracts from cells grown in succinate (D. E. Danley, Senior Thesis, New Mexico State University, Las Cruces, 1975).

Tryptophanase induction has been shown to be severely inhibited by growth with glucose, e.g., conditions of permanent catabolite repression (5, 9). In several experiments, the growth medium was supplemented with 250 µg of L-tryptophan per ml and the tryptophanase activity (5) as well as the cAMP binding activity was determined. It was found that extracts of cells grown with glucose had only 2% of the tryptophanase activity found in extracts of cells grown with succinate.

A second experiment was run to determine the effect of adding glucose to cells growing in succinate. These conditions have been shown to cause severe transient catabolite repression of tryptophanase induction (4). Cells were grown to mid-exponential phase, the culture was split in half, and to one portion glucose was added to give a final concentration of 1%. Both portions received 250 µg of L-tryptophan per ml at this time. After 20 min of incubation, cells were harvested and tryptophanase activity (5) and cAMP binding activity were determined. The binding activity was comparable in both cultures, indicating that the 20-min exposure to glucose was not sufficient to affect the affinity of the CRP for cAMP. However, the culture to which glucose was added made only 7% as much tryptophanase as did the control culture, demonstrating that these conditions did cause transient catabolite repression.

The inhibition of cAMP binding in extracts of cells grown with glucose was found to be relieved by dialysis (Table 2). When the apparent dissociation constant, $K_d$, and the maximal binding were determined, both parameters were found to be influenced by dialysis of extracts from cells grown with glucose, suggesting that the uncompetitive inhibitor of cAMP binding was present in these extracts. Dialysis had much less effect on these parameters in extracts of cells grown with succinate (Table 2).

**Table 1. cAMP binding activity of various Crp phenotypes**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype</th>
<th>Reference</th>
<th>cAMP binding$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5333</td>
<td>Crp⁻</td>
<td>6</td>
<td>0.12</td>
</tr>
<tr>
<td>X7901</td>
<td>Crp⁻</td>
<td>12</td>
<td>0.15</td>
</tr>
<tr>
<td>K-12-1</td>
<td>Wild type</td>
<td>5</td>
<td>1.62</td>
</tr>
<tr>
<td>KLF41/JC1553</td>
<td>Crp⁻/Crp⁺</td>
<td>1</td>
<td>4.55</td>
</tr>
</tbody>
</table>

* Cells were grown in minimal glucose medium as described. Cells were harvested after 15 h of incubation, and extracts were prepared.

* Picomoles of cAMP bound per milligram of protein as determined from slopes of binding curves like that shown in Fig. 1. Protein was determined by the method of Lowry et al. (8).

**Table 2. Effect of carbon source affording growth and dialysis on cAMP binding activity**

<table>
<thead>
<tr>
<th>Extract$^a$</th>
<th>cAMP binding$^b$</th>
<th>$K_c^{c}$ (x10⁻⁴ M)</th>
<th>Maximal$^d$ binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undialyzed</td>
<td>2.26</td>
<td>1.59</td>
<td>13.15</td>
</tr>
<tr>
<td>Dialyzed</td>
<td>2.20</td>
<td>1.24</td>
<td>9.82</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undialyzed</td>
<td>0.95</td>
<td>0.54</td>
<td>2.51</td>
</tr>
<tr>
<td>Dialyzed</td>
<td>1.65</td>
<td>1.65</td>
<td>8.57</td>
</tr>
</tbody>
</table>

* Extracts from cells of strain K-12-1 grown with succinate or glucose extracts. Extracts were dialyzed against 100 volumes of buffer A (1) with three changes.

* Picomoles of cAMP bound per milligram of protein as determined in Fig. 1. These values represent averages from many determinations.

* Values were determined from a plot of 1/cAMP bound versus 1/concentration of cAMP in the reaction mixture and also from a plot of concentration of cAMP/cAMP bound versus cAMP concentration with similar results. In these experiments, the protein concentration was held constant at 0.55 mg of protein in each reaction. Except for the values reported for the dialyzed succinate extracts, all values were obtained from least-square fit plots of the data from several experiments (10 to 30 values each).

* Expressed as picomoles bound per milligram of protein. These values were determined from the plots described in footnote c.
To verify that an inhibitor of cAMP binding is removed from the extracts by dialysis, the putative inhibitor was added back to dialyzed extracts. Extracts of cells grown with glucose were dialyzed against double-distilled water, and the small molecules in the dialysis fluid were concentrated by lyophilization. The concentrate, with a concentration calculated to be half that in the original extracts, was then added to extracts of cells dialyzed against buffer. This resulted in a 30% inhibition of cAMP binding. When the dialysis fluid of extracts from cells grown with succinate was concentrated and added to extracts, no inhibitory effect was observed.

These results suggested that a catabolite unique to glucose metabolism could be responsible for the observed inhibition of cAMP binding. Glycolytic intermediates have been shown to be present in much higher concentration in cells growing with glucose than in cells growing with succinate (7). These intermediates were tested alone and in combination at several concentrations in the physiological range. None were found to have any consistent effect.

Citrate and glutamate are also found to vary dramatically in cells growing with glucose relative to cells growing with succinate (7). The addition of glutamate at physiological concentrations was found to have no effect on cAMP binding. The addition of citrate made the binding erratic. Initially 5 mM citrate was found to inhibit binding about 35% (D. E. Danley and J. L. Botsford, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, K188, p. 167). However, when this was investigated in more detail, no consistent effect could be determined.

In summary, we have shown that the ability of the CRP to bind cAMP in crude cell-free extracts is influenced by a dialyzable compound present in extracts of cells grown with glucose. This differential capacity of the CRP to bind cAMP could influence formation of the cAMP-CRP complex active in transcription.

(Most of this investigation was done by D.E.D. in partial fulfillment of the requirements for the M.S. degree at New Mexico State University.)

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LITERATURE CITED