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A cAMP-dependent protein kinase is present in differentiating *Dictyostelium discoideum* cells

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We demonstrate the occurrence of a cAMP-dependent protein kinase in *Dictyostelium discoideum* cells at the terminal stage of differentiation. A cAMP-binding component was purified to homogeneity by affinity chromatography. This subunit inhibits the activity of purified catalytic subunit from beef heart protein kinase; the inhibition is reversed upon addition of cAMP. The protein is highly specific for cAMP and has a dissociation constant of 4 nM. The isolated regulatory subunit is a monomer of 39 K, with a sedimentation coefficient of 3.5S and a frictional coefficient of 1.24. The differences between this regulatory subunit and regulatory subunits of protein kinases from other sources are discussed.
Key words: Dictyostelium discoideum/cAMP/protein kinase/affinity chromatography

Introduction

Upon starvation, *Dictyostelium discoideum* amoebae enter a developmental sequence that terminates in the formation of a multicellular structure consisting of a stalk supporting a mass of spores (Loomis, 1975). A few hours after depletion of nutrient, amoebae aggregate towards specific centers through the rhythmic emission and relay from cell to cell of a chemoattractant, adenosine 3′:5′cyclic monophosphate (cAMP) (Robertson and Grutsch, 1981). Aside from their role in chemotaxis, the successive cAMP pulses stimulate differentiation of the amoebae from the vegetative state to 'aggregation competence' (Darmon *et al.*, 1975; Gerisch *et al.*, 1975). When cells have aggregated and formed tight contacts, they initiate the differentiation of stalk and spore cells, together with morphogenesis of the fruiting body. This terminal differentiation involves the expression of a large number of new genes (Alton and Lodish, 1977a; Jacquet *et al.*, 1981) and it has been suggested that the establishment of cell-cell contacts is necessary for the initiation of this process (Alton and Lodish, 1977b; Blumberg *et al.*, 1982). However, differentiation into both stalk and spore cells has been observed with isolated cells in the presence of cAMP along with a morphogen (Kay, 1982).

Although there are indications that cAMP ultimately controls gene expression at the transcriptional level (Williams *et al.*, 1980), its precise mode of action remains unknown. Cytoplasmic cAMP-binding proteins that could constitute intracellular targets for the regulatory role of the nucleotide have been described. A high mol. wt. protein that binds cAMP as well as adenosine and its derivatives is present in vegetative and aggregation competent cells (Gunzburg and Véron, 1981; Leichtling *et al.*, 1981a). This protein, which is constitutive throughout the developmental cycle, will be described in detail elsewhere. In contrast, a set of low mol. wt. cAMP-binding proteins appear to be developmentally regulated (Wallace and Frazier, 1979; Cooper *et al.*, 1980; Gunzburg and Véron, 1981; Arents and van Driel, 1982; Leichtling *et al.*, 1982). Although there have been conflicting reports about the occurrence of cAMP-dependent protein kinases in *D. discoideum* (Sampson, 1977; Véron and Patte, 1978; Rahmsdorf and Gerisch, 1978), a cAMP-binding protein present in aggregating cells has recently been shown to be capable of regulating the activity of a catalytic subunit from mammalian protein kinase (Leichtling *et al.*, 1981b). Here we report the presence of a cAMP-protein kinase in *D. discoideum* cells at the stage of terminal differentiation and describe the purification of its regulatory component.

Results

Evidence for a cAMP-dependent protein kinase in differentiating D. discoideum

Exponentially growing *D. discoideum* cells were allowed to differentiate up to the stage of early culmination (see Materials and methods). A crude soluble extract was prepared and chromatographed on DEAE-Sephacel; fractions were analyzed for cAMP binding and protein kinase activities. As shown in Figure 1, all of the cAMP-binding activity was eluted in a single peak at 125 mM NaCl. [^{3}H]cAMP binding was totally abolished by the addition of 0.1 mM non-radioactive cAMP. cGMP (0.1 mM) inhibited cAMP binding by >80%, whereas 0.1 mM adenosine had little effect.

Figure 1 shows that high levels of protein kinase activity were present in the column fractions in our assay conditions (see Materials and methods). The activity was eluted in three

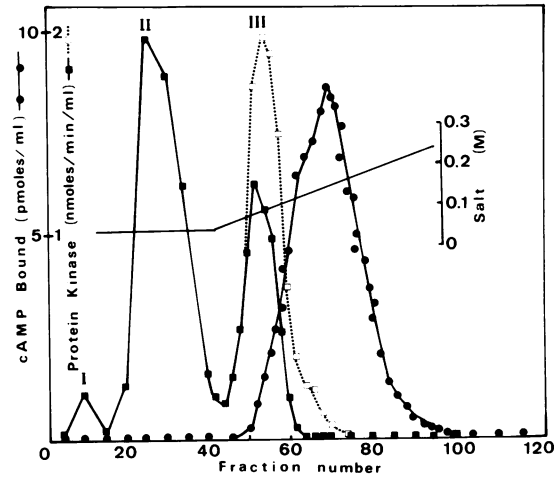

Fig. 1. DEAE-Sephacel chromatography of a soluble extract from 18 h differentiated cells. The crude soluble extract prepared in buffer A5 from 1.5 x 10^{10} cells at the stage of early culmination was chromatographed on a DEAE-Sephacel column (2.5 x 10 cm) developed with a gradient of NaCl (0 – 400 mM). 5 ml fractions were collected, extensively dialyzed against buffer A5 and analyzed for cAMP binding with 0.1 μM [^{3}H]cAMP (43 Ci/mmol) (●) and protein kinase activity in the absence (■) or presence (□) of 0.1 mM cAMP.

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peaks, none of which coincided with the cAMP-binding peak. The phosphorylating activity in peak I, representing <5% of the total, could also be measured in the absence of exogenous substrate (i.e., endogenous proteins of those fractions served as substrates); it was not studied further. In contrast, the activity in peaks II and III could only be measured in the presence of added substrate. Addition of cAMP to fractions from peak II did not affect the activity. However, in those fractions of peak III that co-eluted with the leading part of the cAMP-binding peak (fractions 50–70), the kinase activity was stimulated by cAMP. One explanation is that the cAMP-binding protein is inhibiting the protein kinase, and that inhibition is relieved by cAMP. This hypothesis is supported by the data in Table I. Preincubation of material from the cAMP binding peak, containing no kinase activity, with fractions from peaks II and III devoid of cAMP-binding activity resulted in a substantial inhibition of their phosphorylating activity. This inhibition was reversed by 0.1 mM cAMP. Hence, the cAMP-binding component can exert a cAMP-reversible inhibition of the phosphorylating activity of peaks II and III. The fact that protein kinase activity was eluted in different peaks may not reflect the presence of different protein species. When fractions 20–85 containing cAMP-binding and/or kinase activity were pooled, extensively dialyzed against buffer A5, and rechromatographed on a similar column, all of the kinase activity was eluted in a single peak at 75 mM NaCl and could be regulated by the addition of cAMP-binding component (not shown). Therefore, the activities in peaks II and III (Figure 1) apparently represent the catalytic subunit of a cAMP-dependent protein kinase from *D. discoideum*, and the cAMP-binding peak corresponds to the regulatory subunit. Dissociation of the complex under the conditions of the DEAE-chromatography may merely reflect the presence of cAMP in the crude extract (see Discussion).

Purification and characterization of the cAMP-binding protein

cAMP-binding activity recovered from a DEAE-Sephacel column was adsorbed to 8-HET-cAMP-Sepharose and eluted with cAMP as described in Materials and methods. Figure 2 (lanes 1 and 2) shows that this step resulted in considerable purification. The protein was further purified to homogeneity by a second affinity chromatography on N^6-AEt-cAMP-Sepharose; analysis of the recovered material by polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE) showed a single protein band of mol. wt. 38 000 (Figure 2, lane 3). The purified protein could be specifically labeled with the photoaffinity analogue of cAMP, 8-azido-[^{3}H]cAMP (Figure 2, lane 4); labeling was totally inhibited by the addition of 0.1 mM non-radioactive cAMP prior to photoactivation (Figure 2, lane 5) but not by 0.1 mM 5′-AMP (Figure 2, lane 6). The purified cAMP-binding protein was devoid of protein kinase activity.

The purified protein gave a single peak upon filtration on Ultrogel AcA 44 yielding a Stokes radius of 27.8 Å; the sedimentation coefficient as measured by velocity sedimentation in linear sucrose gradients was 3.5S ± 0.2S (not shown). The presence or absence of 0.01% Triton X-100 in the gradients had no effect on the sedimentation coefficient, neither did preincubation of the protein and sedimentation in the presence of 2 M urea or 1 μM cAMP. Assuming a partial specific volume of 0.72 cm^3/g, a mol. wt. of 39 500 for the native protein and a frictional coefficient of 1.24 were calculated (Siegel and Monty, 1966). Comparison with the subunit mol. wt. measured by SDS-PAGE shows that the protein is a monomer of 39 ± 1.5 K.

The affinity for cAMP was measured with the filter binding assay by titrating the purified protein with cAMP concentrations ranging from 0.1 nM to 5 μM. As shown in Figure 3, the protein contains a single class of binding sites with a dissociation constant for cAMP of 4 nM. Similar results were obtained if the binding measurements were performed by equilibrium dialysis. Table II (column 1) shows the inhibition of [^{3}H]cAMP binding by a number of analogs. Attachment of the phosphate group to both 3′ and 5′ carbons of the ribose moiety of the base is necessary for recognition.

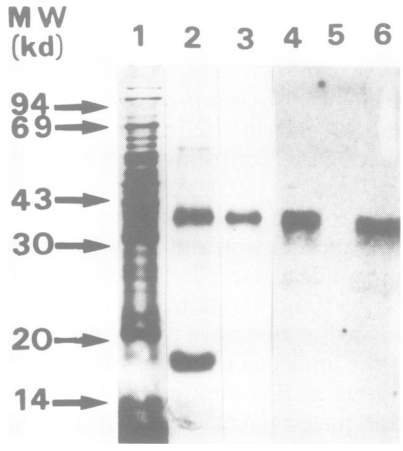

Fig. 2. SDS-PAGE of cAMP-binding protein at various steps of the purification procedure. **(a) Lanes 1, 2, 3**: 10% polyacrylamide gels were stained with silver nitrate according to Morrissey (1981). **Lane 1**: eluate from DEAE; **lane 2**: eluate from 8-HET-cAMP-Sepharose; **lane 3**: eluate from N^6-AEt-cAMP-Sepharose. **(b) Lanes 4, 5, 6**: fluorograph of a gel where purified cAMP-binding protein was labeled with 8-azido-[^{3}H]cAMP **(lane 4)**. Prior to photoactivation, incubations were carried out with 0.1 mM cAMP **(lane 5)** or 0.1 mM 5′AMP **(lane 6)**.

Table I. Inhibition of *D. discoideum* protein kinase activity by the cAMP-binding component[a]

DEAE-Sephacel fraction	No addition	+ 0.1 mM cAMP	+ cAMP-binding component[b]	+ cAMP-binding component + 0.1 mM cAMP
Peak II (fraction 25)	1890	1796	813	2381
Peak III (fraction 50)	677	745	0	819

[a]Samples (20 μl) from fractions of peaks II and III in Figure 1 were preincubated for 1 h at 0°C with the indicated additions. Protein kinase activity was measured as described in Materials and methods except that the final volume was 75 μl and NaF was 20 mM. The data result from duplicate determinations and are expressed as pmol [^{32}P]Kemptide formed/min/ml extract.
[b]The source of cAMP-binding activity was fraction 70 (20 μl) from the column shown in Figure 1. It was devoid of protein kinase activity, whether assayed in the presence or absence of 0.1 mM cAMP.

In contrast, substitution of the adenine ring on the N-6 position (N^6-AEt-cAMP) or on the C-8 position (8-HET-cAMP, 8-azido-cAMP) does not significantly alter binding. Presence of the N-6 amino group is not essential since cIMP is a good competitor of cAMP binding, but further substitution of the carbon-2 with an amino group (cGMP) results in a substantial decrease of affinity.

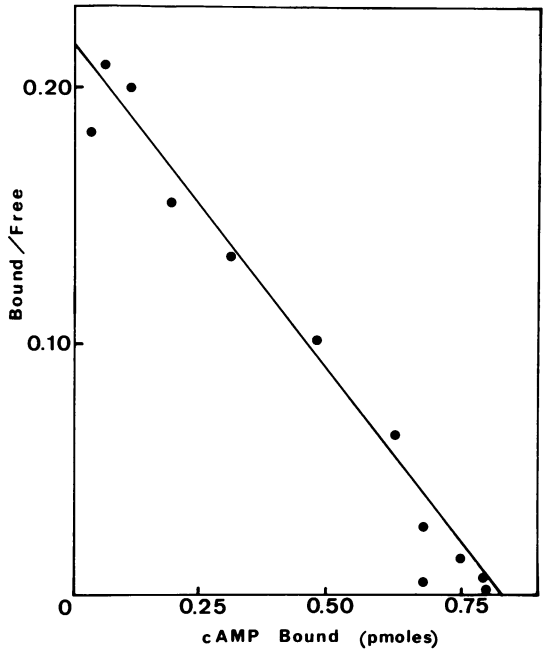

Fig. 3. Scatchard plot of cAMP binding. Purified protein was incubated for 17 h at 0°C in buffer C in the presence of varying [^{3}H]cAMP amounts (43 Ci/mmol or 4.3 Ci/mmol depending on the cAMP concentration). The assay was performed in a total volume of 1 ml at low cAMP and of 0.1 ml when cAMP was 100 nM or higher. Background radioactivity bound to the filters in the absence of protein was subtracted. Free cAMP concentration at equilibrium was calculated from the difference between total radioactivity (measured directly by spotting an aliquot of the incubation mixture on a filter), and bound radioactivity. Average of duplicate determinations are shown.

Table II. Specificity of cAMP binding and activation of protein kinase

Addition[a]	Residual [^{3}H]cAMP binding[b] (%)	Protein kinase activity[c] (%)
none	100	100
3',5'-cAMP	0	259
2',3'-cAMP	100	107
N^6-NEt-cAMP	2.5	258
8-HET-cAMP	0	246
8-azido-cAMP	0	242
5'-AMP	92	102
adenosine	90	75
3',5'-cIMP	0	261
3',5'-cGMP	17	240
2',3'-cGMP	72	119
5'-GMP	81	124

[a]0.1 mM final concentration.
[b]Purified cAMP-binding protein was incubated for 17 h at 0°C with the indicated analog, 2.5 μg BSA and all of the constituents of the protein kinase assay except for ATP and Kemptide. Residual cAMP binding was measured with 0.1 μM [^{3}H]cAMP (43 Ci/mmol).
[c]Incubation was the same as in b except that BSA was replaced by 0.25 pmol purified catalytic subunit from beef heart cAMP-dependent protein kinase. After 17 h at 0°C, the kinase activity was measured.

Characterization of the purified cAMP-binding protein from D. discoideum as a regulatory subunit of a cAMP-dependent protein kinase

Analysis of the data in Figure 1 (see above) showed that cAMP-binding activity is carried by a protein which is capable of regulating protein kinase activity. Further demonstration of the regulatory role of the cAMP-binding component was achieved by a study with purified components. Since no purified catalytic subunit from the *D. discoideum* protein kinase is yet available, the interaction of the purified cAMP-binding protein from differentiating cells was studied with purified catalytic subunit from beef heart cAMP-dependent protein kinase. Figure 4 shows the inhibition of protein kinase activity by purified cAMP-binding protein. The molar ratio of the two components was computed by: (i) calculating the concentration of beef heart catalytic subunit assuming a mol. wt. of 40 K (Erlichman *et al.*, 1973) and; (ii) estimating the amount of cAMP-binding protein from the extrapolation of the Scatchard plot shown in Figure 3, assuming a stoichiometry of one cAMP binding site per 39-K polypeptide. The inhibition of phosphorylating activity was directly proportional to the concentration of cAMP-binding protein until a stoichiometric concentration of regulatory subunit was reached; at that point, inhibition was 70%. When cAMP-binding protein was present in 4-fold excess, 90% inhibition was achieved. In all cases, the inhibition was abolished by the addition of 0.1 mM cAMP.

Figure 5 shows the effect of increasing concentrations of cAMP on the reversal of protein kinase inhibition by the purified *D. discoideum* regulatory subunit. Half-maximal activation occurred at 40 nM cAMP. Table II shows that there is a good correlation between the ability of various cAMP analogs to inhibit cAMP binding and to activate the kinase activity. Compounds that inhibited cAMP binding were also capable of activating phosphorylation. Conversely, those which did not compete with cAMP binding were unable to relieve the inhibition of protein kinase activity by the cAMP-binding protein. These results support our conclusion that the purified cAMP-binding component is the regulatory subunit

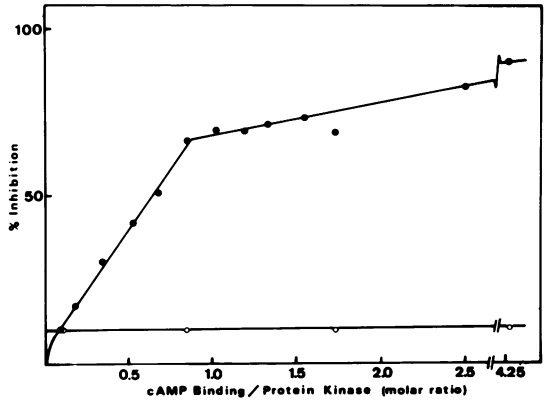

Fig. 4. Interaction of purified cAMP-binding protein from *D. discoideum* with the catalytic subunit of beef heart protein kinase. 0.25 pmol catalytic subunit from beef heart cAMP-dependent protein kinase were incubated for 30 min at 0°C with 10 μl buffer C containing the indicated amount of purified cAMP-binding protein prior to the assay of protein kinase activity. Results are expressed as percent inhibition compared to the control containing only catalytic subunit. Incubations were performed in the absence (●) or presence (○) of 0.1 mM cAMP.

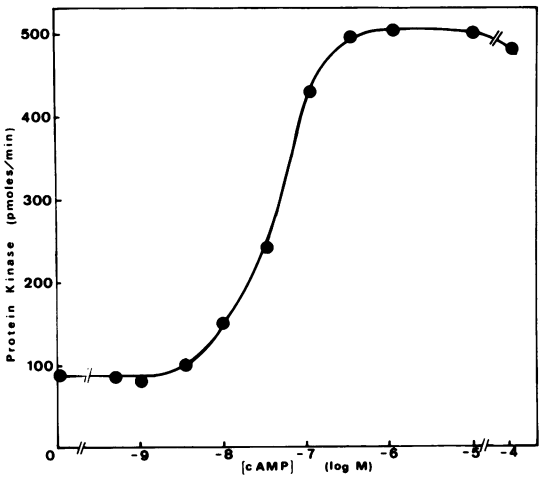

Fig. 5. Activation of the *D. discoideum* cAMP-binding protein-beef heart catalytic subunit complex. 0.25 pmol catalytic subunit from beef heart were incubated for 30 min at 0°C with 0.21 pmol purified cAMP-binding protein in buffer C, together with all of the constituents of the assay for protein kinase activity except for ATP and the peptide substrate. The preformed complexes were incubated overnight with varying amounts of cAMP, and protein kinase activity was measured.

of a cAMP-dependent protein kinase complex in *D. discoideum*.

Discussion

For most of the cAMP-dependent protein kinases reported in eukaryotic systems, the catalytic subunit is fully active unless it is bound to the regulatory subunit. cAMP stimulates the activity by causing dissociation of the inactive catalytic-regulatory subunit complex. We report the presence of high levels of protein kinase activity in differentiating *D. discoideum* cells. More than 95% of the activity elutes in two peaks from a DEAE column (see Figure 1), only a fraction of which is enhanced by cAMP. There have been conflicting reports on cAMP stimulation of protein kinase activity in *D. discoideum* when histones were used as substrates (Sampson, 1977; Véron and Patte, 1978; Rahmsdorf and Gerisch, 1978). It now appears that such discrepancies may have resulted from dissociation of a catalytic-regulatory subunit complex prior to addition of cAMP. This could result both from an effect of the histones themselves, which have been shown to have dissociating effects (Miyamoto *et al.*, 1973), and from the presence of cAMP in the extracts of *D. discoideum*. Indeed, the crude extract that was chromatographed in Figure 1 contained endogenous cAMP which was retained on the DEAE and eluted at 80 mM NaCl, at the position of peak II (not shown). In our experiments, the presence of a cAMP-dependent protein kinase activity has thus been demonstrated, not by direct cAMP stimulation of the phosphorylating activity but by showing that the cAMP-binding component was able to inhibit this activity and that this inhibition was abolished by cAMP (Table I).

The cAMP-binding protein was purified to homogeneity by affinity chromatography. Additional evidence for its role as a regulatory subunit of a cAMP-dependent protein kinase comes from its capacity to regulate the activity of the catalytic subunit of the kinase from beef heart. Similar results were obtained with partially purified cAMP binding activity from aggregating cells by Leichtling *et al.* (1981b) and we have extended these observations to differentiating cells. The use of a purified cAMP-binding component has enabled us to show that it interacts stoichiometrically with the mammalian catalytic subunit, assuming one cAMP binding site per 39-K polypeptide. There is a good correlation between the ability of cAMP analogs to compete with cAMP binding and to activate the *D. discoideum*/beef heart hybrid molecule. Half-maximal activation is obtained at 40 nM cAMP, whereas the purified regulatory subunit has a dissociation constant of 4 nM for cAMP. Such differences between the affinity for cAMP of the regulatory subunit and the concentration necessary to dissociate the complex have also been observed with the mammalian systems (Rannels and Corbin, 1981).

Regulatory subunits of mammalian type I and type II cAMP-dependent protein kinases contain two non-identical binding sites for cAMP (Corbin *et al.*, 1978), only one of which is detectable when cAMP binding is assayed by a membrane filtration method (Builder *et al.*, 1980). Furthermore, the two binding sites exhibit different specificities towards cAMP derivatives substituted on the N-6 or C-8 positions (Rannels and Corbin, 1980). We were not able to determine the stoichiometry of cAMP binding to the purified protein due to the difficulty of precisely measuring protein concentration in the very dilute purified fractions. However, heterogeneity among cAMP binding sites was not detected with the protein purified from differentiating *D. discoideum* cells, and similar binding isotherms are found when measured by membrane-filtation or equilibrium dialysis. Moreover, there were no significant differences when the binding of [^{3}H]cAMP was competed with non-radioactive cAMP, N^6-AEt-cAMP or 8-HET-cAMP.

Another difference with mammalian regulatory subunits lies in the smaller mol. wt. of the protein that we have isolated compared to the values of 47 K and 55 K respectively, for type I and type II subunits from mammalian tissues (Zoller *et al.*, 1979). The possibility that limited proteolysis is responsible for this difference must be considered. However, although regulatory subunits of mammalian protein kinases are susceptible to partial proteolytic degradation (Potter and Taylor, 1980), the proteolyzed subunits are usually incapable of regulating the activity of the catalytic subunit (Weber and Hilz, 1978). The capacity of our isolated cAMP-binding component to interact with beef heart catalytic subunit thus argues for it being in its native state.

While definitive proof of this point will depend on future experiments using antibody against the purified cAMP-binding protein, the present paper demonstrates the presence of a cAMP-dependent protein kinase in differentiating *D. discoideum* cells. Recently, Kay (1982) showed that cAMP could promote the differentiation into prespore cells in the absence of cell contacts. It is thus tempting to speculate on a specific role for this cAMP-dependent protein kinase in the prespore differentiation pathway.

Materials and methods

Chemicals and buffers

Buffer A5 was 5 mM sodium phosphate buffer pH 6.6 containing 5 mM $MgCl_2$, 2 mM Mg-EDTA, and 15 mM 2-mercaptoethanol. Buffer A25 was 25 mM sodium phosphate buffer pH 6.5 containing 5 mM $MgCl_2$ and 1 mM dithiothreitol. Buffer B was buffer A25 containing 0.01% Triton X-100. Buffer C was 10 mM Tris-HCl buffer pH 7.0 containing 5 mM $MgCl_2$, 2 mM Mg-EDTA, 50 mM NaCl, 5 mM 2-mercaptoethanol, and 0.01% Triton X-100. DEAE-Sephacel, CNBr-activated Sepharose 4B and epoxy-activated Sepharose 6B were obtained from Pharmacia (Uppsala, Sweden), Ultrogel

AcA 44 from LKB (IBF, France), and Dowex I-X8 (200–400 mesh) from Bio-Rad (Richmond, CA). Kemptide and purified catalytic subunit of cAMP-dependent protein kinase from beef heart were purchased from Sigma.

Strains, conditions for growth, and differentiation

Strain AX2 of *D. discoideum* was grown at 22°C in HL5 broth (Watts and Ashworth, 1970). Exponentially growing cells were collected when they reached a density of 4–7 x 10^6 cells/ml and washed free of nutrient medium in 20 mM potassium phosphate buffer pH 6.2. The cells were plated at a density of 4–5 x 10^6 cells/cm^2 on 50 x 60 cm trays containing 2% agar in 40 mM potassium phosphate buffer pH 6.2 supplemented with 20 mM KCl and 2.5 mM $MgCl_2$, and were allowed to differentiate for 18 h at 22°C. At this time, all of the cells had aggregated and the cellular masses were either at the finger stage or starting to culminate. They were washed twice with 20 mM potassium phosphate buffer pH 6.2 and either used immediately, or stored as a frozen pellet at −70°C.

Preparation of the crude soluble extract

Cells were resuspended at a density of 3 x 10^8 cells/ml in ice-cold buffer A5 or A25 containing 0.1 mM phenylmethane-sulfonyl fluoride (PMSF) and disrupted by three strokes of the tight-fitted pestle in a Dounce homogenizer followed by sonication for 3 x 45 s at 50 W with a Branson S 75 sonifier. This procedure resulted in disruption of all the cells, with the exception of mature spores. Large debris and spores were eliminated by a 20 min centrifugation at 10 000 *g* and the crude soluble extract was prepared by centrifugation at 140 000 *g* for 1 h. All subsequent procedures were carried out in the cold.

Purification of the cAMP-binding protein by affinity chromatography

For preparative purposes, a soluble extract was prepared from 6.4 x 10^{10} cells that had developed as described above and chromatographed on DEAE-Sephacel as described in Figure 1 except that the column buffer was A25. A minor fraction (10–20%) of the cAMP binding activity recovered from the DEAE could be inhibited by adenosine but not by cGMP. This protein is neither able to regulate protein kinase activity nor is it retained on the affinity resins. Its characteristics will be described elsewhere.

The peak containing cAMP-binding activity was pooled and further purified by affinity chromatography. Advantage was taken of the possibility to substitute the purine ring of cAMP on different positions without affecting its binding properties (see Table II, column 1); 8-(β-hydroxylethylthio)-cAMP (8-HET-cAMP) which carries a substitution on the C-8 position was synthesized according to Muneyama *et al.* (1971). The analog was linked to epoxy-activated Sepharose 6B through a long hydrophilic spacer (Weber *et al.*, 1979). Conversely, substitution of the N-6 position of cAMP by an aminoethyl group (N^6-AEt-cAMP) by the method of Dills *et al.* (1975) permitted the derivative to be coupled to CNBr-activated Sepharose 4B with a short hydrophobic linker. The pool from the DEAE column (1.9 l) was stirred with 3.5 ml of 8-HET-cAMP-Sepharose at 4°C for 24 h and poured into a glass column (1 x 4.5 cm). 60–80% of the cAMP-binding activity was retained by the resin. The column was washed successively with 17.5 ml buffer B, 17.5 ml buffer B containing 2 M NaCl, and 3.5 ml buffer B; no activity was recovered in the washes. The matrix was overlayed with 10.5 ml buffer B containing 30 mM cAMP and the flow was stopped for 24 h. After elution, excess cAMP was removed by extensive dialysis against buffer C. Under these conditions, 25–45% of the starting activity was recovered from the column; repeating this treatment did not allow any more activity to be recovered, nor did elution with 6 M urea or 6 M guanidine-HCl.

A second affinity chromatography step was performed. The material eluted from the first affinity column (10.5 ml) was stirred overnight with 0.4 ml of N^6-AET-cAMP-Sepharose at 4°C and a column was formed in an automatic pipette tip; no cAMP-binding activity was recovered in the effluent. The matrix was washed successively with 3 ml of buffer C, 6 ml of buffer C containing 2 M NaCl and 1 ml of buffer C. Elution was carried out by soaking the matrix in three bed volumes of buffer C containing 30 mM cAMP for 24 h at 4°C. The effluent was then collected and extensively dialysed to remove excess cAMP. In this step, 70% of the activity loaded onto the column was recovered. No further cAMP-binding activity was eluted by repeating this procedure or treating the column with buffer C containing 6 M urea or 6 M guanidine-HCl.

Photoaffinity labeling

Samples of purified cAMP-binding protein (5 μl) were incubated in the wells of a microtiter plate at 0°C for 30 min in the dark with 0.1 μM 8-azido-[^{3}H]cAMP (13.5 Ci/mmol; New England Nuclear), in a total volume of 25 μl. U.v. irradiation at 254 nm was carried out for 5 min at 0°C with a Mineralight UVSL-58 lamp at a distance of 6 cm. Following irradiation, the proteins were denatured by boiling 2 min in 0.1% SDS and subjected to SDS-PAGE. Fluorography was performed according to Bonner and Laskey (1974).

Assays

cAMP binding was measured using a filter assay with [5′,8-^{3}H]cAMP (43 Ci/mmol; Amersham International Ltd.) as previously described (Gunzburg and Véron, 1981).

Protein kinase activity was determined, using the synthetic peptide Leu-Arg-Arg-Ala-Ser-Leu-Gly (Kemptide) as substrate (Kemp *et al.*, 1977). The assay for *D. discoideum* protein kinase activity (50 μl) contained 50 mM MES buffer pH 6.5, 0.2 mM [γ-^{32}P]ATP (250–400 c.p.m./mol), 5 mM $MgCl_2$, 0.5 mM EGTA, 10 mM NaF, 0.2 mM Kemptide and, when indicated, 0.1 mM cAMP. The assay for catalytic subunit of beef heart protein kinase was also in 50 mM MES buffer pH 6.5 with 0.1 mM [γ-^{32}P]ATP, 0.5 mM EGTA, 10 mM NaF, 2.5 mM dithiothreitol, 20 μM Kemptide, and 0.25 pmol of catalytic subunit [in 50 mM MES buffer containing 0.5 mg/ml bovine serum albumin (BSA)]. The reaction was started by the addition of [^{32}P]ATP and Kemptide. After incubation at 30°C for 5 min, the reaction was stopped by adding 0.5 ml of 30% acetic acid. Under these conditions, the assay was linear with respect to time and enzyme concentration. The phosphopeptide was recovered by ion exchange chromatography on Dowex I-X8 (Kemp *et al.*, 1976) and the extent of phosphorylation measured by liquid scintillation counting.

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