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The Cyclic Nucleotide Specificity of Eight cAMP-binding Proteins in *Dictyostelium discoideum* Is Correlated into Three Groups*

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cAMP is a mediator of inter- and intracellular events in *Dictyostelium discoideum* and is thought to act through specific receptors. Eight forms of cAMP-binding proteins have been described in this organism: four forms of a cell surface receptor, a cell surface and extracellular phosphodiesterase, an intracellular cAMP-dependent protein kinase (CAK), and a recently identified cAMP-binding protein (CABP1) that is present on the cell surface, in the cytoplasm, and in the nucleus. In this study we have analyzed the cyclic nucleotide specificity of these cAMP-binding proteins using 13 derivatives of cAMP with modifications in the adenine, ribose, and phosphate moiety.

The results suggest that the cAMP-binding proteins belong to three groups: (i) four forms of the cell surface receptor, (ii) two forms of an intracellular receptor (CABP1 and CAK), and (iii) cell surface and extracellular phosphodiesterase. cAMP is probably bound to the surface receptors in the *anti* conformation in a hydrophobic cleft of the receptor with essential interactions at N^6H_2 and $O^{3'}$. In contrast, cAMP is probably bound to CAK and CABP1 in the *syn* conformation with essential interactions at $O^{2'}$, $O^{3'}$, $O^{5'}$, and exocyclic oxygen. Finally, binding of cAMP to phosphodiesterase involves only $O^{3'}$ and exocyclic oxygen.

The cyclic nucleotide specificity of cAMP-induced processes in *D. discoideum* indicates that the cell surface receptors participate in the transduction of the cAMP signal during chemotaxis and cell differentiation. Functions for CABP1 and CAK in these processes are presently elusive.

Vegetative amoebae of *Dictyostelium discoideum* feed on bacteria. When deprived of their food source the amoebae enter a process of development and differentiation. cAMP is known to play an important role in this process and may act both as first and second messenger. After starvation, some cells start to secrete pulses of cAMP and surrounding cells detect this cAMP by means of cell surface receptors. These cells respond by chemotaxis to the cAMP source and eventually start to secrete cAMP themselves. The cells aggregate and form multicellular structures which are called slugs. Cells in these slugs differentiate into two types, stalk and spore cells. Extracellular cAMP acts as a morphogen in this stage of the life cycle, inducing cell type-specific gene expression (1–5).

Extracellular cAMP binds to cell surface receptors, which have been investigated extensively (6–8). Kinetic studies revealed four forms (A^H, A^L, B^S, and B^{SS}), which differ in their respective dissociation constants and dissociation rates (7, 9). The addition of cAMP to sensitive cells leads to the activation of adenylate cyclase and the formation of intracellular cAMP. Besides the cell surface receptor, which detects cAMP as first messenger, other cAMP-binding proteins have been described that could detect cAMP as the second messenger: an intracellularly localized cAMP-dependent protein kinase (CAK)[1] (10) and a recently identified cAMP-binding protein (CABP1) (11, 12) that is present on the cell surface, in the cytoplasm, and in the nucleus. Finally, *D. discoideum* cells contain a cyclic nucleotide phosphodiesterase that is present on the cell surface (sPDE) and in the extracellular medium (ePDE) (13, 14).

In what way extracellular cAMP mediates intracellular responses is not fully understood. One approach has been the determination of the cyclic nucleotide specificity of cAMP-binding proteins and the specificity of cAMP-induced responses (15, 16). However, the detection of four forms of cAMP binding to intact cells (7) and the detection of CABP1 on the cell surface (12) complicate conclusions on how the cAMP signal is transduced.

Therefore, we investigated the cyclic nucleotide binding specificity of CABP1, extracellular PDE, and the four binding forms of the cell surface receptor and compared them with each other and with published data on CAK and sPDE. We conclude that cyclic nucleotide specificity of the eight cAMP-binding proteins are related in three groups: (i) the four forms of the cell surface receptor have essentially identical binding specificity. (ii) The binding specificity of CABP1 and CAK are similar to each other but very dissimilar to that of the cell surface receptor. (iii) The binding specificity of ePDE and sPDE are essentially identical but not related to any of the other proteins.

EXPERIMENTAL PROCEDURES

Materials—[8-^{3}H]cAMP (1.92 TBq/mmol) was obtained from Amersham Corp.; glycerol was from Merck; dithiothreitol (DTT) and snake venom (*Ophiophaga hannah*) were obtained from Sigma; Dowex AG 1-X2 was from Serva. DE52 cellulose was obtained from Whatman; nitrocellulose membrane filters (BA85) were from Schleicher & Schüll. cAMP derivatives 1, 3, 5, 6, 13, and 14 were obtained from Boehringer Mannheim; compound 4 was a generous

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[1] The abbreviations used are: CAK, intracellular cAMP-dependent protein kinase; sPDE, surface cyclic nucleotide phosphodiesterase; ePDE, extracellular cyclic nucleotide phosphodiesterase; CABP1, intracellular cAMP-binding protein-1; DTT, dithiothreitol; (S_p)-cAMPS and (R_p)-cAMPS, S_p and R_p isomers of adenosine 3′:5′-monophosphorothioate.

gift of Dr. R. Hanze (The Upjohn Co.); compounds 2, 7, 8, 11, and 12 were kindly supplied by Dr. B. Jastorff (University of Bremen, Federal Republic of Germany); the synthesis of these derivatives has been described previously (17–21).

Conditions for Growth and Development—*D. discoideum* strain NC-4 was grown in association with *Escherichia coli* 281 on a solid medium containing 3.3 g of peptone, 3.3 g of glucose, 4.5 g of KH_2PO_4, 1.5 g of $Na_2HPO_4 \cdot 2H_2O$, and 15 g of agar/liter. Cells were harvested with 10 mM sodium-potassium phosphate buffer, pH 6.5 (PB), when the lawn of bacteria was just starting to clear and washed free from bacteria by repeated centrifugation at 100 g. For the cAMP cell surface receptor-binding studies, the cells were starved in suspension for 5 h, at a cell density of 10^7/ml, washed in PB buffer, and resuspended in this buffer at a density of 10^8 cells/ml.

To obtain slugs for the isolation of CABP1, vegetative cells were distributed on non-nutrient agar (1.5% agar in PB) at approximately 3×10^6 cells/cm^2. The agar plates were placed at 22 °C and cells were allowed to develop for 16–18 h.

Isolation of CABP1—The CABP1 of *D. discoideum* was isolated from slugs as described by Tsang and Tasaka (11) with a few alterations. The slugs were harvested, washed once in 20 mM PB buffer, pH 6.5, and dissociated into single cells by a 15-min treatment with cellulase (5 mg/ml) and 2 mM EDTA in 20 mM PB buffer at 22 °C (22). All subsequent steps were performed at 4 °C. Cells were washed twice in 50 mM Tris-HCl, pH 7.2, which contained 2 mM DTT, 0.1 mM phenylmethylsulfonyl fluoride, 150 μg/ml benzamidine, 50 μg/ml leupeptin, and 10% glycerol (buffer A). The cells were disrupted by sonication with a Branson Sonifier five times for 5 s at 100 watts with a 20-s cooling interval. Unbroken cells and membranes were removed by centrifugation for 10 min at 10,000 × *g*. The supernatant was centrifuged again in a Beckman Airfuge at 100,000 × *g* for 5 min. The supernatant derived from 5×10^9 cells was applied immediately to a DEAE-cellulose column (1.2 × 10 cm), which was eluted with buffer A at a flow rate of 15 ml·h^{-1}. The first peak of cAMP binding activity (see Fig. 2) was pooled and used to test the binding specificity of cAMP derivatives to CABP1.

cAMP Binding Assay for CABP1—The binding assay contained 50 mM sodium acetate, pH 5.0, 10 mM DTT, 5% glycerol, 20 nM [^{3}H]cAMP, 1 μM 5′-AMP, approximately 50 μg of cellular protein, and varying concentrations of cAMP or derivatives in a final volume of 50 μl. Samples were incubated at 0 °C for 15 min and filtered through a Millipore membrane filter (nitrocellulose, pore size 0.45 μm). After rinsing the filters three times with 3 ml of ice-cold Na^+ acetate buffer (50 mM, pH 5.0) the filters were dropped into 3 ml of scintillation fluid (emulsifier scintillator 299, Packard United Technologies) and radioactivity was determined. Nonspecific binding was measured by adding 0.1 mM cAMP to the incubation mixture.

cAMP Binding Assay for cAMP Cell Surface Receptors—The binding of cAMP to cells was performed according to the method as described by Van Haastert (23). In short, 8×10^6 cells, PB buffer, 10 mM DTT, 2 or 100 nM [^{3}H]cAMP and different concentrations of cAMP or derivatives were incubated in a total volume of 100 μl. After an incubation of 45 s at 22 °C the binding reaction was terminated either by direct centrifugation of the mixture through silicone oil or by centrifugation at 10 s or 2 min after the addition of 1 ml of 0.1 mM cAMP in PB buffer. Nonspecific binding was measured by adding 0.1 mM cAMP to the incubation mixture.

Assay for Extracellular Phosphodiesterase—Phosphodiesterase activity was determined at 25 °C in a total volume of 100 μl containing 1 μM [^{3}H]cAMP, 10 mM PB, pH 7.25, 8 ng of extracellular PDE, and varying concentrations of cAMP derivatives. Extracellular PDE was prepared as described by Van Haastert and Van der Heijden (24). The incubation was terminated after 15 min by boiling the samples for 2 min. After cooling, 50 μg of snake venom (*O. hannah*) was added. Nonhydrolyzed cAMP was removed after 30 min by the addition of 0.5 ml of anion exchanger (1 part AG 1-X2 and 2 parts H_2O). Samples were shaken for 2 min, centrifuged, and the radioactivity of the supernatant was determined. Nonspecific binding was determined by boiling ePDE prior to the start of the incubation.

Protein Determination—Protein concentration was determined according to the method of Bradford (25).

RESULTS AND DISCUSSION

Selection of cAMP Derivatives—cAMP can form several interactions with its surrounding medium (water or receptors), such as hydrogen bonds, ionic bonds, and hydrophobic interactions. A set of cAMP derivatives was selected (Fig. 1 and Table I) which may reveal the importance of these interactions for binding to receptor proteins. Hydrogen bonding is prevented at the modified atoms or atom groups in derivatives 2, 3, 4, 6, 7, and 8. In derivatives 9 and 10 one of the exocyclic oxygen atoms is replaced by a sulfur atom (axial or equatorial), which fixates the negative charge on sulfur (26); these derivatives may reveal ionic and/or stereospecific interactions at phosphorus between cAMP and its receptor. The cAMP molecule has two favorable conformations, *syn* and *anti*; the distribution of 1:1 in cAMP is changed to 95% *syn* conformation in derivative 5 (27, 28). The differences in polarity of the derivatives (14) (see Table II) may reveal hydrophobic interactions such as π-electron stacking by dipole-induced dipole interactions between the adenine moiety of the cAMP molecule and an aromatic group of a receptor. Finally, derivatives 1, 11, 12, 13, and 14 have the following sequence in increasing polarizability: 13, 14, 12, 1, 11, and increasing polarizing power: 11, 12, 1, 13, 14 (29).

In order to compare the present results with previous experiments and other systems, the following standardization has been applied (30).

$$\delta\Delta G = RT \ln K_{0.5}\ \text{derivative}/K_{0.5}\text{cAMP}$$

$K_{0.5}$ is defined as the concentration of derivative that results in a 50% inhibition of binding of [^{3}H]cAMP. $\delta\Delta G$ values represent the reduction in binding of a cAMP derivative compared to cAMP (in kJ/mol).

Specificity of CABP1—Fig. 2 represents a typical isolation of CABP1 and the regulatory subunit of CAK from developing *D. discoideum* cells on an ion exchanger column. The peak of cAMP binding activity in the flow-through was pooled and used as source for CABP1 (11). The second peak of cAMP binding activity eluted from the column with a NaCl gradient (0–0.8 M); this peak was described by Tsang and Tasaka as CABP2 (11); it has the same chromatographic properties and cyclic nucleotide specificity as CAK (see below).

The cyclic nucleotide specificity of CABP1 was investigated using several preparations of CABP1 which were isolated

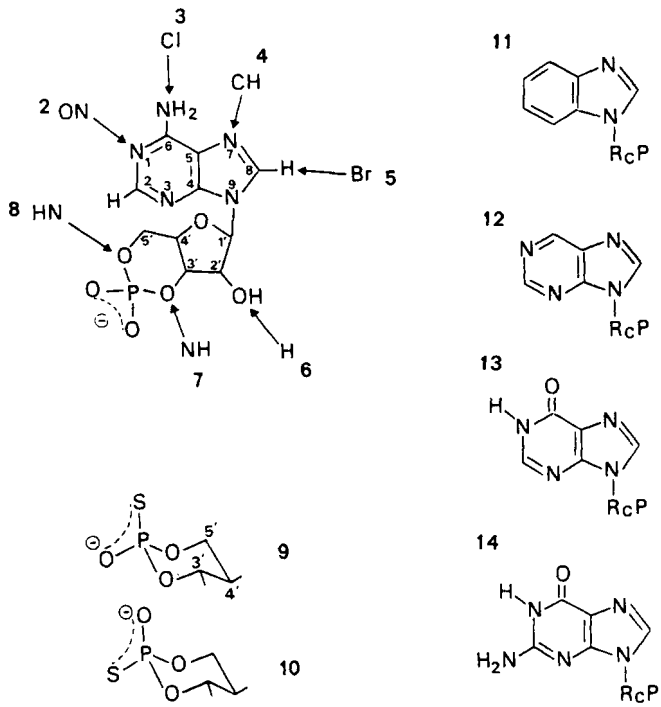

FIG. 1. **Structures of the cAMP derivatives.**

TABLE I

List of cAMP derivatives

Cyclic nucleotide derivatives used in this study

No.	Name	Abbreviation
1.	Adenosine 3′:5′-monophosphate	cAMP
2.	Adenosine-N^1-oxide 3′:5′-monophosphate	N^1-*O*-cAMP
3.	6-Chloropurineriboside 3′:5′-monophosphate	6-Cl-PuRMP
4.	7-Deazaadenosine 3′:5′-monophosphate	7-CH-cAMP
5.	8-Bromoadenosine 3′:5′-monophosphate	8-Br-cAMP
6.	2′-Deoxyadenosine 3′:5′-monophosphate	2′-H-cAMP
7.	3′-Deoxy-3′-aminoadenosine 3′:5′-monophosphate	3′-NH-cAMP
8.	5′-Deoxy-5′-aminoadenosine 3′:5′-monophosphate	5′-NH-cAMP
9.	Adenosine 3′:5′-monophosphorothioate, S_p isomer	(S_p)-cAMPS
10.	Adenosine 3′:5′-monophosphorothioate, R_p isomer	(R_p)-cAMPS
11.	Benzimidazoleriboside 3′:5′-monophosphate	cBIMP
12.	Purineriboside 3′:5′-monophosphate	PuRMP
13.	Inosine 3′:5′-monophosphate	cIMP
14.	Guanosine 3′:5′-monophosphate	cGMP

TABLE II

Specificity of eight cAMP-binding proteins in D. discoideum

$\delta\Delta G$ values of CAK were retrieved from Ref. 32; values for total binding were measured in ammonium sulfate and came from Ref. 15; $\delta\Delta G$ values for the cell surface phosphodiesterase were derived from Ref. 14. The $\delta\Delta G$ values for the cell surface receptors, CABP1 and extracellular phosphodiesterase were determined as described under "Experimental Procedures."

Derivative[a]	$\delta\Delta G$									α[c]
	Cell surface receptors					Intracellular binding proteins		Phosphodiesterases		
	Total[b]	A^H	A^L	B^S	B^{SS}	CABP1	CAK	ePDE	sPDE	
	kJ/mol									
1. cAMP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2. N^1-*O*-cAMP	9.1	8.5	11.0	9.9	10.4	3.9	4.7	3.2	3.9	−4.49
3. 6-Cl-PuRMP	14.6	17.8	17.5	16.4	16.9	−0.2	1.8	2.4	2.6	2.06
4. 7-CH-cAMP	13.3	12.4	10.7	13.5	15.8	−0.72	0.7	0.0	−0.6	0.36
5. 8-Br-cAMP	15.1	17.5	16.1	15.5	17.8	−0.5	−2.6	5.4	5.1	1.98
6. 2′-H-cAMP	5.6	6.2	6.5	5.6	5.9	19.0	22.0	4.4	4.3	−0.46
7. 3′-NH-cAMP	15.2	15.2	13.5	15.0	17.5	17.1	13.0	≥16.0	≥22.0	−0.02
8. 5′-NH-cAMP	4.5	3.4	3.7	4.0	4.5	16.9	17.5	−6.0	−7.5	−1.65
9. (S_p)-cAMPS	10.7	11.9	11.3	13.3	12.1	6.9	4.5	10.2	15.3	1.63
10. (R_p)-cAMPS	10.8	14.6	13.3	14.9	15.3	17.0	12.0	≥16.0	≥22.0	0.66
11. cBIMP	11.5	14.6	13.8	15.8	13.5	7.2	6.0	8.3	5.7	2.75
12. PuRMP	16.0	–[d]	–	–	–	2.2	3.9	8.3	3.0	−0.46
13. cIMP	21.7	–	–	–	–	3.4	3.9	4.5	7.0	−3.41
14. cGMP	22.7	–	–	–	–	12.3	13.9	4.4	2.2	−3.21

[a] See Table I.
[b] Binding of A^H, A^L, B^S, and B^{SS}.
[c] α, polarity.
[d] –, not determined.

from slugs or aggregation-competent cells and purified by DEAE-cellulose chromatography as described above or a subsequent purification step by an affinity column with Affi-Gel Blue. The affinity of derivatives 1, 3, 5, 6, 13, and 14 was essentially the same for all preparations of CABP1 (data not shown).

The inhibition of binding of 20 nM [^{3}H]cAMP by cAMP and several derivatives to CABP1 is shown in Fig. 3. The $K_{0.5}$ values were derived and the $\delta\Delta G$ values were calculated (Table II). The derivatives with alterations in the base moiety do not show a strong reduction in binding affinity. This suggests that no hydrogen bonds are formed between the base moiety of the cAMP molecule and CABP1. The polarizing power of several derivatives increases according to the following sequence: 14 > 13 > 1 > 12 > 11; the polarizability increases according to 11 > 1 > 12 > 14 > 13 (29). The sequence of binding affinity is different from both: 1 > 12 = 13 > 11 > 14. In addition, no correlation was found between the polarity of adenine-substituted cAMP derivatives (14) and their binding affinities (correlation coefficient: −0.13). These results suggest that no hydrophobic interaction exists between the adenine molecule and CABP1.

Derivative 5 exists mainly in the *syn* conformation and has approximately the same binding affinity for CABP1 as cAMP itself, suggesting that binding of cAMP to CABP1 takes place preferably in the *syn* conformation.

The ribose moiety of the cAMP molecule appears to be bound to CABP1 by at least three hydrogen bonds. The $\delta\Delta G$ values are, respectively, 19.0, 17.2, and 17.2 kJ/mol when no hydrogen bonds with oxygen at the 2′-, 3′-, or 5′-position can be formed (derivatives 6, 7, and 8). The different $\delta\Delta G$ values of derivatives (S_p)-cAMPS and (R_p)-cAMPS (derivatives 9 and 10) point to a stereoselective recognition of the exocyclic oxygen atoms and may identify the need for a salt bridge between cAMP and CABP1. The negative charge is either fixed on the equatorial or axial exocyclic sulfur atom (26). Compound 10 (charge is fixed on the equatorial sulfur atom) has a high $\delta\Delta G$ value, which may indicate that a salt bridge is preferentially formed with the axial exocyclic oxygen atom.

Summarizing these binding data we propose that cAMP is

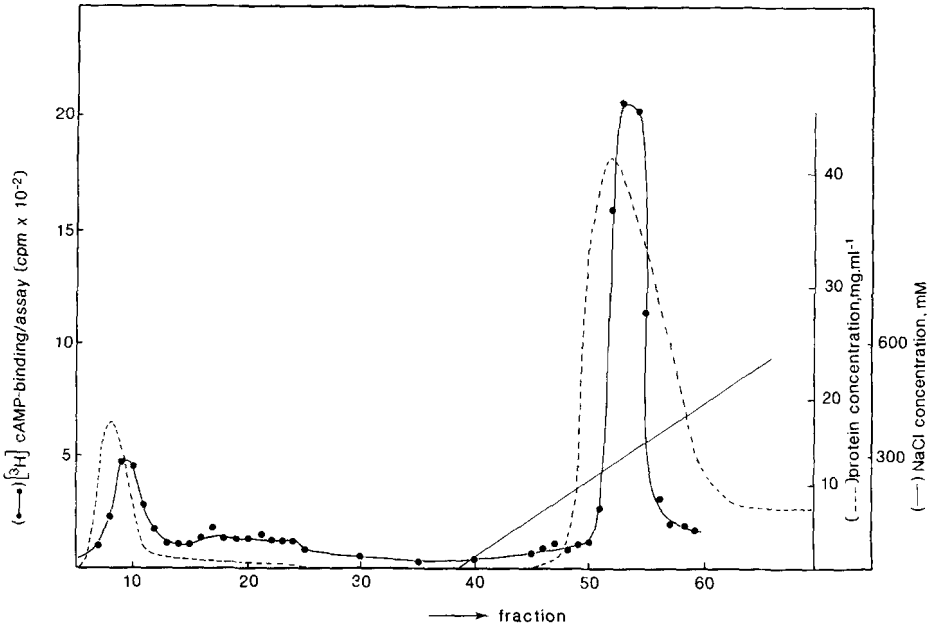

FIG. 2. **Elution profile of cAMP binding activity from a DEAE-cellulose column.** Crude soluble extract prepared from approximately 5×10^9 *D. discoideum* cells was applied to a DEAE-cellulose column (1.2 × 10 cm). The column was washed with Buffer A at a flow rate of 15 ml·h^{-1}, and the bound proteins were eluted with a linear gradient (0–0.8 M NaCl in Buffer A). Fractions of 0.9 ml were collected. ●, cAMP binding; – – –, protein concentration; —, NaCl concentration.

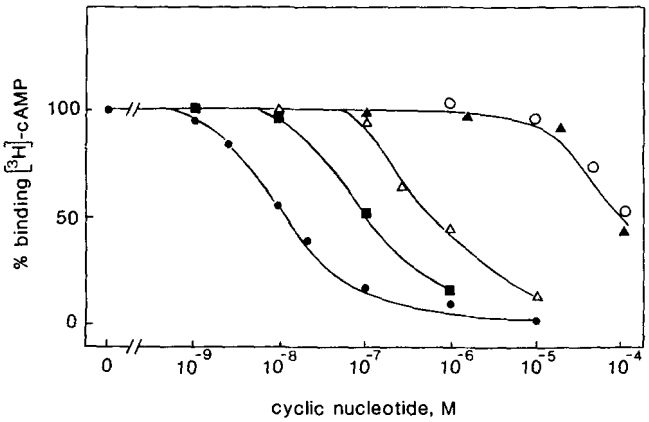

FIG. 3. **Inhibition of the binding of [^{3}H]cAMP by cAMP and four cAMP derivatives by CABP1 of *D. discoideum*.** The binding of 20 nM [^{3}H]cAMP in the absence of cAMP or cAMP derivatives was set at 100%. ●, cAMP; △, compound *9*; ▲, compound *10*; ○, compound *6*; ■, compound *12*.

bound to CABP1 in a very specific way. Hydrogen bonds are formed at the $O^{2'}$-, $O^{3'}$-, and $O^{5'}$-positions of the base moiety, the molecule preferentially binds in a *syn* conformation, and a salt bridge with the phosphate group is necessary.

The Specificity of the Four Forms of the Cell Surface Receptor—Previously, it was demonstrated that *D. discoideum* cells may contain multiple forms of the cell surface receptor, designated A^H, A^L, B^S, and B^{SS}, which have different affinities and rate constants of dissociation (7, 9). The specificity of these four forms (A^H, A^L, B^S, and B^{SS}) was investigated in this study according to the method described by Van Haastert (31).

Table II shows the $\delta\Delta G$ values of 14 cAMP derivatives for the four surface cAMP binding forms. No apparent differences of $\delta\Delta G$ values are noticeable when the four binding forms are compared. In a previous report binding specificity for the cell surface receptor was investigated in the ammonium sulfate stabilizing assay (15). For all derivatives the $\delta\Delta G$ values in both the ammonium sulfate assay and the present assays are approximately the same, with the exception of the antagonist (R_p)-cAMPS, which has higher affinity in ammonium sulfate than in phosphate buffer. We reach the conclusion that the four forms of the cell surface receptor bind cAMP by the same molecular interactions. cAMP is bound to the cell surface receptor via hydrogen bonds at N^6H_2 and $O^{3'}$ and the adenine moiety is bound in a hydrophobic cleft of the receptor. cAMP preferably binds in the *anti* conformation, and a stereospecific interaction between the receptor and the phosphate moiety of cAMP is not necessary for binding.

The Specificity of CAK, ePDE, and sPDE—The specificity of the binding of cAMP to the regulatory subunit of CAK has been described by De Wit *et al.* (32). The chromatographic properties and cyclic nucleotide specificity of CAK is very similar to that of the second peak of cAMP binding activity eluted from the DEAE-cellulose column (data not shown), suggesting that this CABP2 and CAK are identical.

In addition to the cAMP-binding proteins described above, *D. discoideum* cells also contain a cell surface and extracellular localized cAMP-hydrolyzing phosphodiesterase (sPDE and ePDE, respectively). The specificity of the enzyme on the cell surface has been described (14); the apparent K_m values of the derivatives were transformed to $\delta\Delta G$ values for a comparison with the other cAMP-binding proteins (Table II). The specificity of extracellular PDE was determined in the present study (Table II). The results show that the cyclic nucleotide specificity of sPDE and ePDE are nearly identical; for nearly all derivatives the $\delta\Delta G$ values for sPDE and ePDE are alike. Only derivative 9, (S_p)-cAMPS, seems to have a slightly higher affinity for ePDE than for sPDE. Derivatives 11, 13, and 14 have somewhat higher $\delta\Delta G$ values (lower affinity) for ePDE, when compared with the $\delta\Delta G$ values of sPDE. Both phosphodiesterases preferably bind to the cAMP molecule through a hydrogen bond at the $O^{3'}$ atom of the ribose moiety of cAMP and form a salt bridge with an exocyclic oxygen atom.

Comparison of the Different cAMP Receptors—The cyclic nucleotide specificities and affinities of all eight cAMP-binding proteins are compared in Tables III and IV, respectively. Apparently, the binding proteins form three groups: 1) four forms of the surface receptor, 2) CABP1 and CAK, and 3) sPDE and ePDE.

The four forms of the surface cAMP receptor that have been detected by kinetic methods (32) have essentially identical cyclic nucleotide specificity. Apparently, the molecular

TABLE III

Correlation matrix of the $\delta\Delta G$ values of the different derivatives tested on cAMP-binding proteins in D. discoideum

	A^H	A^L	B^S	B^{SS}	CABP1	CAK	sPDE	ePDE
A^H	1.0	0.98	0.94	0.96	−0.15	−0.30	0.37	0.22
A^L		1.0	0.94	0.94	−0.18	−0.30	0.45	0.53
B^S			1.0	0.95	−0.14	−0.30	0.48	0.57
B^{SS}				1.0	−0.14	−0.30	0.37	0.44
CABP1					1.0	0.95	0.34	−0.16
CAK						1.0	−0.26	−0.22
sPDE							1.0	0.88
ePDE								1.0

TABLE IV

Kinetics of cAMP-binding proteins

The K_D and k_{-1} values of A^H, A^L, B^S, and B^{SS} were retrieved from Ref. 9 and data for CAK are from Ref. 32. The K_D and k_{-1} values of CABP1 were determined as described for CAK.

Kinetics (cAMP)	Cell surface receptor				CABP1	CAK
	A^H	A^L	B^S	B^{SS}		
Apparent K_D (nM)	60	450	15	15	3.1	2.2
k_{-1} (s^{-1})	0.7	1.0	0.05	0.005	0.021	0.001

interactions between cAMP and the receptor protein are the same in the four binding forms (Table III), and are thus not the cause of the 30-fold difference in affinity or 150-fold difference in the dissociation rate between B^{SS} and A^L (Table IV). The structural differences between the four forms of the surface cAMP receptor are still largely unknown. Different gene products or posttranslational modification could be the fundamental difference. Alternatively, the embedding of the same protein in a different environment could be the reason for the different kinetic properties. It has been proposed that the four forms represent different states of interaction with G-proteins (9) or the cytoskeleton (33, 34) and are involved in the transduction of the cAMP signal to different effectors. In support of this hypothesis is the observation that a mutant (*fgd* A) which lacks a G_α subunit still possesses A^H, A^L, and B^S but lacks B^{SS} (35). Mutants in the cAMP receptor gene(s) should help to elucidate the divergence of transmembrane signal transduction at the cAMP receptor.

The high correlation ($r = 0.95$) between the cyclic nucleotide specificity of the regulatory subunit of CAK and that of CABP1 points to a structural relationship between these two cAMP-binding proteins in the parts of the protein that bind cAMP. The 20-fold difference which exists between the dissociation rates of CABP1 and CAK (see Table IV) does not favor the idea that these proteins might be the same. It is unlikely that CABP1 is a degradation product of CAK, because it has been described that these proteins are immunologically distinct (11). The exact relationship between CABP1 and CAK awaits the elucidation of the primary sequence of CABP1 and the comparison with that of CAK (36).

No correlation was found between the specificity of the cell surface receptors and the specificity of the other proteins, CAK, CABP1, sPDE, and ePDE. Although CABP1 has been detected on the surface by immunological methods, it seems unlikely that CABP1 is one of the surface receptors that binds extracellular cAMP. Possibly, the cAMP binding site of CABP1 is not directed to the extracellular but to the cytoplasmatic face of the plasma membrane.

Finally, the cyclic nucleotide specificity of sPDE correlates very well with that of ePDE (correlation coefficient: 0.88), but is not closely correlated with any of the other cAMP-binding proteins (see Table III). The kinetic properties of these enzymes are similar (37) and the functional relationship between sPDE and ePDE was studied with a mutant defective in PDE production showing that both sPDE and ePDE were reduced (38).

Relationship with cAMP-induced Responses and Differentiation—Extracellular cAMP induces several fast responses in *D. discoideum* including the activation of adenylate and guanylate cyclase and chemotaxis. The cyclic nucleotide specificities of these responses are very similar to those of the cell surface receptors (15, 16, 23) but are different from the specificity of the regulatory subunit of CAK, PDE, and CABP1, suggesting that these processes are mediated by the cAMP cell surface receptor.

Submicromolar concentrations of extracellular cAMP also induces postaggregative gene expression (39). The intracellular cAMP-binding proteins CABP1 and CAK are both developmentally regulated. CABP1 has its maximal appearance during the aggregation and culmination stage (11, 12) and CAK has maximal activity during the slug and culmination stage (40, 41). During development both CABP1 and CAK seem to be translocated from the periphery to the nucleus of the cell (12). These characteristics make CABP1 and CAK likely candidates for the targets of cAMP-induced gene expression. However, the cyclic nucleotide specificity for the induction of specific markers of postaggregative differentiation does not match the specificity of CABP1 or CAK (5, 42–44); cell treatment with a derivative that has high affinity for CAK and CABP1 (8-Br-cAMP) did not result in prespore gene expression, whereas treatment of cells with a derivative that has high specificity for cell surface receptors (2′-deoxy-cAMP) induced prespore-specific genes.

The present and previous results suggest that CABP1 and CAK are not the primary targets of cAMP for the induction of prespore gene expression. It should be stressed, however, that the present specificity data cannot exclude the involvement of these proteins in differentiation. First, the specificity for gene expression has been investigated with only a few derivatives. Second, the specificity for prestalk gene expression is different from the specificity for prespore gene expression and more similar (but not identical) to that of CABP1 or CAK (45). Finally, no experiments have been reported that probe the effect on gene expression after specific activation or inactivation of CAK or CABP1, without activation of the cAMP cell surface receptor.

CONCLUSIONS

A comparison of the cyclic nucleotide specificity of eight cAMP-binding proteins in *D. discoideum* suggests that they belong to three groups: four forms of the cell surface receptor, two forms of an intracellular receptor (CABP1 and CAK), and two forms of phosphodiesterase. The cyclic nucleotide specificity of cAMP-induced processes implies that the cell surface receptors participate in the transduction of the cAMP signal during chemotaxis and cell differentiation. Functions for CABP1 and CAK in these processes are presently elusive but have not been excluded. To target the functions of these proteins during chemotaxis, cell aggregation, and differentiation, apparently cAMP derivatives are required with higher specificity, to discriminate between the cAMP-binding proteins. cAMP derivatives which are hydrolysis-resistant, cell-permeable, and with a very high selectivity for CABP1 and CAK *versus* the surface receptor may be useful.

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