Dictyostelium discoideum cell membranes contain masked chemotactic receptors for cyclic AMP

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Aggregating *Dictyostelium discoideum* cells possess highly specific receptors for the chemoattractant cAMP on their cell surface. Isolated membranes as well as intact cells are shown to contain a large number of latent cAMP receptors. These are reversibly unmasked in the presence of a high salt concentration (0.1–2 M) or in the presence of millimolar concentrations of Ca^{2+}.

Slime mould Dictyostelium discoideum *cAMP receptor* *Chemotactic receptor*

1. INTRODUCTION

Food deprivation induces solitary *Dictyostelium discoideum* amoeba to differentiate, resulting in the formation of a multicellular organism. Cell aggregation is mediated by the chemoattractant cAMP which is detected by specific receptors on the cell surface [1]. Binding of cAMP to these receptors induces a chemotactic response and activation of a signal-relay mechanism, transmitting the aggregation signal (cAMP) to neighbouring cells [1]. Here, we show that the number of accessible cAMP receptors on isolated cell membranes and intact cells can be increased 2- to 3-fold in the presence of various salts or Ca^{2+}. The effect of Ca^{2+} on cAMP binding to intact cells has been described earlier [2].

2. MATERIALS AND METHODS

2.1. *Materials*

[5′,8-^{3}H]cAMP was purchased from Amersham International, cAMP from Serva (Heidelberg), dithiothreitol from Calbiochem-Behring (San Deigo, CA), 5′ AMP from Boehringer (Mannheim) and cGMP, cIMP and 2′-deoxy-cAMP from Sigma (St. Louis, MO). Polycarbonate filters (0.2 μm pore size) were obtained from Nuclepore Corp. (Pleasanton, CA).

2.2. *Cells and membrane preparations*

D. discoideum cells, strain AX2, were grown and developed by 6-h starvation (aggregation-competent cells) as described previously [3]. Cells were homogenised by nitrogen cavitation [4]; subsequently, a membrane fraction, which was enriched in cAMP receptors, was isolated by flotation through 30–55% (w/v) sucrose gradients in 40 mM Hepes/NaOH (pH 7.7). Gradients were centrifuged at 100 000 × g for 15 h at 4°C. Fractions containing cAMP-binding activity (banding at a density of 1.6 g/ml) were collected and stored in liquid nitrogen. Details of the isolation procedure will be published elsewhere. Protein was determined according to the method of [5], as modified in [6].

2.3. *Cyclic AMP-binding assays*

Cyclic AMP-binding was measured at 0°C in 20 mM Hepes/NaOH (pH 7.0), 10 mM dithiothreitol, 20 μM 5′ AMP (cAMP-binding medium), containing [^{3}H]cAMP at concentrations given in the text. If indicated, $(NH_4)_2SO_4$ up to 2.4 M, $CaCl_2$ (5 mM) or other salts were present. The binding

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reaction was initiated by the addition of membranes (~20 μg protein, 75–200 fmol cAMP-binding sites) or 4×10^6 aggregation-competent cells. Membranes or cells were equilibrated with cAMP for 5 min, which was sufficient to reach equilibrium, both in the presence and absence of 2 M ammonium sulphate.

Separation of cAMP bound to membranes and free cAMP was achieved by filtration through 0.2 μm pore-size polycarbonate filters, or by centrifugation for 5 min at 180000 × g in an air-driven micro-ultracentrifuge (Beckman, Geneva). In a number of experiments, centrifugation for 2 min at 10000 × g in a microcentrifuge (Eppendorf Gerätebau, Hamburg) was used. All methods gave essentially the same results. Non-specific binding of [^{3}H]cAMP was estimated in the presence of 25 μM unlabelled cAMP. cAMP bound to intact cells was measured by centrifugation for 1 min at 10000 × g.

3. RESULTS AND DISCUSSION

3.1. *Ammonium sulphate and other salts increase the binding of cAMP to Dictyostelium membranes*

Ammonium sulphate precipitation is employed to facilitate the separation of bound and free ligand in hormone-receptor assays [7]. We (and others [8]) have used this method for measuring the binding of cAMP to cell surface chemotactic receptors on intact *Dictyostelium* cells and in isolated membranes. We found that ammonium sulphate induced a dramatic increase in cAMP-binding to membranes (fig.1a). The effect of ammonium sulphate was biphasic, showing a steep increase in cAMP-binding up to 100 mM followed by a smaller rate of increase at higher concentrations. Under the prevailing conditions (10 nM cAMP), 20 mM of the salt already resulted in a more than 2-fold increase in binding of cAMP. The same result was obtained if bound and free cAMP were separated by filtration through a 0.2 μm pore size polycarbonate filter or by centrifugation for 5 min at 180000 × g in an air-driven micro-ultracentrifuge.

A number of salts other than ammonium sulphate induced a comparable increase in cAMP binding to *Dictyostelium* membranes (table 1). Some ions, particularly nitrate and thiocyanate, at high concentrations strongly reduce binding, probably due to their chaotropic properties. Inspection of table 1 does not yield evidence for specific effects of certain ions, which would suggest that ionic strength is the important parameter.

Cyclic AMP binding to intact *Dictyostelium* cells displayed a similar salt-induced increase as found for membranes (fig.1b, table 1). Authors in [2] have shown that millimolar Ca^{2+} concentrations

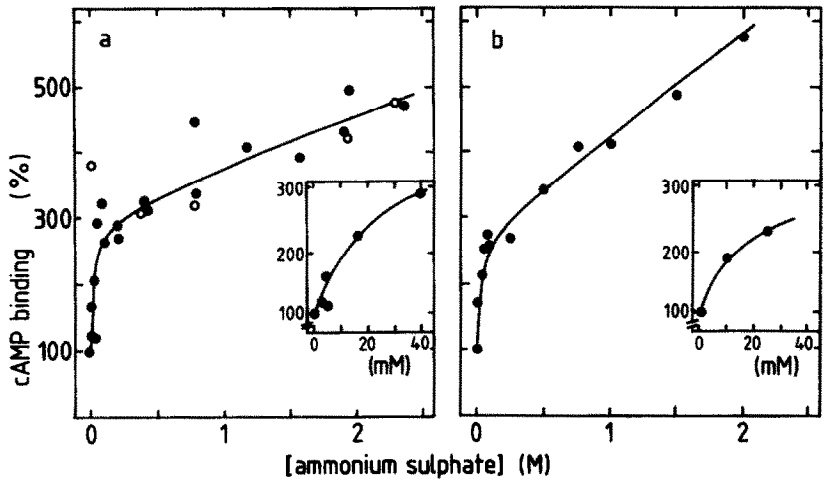

Fig.1. Effect of ammonium sulphate and Ca^{2+} on the binding of [^{3}H]cAMP (10 nM) to membranes (a) and intact cells (b). Binding in the absence of ammonium sulphate or Ca^{2+} was taken as 100%. cAMP binding in the absence (●) and presence (○) of 5 mM $CaCl_2$ is displayed.

Table 1

Effect of various salts on the binding of [^{3}H]cAMP (10 nM) to isolated membranes and intact cells

Addition	Relative amount of cAMP bound (%)[a]					
	Salt concentration (M)					
	Membranes			Intact cells		
	0.10	0.25	1.0	0.10	0.25	1.0
$(NH_4)_2SO_4$	260	302	375	259	298	413
NH_4 acetate	141	–	104	208	–	90
NH_4NO_3	104	–	9	154	–	25
NH_4CNS	26	–	0	–	–	–
NH_4Cl	145	–	100	185	–	86
KCl	144	–	98	–	–	–
K_2SO_4	223	273	–	248	245	–
Na_2SO_4	219	282	–	226	246	–
$MgSO_4$	237	–	331	–	–	–
KH_2PO_4/K_2HPO_4	235	–	99	198	–	188
Na_2 succinate	188	239	–	292	–	491
Na_3 citrate	234	–	55	259	–	413

[a] 100% binding is measured in 20 mM Hepes/NaOH (pH 7.0) in the presence of 10 mM dithiothreitol and 20 μM 5′ AMP. Bound and free cAMP were separated by centrifugation

enhance the binding of cAMP to intact cells about 4-fold. We found the same for isolated membranes (fig.1a). The effects of Ca^{2+} (5 mM) and $(NH_4)_2SO_4$ (2 M) are quantitatively similar and are not additive, suggesting that they affect cAMP receptors via a similar mechanism. Mg^{2+} (5 mM) had the same stimulatory effect as Ca^{2+} on cAMP receptors in isolated membranes (not shown).

3.2. *Ammonium sulphate increases the number and the affinity of cAMP receptors*

Fig.2 shows the equilibrium cAMP-binding properties of isolated membranes in the presence and absence of 2 M ammonium sulphate. The salt induced increased affinity for cAMP, reflected by a decrease of $K_{0.5}$ from 60 to 20 nM. A Scatchard analysis indicates a 2–3-fold increase in the number of receptors. The shape of the cAMP-binding curve found for isolated membranes was the same as that observed for intact cells [9,10], reflecting apparent positive cooperativity of binding at low receptor occupation levels and negative cooperativity or heterogeneity at higher cAMP concentrations.

3.3. *Identification of cAMP receptors unmasked by ammonium sulphate*

D. discoideum cells contain various cAMP-binding proteins, the specificities of which are known in detail: cell-surface chemotactic receptors [8,11], cyclic nucleotide phosphodiesterase which is present on the cell surface [12] and cAMP-dependent protein kinase [13]. The data in table 2 show that the cAMP-binding sites on membranes, isolated by sucrose gradient centrifugation, had the same cyclic nucleotide specificity as cAMP receptors on the cell surface. A more detailed investigation of cAMP-binding sites on the isolated *D. discoideum* membranes will be published elsewhere. In order to determine the nature of the cAMP receptors that were unmasked by ammonium sulphate, we measured the relative affinity for 3 cAMP analogues that have typically different affinities for the 3 above-mentioned cAMP-binding proteins [8,11–13]. As can be seen from table 2, the specificity of the receptors exposed by ammonium sulphate showed good correlation with the specificity of cAMP receptors that are detected on membranes in the absence of ammonium sulphate or $CaCl_2$ and with that of the cell-surface cAMP receptors [8,11].

3.4. *Mechanism of exposure of latent cAMP receptors*

The increase in number of cAMP receptors on intact cells induced by Ca^{2+} and several salts is not due to the exposure of intracellular, spare receptors, because the same phenomenon was observed with isolated membranes. We have examined the possibility that high salt concentrations release some effector molecule blocking or modulating cAMP binding. Table 3 shows that the effect of 2 M ammonium sulphate was readily reversible. When membranes were washed with buffer containing 2 M ammonium sulphate and cAMP binding was measured subsequently in the absence of salt, a non-increased cAMP-binding level was observed. This shows that high salt concentrations do not remove a factor that modulates cAMP binding from membranes. When ammonium sulphate was removed in the continuous presence of

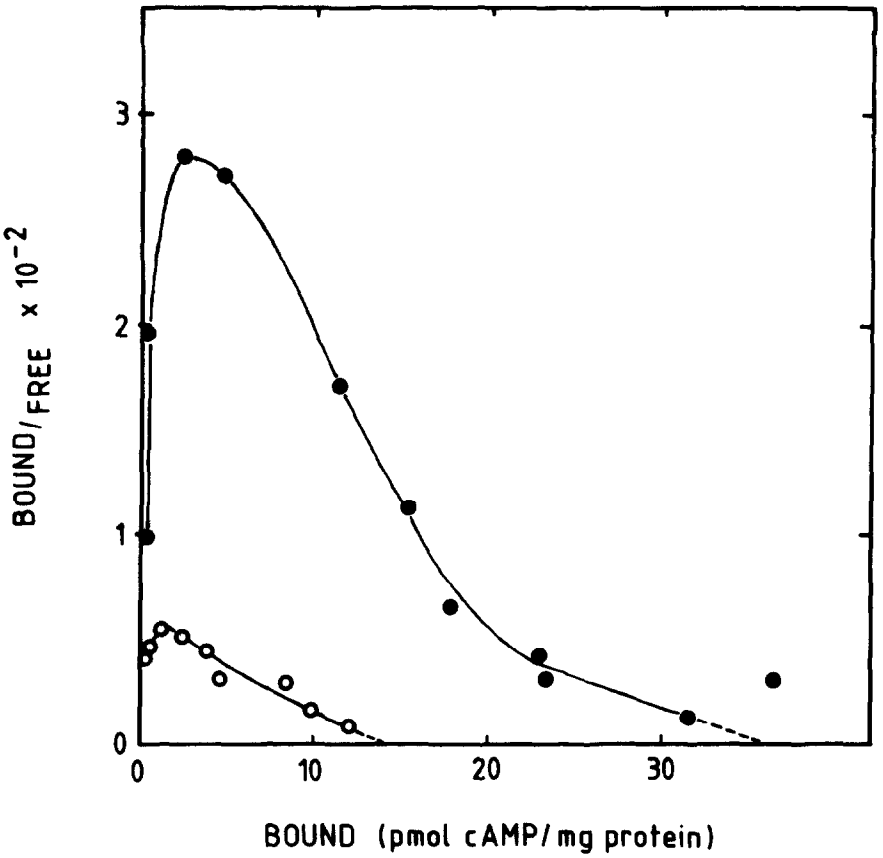

Fig.2. Binding of cAMP to isolated membranes in the presence (●) and absence (○) of 2 M ammonium sulphate. The ligand concentration ranged from 0.2 to 360 nM.

10 nM [^{3}H]cAMP, no ligand was trapped in a non-dissociating form (table 3). This makes it unlikely that cAMP receptors shift from a cAMP-accessible to a cAMP-inaccessible compartment upon removal of ammonium sulphate.

These results show that increased salt concentrations and probably also Ca^{2+} either affect the cAMP receptor directly, or modulate the interaction with a regulatory component which remains bound to the membrane. No information is available on the physiological role of these masked receptors. It is conceivable that their exposure is controlled in the multicellular organism by controlled changes in the ionic composition of the intercellular fluid. From the data of [2] it can be seen that the relative effect of Ca^{2+} on cAMP binding increases during cell aggregation. This supports the idea of a role of masked receptors in the cell aggregate.

Whatever the physiological role of the latent receptors may be, it is important to note that apparent receptor numbers are quite sensitive to the prevailing ionic conditions.

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Table 2

Specificity of cAMP receptors on isolated membranes unmasked by ammonium sulphate; comparison with the specificity of receptors detected in the absence of ammonium sulphate and 3 *D. discoideum* cAMP-binding proteins

Cyclic nucleotide	Affinity for cyclic nucleotide relative to cAMP ($\delta\Delta G$, kJ·mol^{-1})[a]				
	cAMP receptors exposed by ammonium sulphate	cAMP receptors detected in the absence of ammonium sulphate	Chemotactic cAMP receptors[b]	Phospho-diesterase[c]	cAMP-dependent protein kinase[d]
Inosine 3′:5′ monophosphate	22.9	16.7	21.7	6.3	3.9
Guanosine 3′:5′ monophosphate	25.3	23.4	22.7	2.0	13.9
2′-Deoxy-adenosine 3′:5′ monophosphate	6.3	4.6	5.6	3.8	22.0

[a] The relative affinity for cIMP, cGMP and 2′-deoxy-cAMP of receptors that were exposed by ammonium sulphate was calculated using: $\delta\Delta G = RT\ln(IC_{50}$ analogue$/IC_{50}$ cAMP) [8]. IC_{50} values were obtained by estimating the cAMP-analogue concentration at which the binding of [^{3}H]cAMP, present at a concentration of 10 nM, was inhibited by 50%
[b] From [11]
[c] Calculated from the apparent K_m in [12]
[d] From [13]

Table 3

Reversibility of the effect of ammonium sulphate[a]

	Preincubation medium		[^{3}H]cAMP binding (%) in:	
	2 M $(NH_4)_2SO_4$	10 nM [^{3}H]cAMP	Buffer	Buffer + 2 M $(NH_4)_2SO_4$
(a)	−	+	100	396
(b)	+	−	77	304
(c)	+	+	137	403

[a] Membranes were preincubated in binding medium with or without 2 M $(NH_4)_2SO_4$, in the absence or presence of 10 nM [^{3}H]cAMP. After 4 min, $(NH_4)_2SO_4$ was removed by 2 min centrifugation at 10000 × *g*. Sediments in (a) and (b) were washed 3 times with 20 mM Hepes/NaOH (pH 7). Sediments in (c) were not washed. Subsequently, all sediments were taken up in binding medium containing 10 nM [^{3}H]cAMP with or without 2 M $(NH_4)_2SO_4$. Binding of cAMP to the sediments was determined by filtration through polycarbonate filters

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