Modulation of the interaction between chemotactic cAMP-receptor and N-protein by cAMP-dependent kinase in *Dictyostelium discoideum* membranes

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Dictyostelium discoideum cells contain kinetically distinguishable surface cAMP receptors. Both GTP and GDP lower the receptor affinity by inducing conversion of slowly dissociating sites to fast dissociating sites, presumably by binding to a N-protein [(1985) Mol. Cell. Biochem. 67, 119–124, and (1986) Biochemistry 25, 1314–1320]. In this paper we show that treatment of isolated membranes with cAMP-dependent protein kinase abolished the GTP-induced receptor transition, but not the one induced by GDP. The effect of GTP on the receptor kinetics could be restored by treatment of the membranes with alkaline phosphatase. These results indicate that in *D. discoideum* membranes phosphorylation of a signal-transduction component reversibly abolishes the interaction of the cAMP receptor with the N^{GTP} complex, but not that with the N^{GDP} complex.

(Dictyostelium discoideum) *cyclic AMP receptor* *N-protein* *cyclic AMP dependence* *Protein kinase* *Desensitization* *Adaptation*

1. INTRODUCTION

Extracellular cAMP functions as a signal molecule in *Dictyostelium discoideum*. It binds to specific cell-surface receptors, which are functionally linked to adenylate cyclase and guanylate cyclase. In intact cells, binding of cAMP induces a transient activation of both enzymes within seconds [1,2]. Prolonged stimulation with constant cAMP concentrations induces desensitisation [3,4].

Kinetic studies revealed at least three forms of cAMP receptors in *D. discoideum*: one form, called SS, with a $k_{-1} = 0.9 \times 10^{-3}\ s^{-1}$ and a K_d of 6.5 nM; another form, called S, with a $k_{-1} = 1.3 \times 10^{-2}\ s^{-1}$ and a K_d of about 6 nM; and one or more forms, called F, with a $k_{-1} > 0.1\ s^{-1}$ and a K_d between 60 and 450 nM [5,6]. Receptor-binding studies have demonstrated a ligand-dependent interconversion of the different receptor forms [6,7]. GTP and GDP were shown to induce a conversion of the slowly dissociating receptor forms (SS and S) to faster dissociating forms [5,8,9]. Analogous to vertebrate receptor-effector systems [10], this has been interpreted as evidence for the involvement of a regulatory, guanine-nucleotide binding protein (N protein) in signal transduction in *D. discoideum*. Consequently, the different receptor forms and their interconversions have been explained by different complexes between receptor and N protein [8].

The molecular mechanism of the regulation of signal transduction in *D. discoideum* by the N protein has not been elucidated. Until now, no difference was observed between GTP and GDP in modulating receptor-binding properties. Moreover, in isolated membranes functional coupling between receptor and adenylate cyclase or

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guanylate cyclase has not yet been proved.

Uncoupling of the receptor-N protein interaction is an essential step in the desensitisation mechanism of several vertebrate receptor-effector systems [11–13]. The uncoupling is associated with phosphorylation of the receptor [11,14–17] and possibly the N protein [18]. Several protein kinases have been found capable of phosphorylation of receptors in vitro, including the cAMP-dependent kinase [11,17] and protein kinase C [15,16]. The identity of the protein kinase responsible for the uncoupling in vivo of receptor and N protein during desensitisation is in most cases not known.

To elucidate the regulatory role of the N protein in signal transduction and desensitisation in *D. discoideum*, we have investigated the effect of cAMP-dependent protein kinase on the coupling of the cAMP receptor to the N protein in isolated *D. discoideum* membranes. Our results show that cAMP-dependent phosphorylation of a signal-transduction component reversibly abolished the interaction of the receptor with the N^{GTP} complex. In contrast, the interaction with the N^{GDP} complex was not affected by the phosphorylation. The results are discussed in terms of a possible desensitisation mechanism.

2. MATERIALS AND METHODS

2.1. *Materials*

[5′,8-^{3}H]cAMP (1.55 TBq · mmol^{-1}) was purchased from Amersham (England), cAMP and dithiothreitol from Serva (Heidelberg, FRG) and 5′-AMP, ATP, GDP and GTP from Boehringer (Mannheim, FRG). cIMP, cAMP-dependent protein kinase (from rabbit muscle, peak II), the catalytic subunit of cAMP-dependent kinase (from bovine heart) and alkaline phosphatase (from bovine intestinal mucosa type VII-S) were obtained from Sigma (St. Louis, MO). Nitrocellulose filters (type BA 85) were from Schleicher and Schüll (Dassel, FRG).

2.2. *Culture conditions and membrane isolation*

Dictyostelium discoideum cells (strain AX2) were grown and developed by 6 h starvation as described before [19]. Membranes enriched in cAMP receptors were isolated by sucrose-gradient centrifugation as described by Janssens and Van Driel [20].

2.3. *Incubation with cAMP-dependent protein kinase*

Membranes (final concentration, 0.5 mg/ml protein), suspended in 50 mM K phosphate buffer (pH 7.0), were incubated for 15 min at 22°C with cAMP-dependent kinase (final concentration, 42 μg/ml), or its catalytic subunit (final concentration, 2.1 μg/ml) in the presence of 1 mM ATP, 1 μM cIMP or cAMP and 1 mM $MgCl_2$. cIMP was used if kinase activation was to be carried out under conditions of minimal cAMP receptor occupation. cAMP-dependent protein kinase is activated by cIMP and cAMP equally well [21]. The chemotactic receptor binds cAMP about 1000-fold better than cIMP [22]. The incubation was chilled to 0°C and centrifuged for 2 min at 10000 × *g* in a microfuge at 4°C. The pellet was washed once with 50 mM K phosphate buffer (pH 7.0, 0°C) and resuspended in the same buffer.

2.4. *Incubation with alkaline phosphatase*

Membranes (final concentration, 0.5 mg/ml), suspended in 50 mM K phosphate buffer (pH 8.2), were incubated with alkaline phosphatase (final concentration, 1.4×10^3 U/ml) for 30 min at 22°C. The incubation was transferred to ice and centrifuged for 2 min at 10000 × *g* in a microfuge at 4°C. The pellet was washed once and resuspended in 50 mM K phosphate buffer (pH 7.0, 0°C).

2.5. *cAMP binding assays*

cAMP binding and dissociation were measured, as described by Janssens et al. [5].

3. RESULTS

Janssens et al. [5,8] and Van Haastert et al. [9] have shown that GTP and GDP decrease the cAMP receptor affinity by inducing a conversion of slowly dissociating, high-affinity receptor forms to faster dissociating forms with lower affinities. The total number of receptors is not changed by GTP and GDP. The effect is most likely exerted via a guanine-nucleotide binding protein (N protein).

Fig.1 shows that incubation of isolated membranes with cAMP-dependent protein kinase (cAMP-Prk), either the holoenzyme plus cIMP or only the catalytic subunit, completely abolished the effect of GTP on cAMP binding, but not that

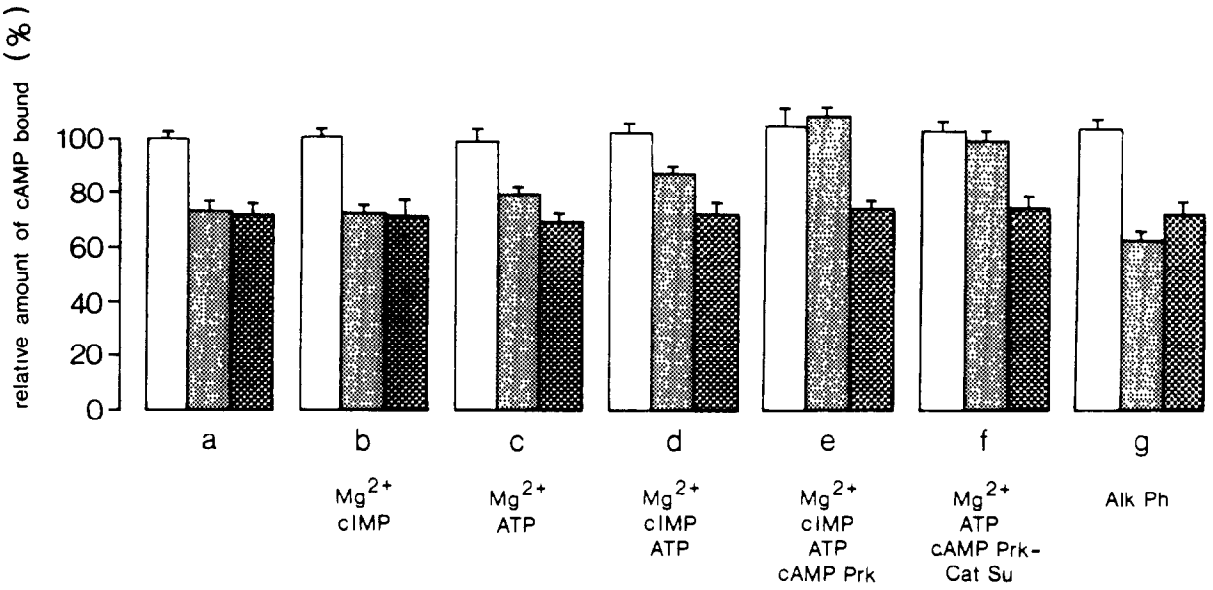

Fig.1. Effect of cAMP-dependent protein kinase on the binding of cAMP to membranes in the absence and presence of GTP and GDP. Membranes were incubated for 15 min at 22°C with additions as indicated in the figure. cAMP PrK, cAMP-dependent protein kinase; cAMP Prk-Cat Su, catalytic subunit of cAMP-dependent protein kinase; AlK Ph, alkaline phosphatase. Subsequently, cAMP binding was measured in the presence of 0.1 mM GTP (light grey bars), in the presence of 0.1 mM GDP (dark grey bars) and in the absence of guanine nucleotides (open bars). The results shown are the means and standard deviations of triplicate determinations from an experiment reproduced four times.

of GDP (fig.1e,f). Maximal effect was reached within 2 min of incubation at 22°C. Incubations at 0°C for up to 1 h did not induce any changes in cAMP binding.

The protein kinase action could be completely reversed by incubation with alkaline phosphatase (fig.1g). Treatment of membranes with Mg ATP, without exogenous kinase, resulted in a small but

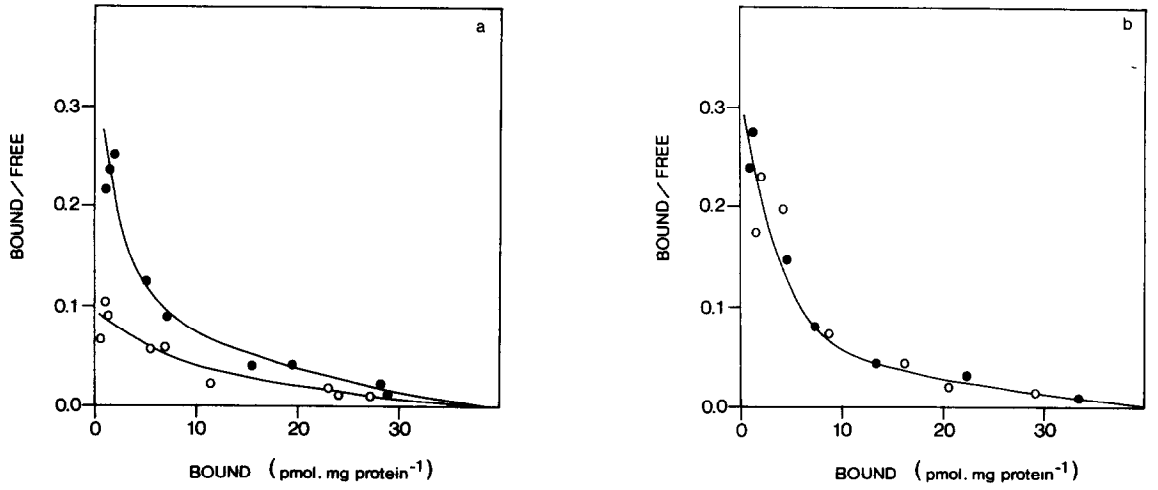

Fig.2. Scatchard analyses of equilibrium binding of cAMP to membranes, in the presence (○) and absence (●) of 0.1 mM GTP. cAMP binding was measured in control membranes (a) and in membranes treated with cAMP-dependent protein kinase (b). Each point is the means of a determination in triplicate of an experiment reproduced twice.

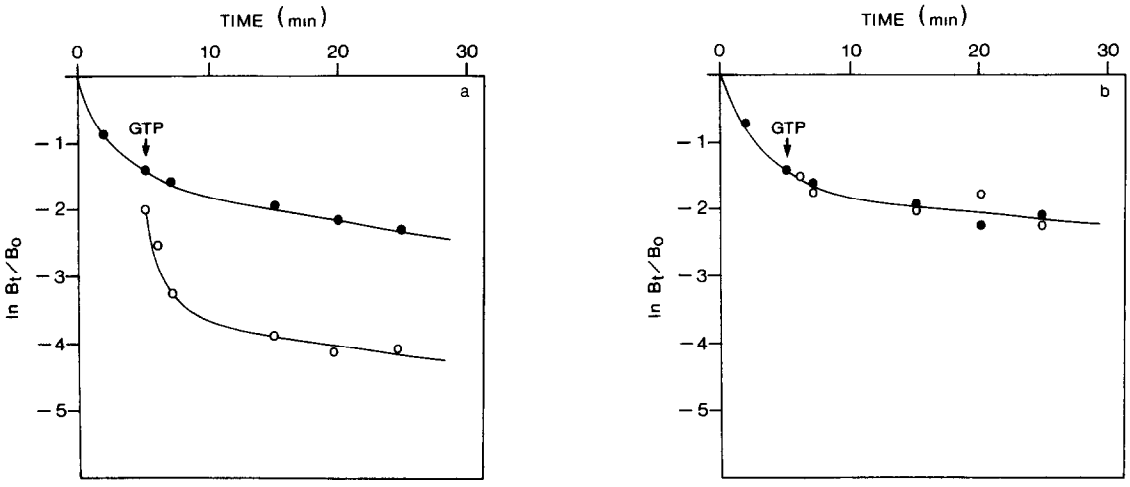

Fig.3. Effect of cAMP-dependent protein kinase on the dissociation of bound cAMP in the presence (●), added after 5 min dissociation, and absence (○) of 0.1 mM GTP. The dissociation was measured in control membranes (a) and in membranes treated with cAMP-dependent protein kinase (b). The experiment was reproduced three times.

significant suppression of the GTP effect (fig.1c). This was found to be slightly enhanced by the addition of cIMP (fig.1d). These data suggest that endogenous protein kinase activity, possibly cAMP-dependent kinase activity, is present in the membrane preparation. No effect on cAMP binding was observed after incubations without ATP (fig.1b).

A Scatchard analysis (fig.2) revealed that treatment of the membranes with cAMP-Prk completely abolished the GTP-induced decrease in receptor affinity. The cAMP binding in the absence of GTP was not changed by the phosphorylation.

In membranes of *D. discoideum*, in the absence of guanine nucleotides, three dissociation processes can be resolved, with first-order rate constants of $0.9\ (\pm\ 0.3) \times 10^{-3}$, $1.3\ (\pm\ 0.4) \times 10^{-2}$ and higher than $0.1\ s^{-1}$ [8,9]. Fig.3 shows that treatment of membranes with cAMP-Prk prevented the GTP-induced transition of the slowest dissociating receptor form SS and possibly also the S form to faster dissociating forms. The dissociation rate of the SS receptor form ($0.7\ (\pm\ 0.2) \times 10^{-3}\ s^{-1}$) was not significantly changed upon phosphorylation.

4. DISCUSSION

Several vertebrate receptor systems have been described where N proteins control agonist binding [24–27]. The same has been found for the chemotactic cAMP receptor of *D. discoideum* [8]. GTP and GDP are equally potent in inducing a transition from a high-affinity, slowly dissociating receptor form (SS form) to a low-affinity, fast dissociating receptor form [5,8,9].

The present data show that phosphorylation of membranes with exogenous cAMP-dependent protein kinase completely suppressed the effect of GTP, but not that of GDP. Membranes seem to contain a small, but significant endogenous kinase activity. The effect of cAMP-dependent protein kinase could be reversed by incubation with alkaline phosphatase. To our knowledge this is the first time that a difference is observed between GTP and GDP in modulating cAMP-receptor properties in *D. discoideum*.

Analogous to the vertebrate receptor-N protein-adenylate cyclase system, a cyclic scheme (fig.4) has been proposed to explain the various interactions between the components of the signal-transduction system in *D. discoideum* [8]. According to this scheme and our data, phosphorylation inhibits formation of LRN^{GTP}, but not of LRN^{GDP}, both being fast dissociating, low-affinity receptor forms. Since all steps in the scheme of fig.4 seem to be reversible, except the hydrolysis of GTP bound to N, the most likely interpretation is that phosphorylation changes the guanine-nucleotide

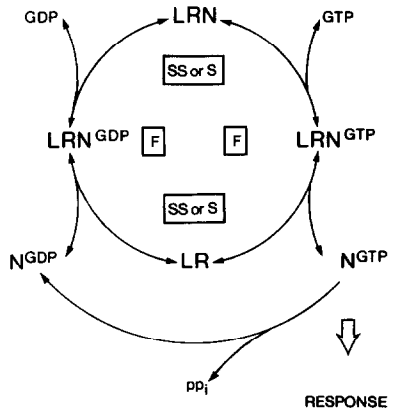

Fig.4. Model of the interaction of cAMP receptors of *D. discoideum* with a guanine-nucleotide binding protein (N protein) to explain the occurrence of F, S and SS receptor forms and their interconversions. L, ligand; R, receptor, N, N protein. Adapted from [8].

specificity of N or LRN, reducing its affinity for GTP but not for GDP.

Recently, GDP was shown to be 10-times more potent than GTP in lowering the affinity of the hepatic glucagon receptor [23]. In contrast, the myocardial muscarinic receptor was equally affected by GTP and GDP [24]. It is unknown whether in these systems the guanine-nucleotide specificity can be modified by phosphorylation or dephosphorylation.

Because N^{GTP} is thought to transduce the signal to adenylate cyclase or guanylate cyclase, the phosphorylation clearly would block signal transduction. Recent work of Devreotes and coworkers [27,28] has indicated that in *D. discoideum* desensitisation parallels a covalent, reversible modification (presumably phosphorylation) of the cAMP receptor. In many vertebrate receptor-effector systems desensitisation is found to be associated with phosphorylation of the receptor [11,14–17] and possibly the N protein [18].

We suggest that in *D. discoideum*, phosphorylation of the receptor or the N protein, or both, is the molecular basis of desensitisation by preventing formation of an active transducer complex N^{GTP}. However, whether the cAMP-dependent kinase, present in *D. discoideum* [22], is responsible for this phosphorylation in vivo remains to be seen.

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