# Specificity of the Cyclic GMP-Binding Activity and of a Cyclic GMP-Dependent Cyclic GMP Phosphodiesterase in Dictyostelium discoideum

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# SPECIFICITY OF THE CYCLIC GMP-BINDING ACTIVITY AND OF A CYCLIC GMP-DEPENDENT CYCLIC GMP PHOSPHODIESTERASE IN *Dictyostelium discoideum*

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The nucleotide specificity of the cyclic GMP-binding activity in a homogenate of *Dictyostelium discoideum* was determined by competition of cyclic GMP derivatives with [8-$^3$H]-cyclic GMP for the binding sites. The results indicate that cyclic GMP is bound to the binding proteins by hydrogen bonds at $N^2H_2$, $N^1$-H and/or $C^6$ = O, $N^7$, $2^1$-OH and $3^1$-O and possibly via a charge–charge interaction with the phosphate moiety of cyclic GMP. Cyclic AMP only competes with cyclic GMP for binding at a 20 000-fold higher concentration.

The same cyclic GMP derivatives were used to modify the hydrolysis of [8-$^3$H] cyclic GMP by phosphodiesterase. The phosphodiesterase is activated by cyclic GMP. The nucleotide specificity for activation of the enzyme differs from the specificity of the enzyme for hydrolysis. This indicates that activation by cyclic GMP and hydrolysis of cyclic GMP occur at different sites of the enzyme. Cyclic AMP neither activates the cyclic GMP phosphodiesterase nor competes with cyclic GMP for hydrolysis. This indicates that intracellular cyclic AMP does not interfere with the action of intracellular cyclic GMP in *D. discoideum*.

*Keywords:* cyclic GMP-dependent cGMP phosphodiesterase; cyclic GMP-binding protein; *Dictyostelium discoideum*; cyclic GMP derivatives.

Vegetative cells of *Dictyostelium discoideum* react chemotactically with folic acid [1], which probably acts as a food-seeking device. Aggregation-competent cells react chemotactically with cyclic AMP [2], which is excreted in pulses by neigh-

*Abbreviations:* cyclic AMP or cAMP, adenosine 3′,5′-monophosphate; cyclic GMP or cGMP, guanosine 3′,5′-monophosphate; cIMP, inosine 3′,5′-monophosphate; cXMP, xanthosine 3′,5′-monophosphate; 8-Br-cGMP, 8-bromoguanosine 3′,5′-monophosphate; 8-$NH_2$-cGMP, 8-aminoguanosine 3′,5′-monophosphate; 8-OH-cGMP, 8-hydroxyguanosine 3′,5′-monophosphate; 8-BA-cGMP, 8-benzylaminoguanosine 3′,5′-monophosphate; 8-M-cGMP, 8-morpholinoguanosine 3′,5′-monophosphate; 2′-H-cGMP, 2′-deoxyguanosine 3′,5′-monophosphate; 5′-GMP, guanosine 5′-monophosphate; Gua, guanosine; DTT, dithiothreitol; Tris, tris(hydroxymethyl)aminomethane.

boring cells [3]. Cyclic GMP levels are transiently elevated by cyclic AMP in aggregative cells of *D. discoideum* [4,5], by folic acid in vegetative cells of *D. discoideum* [4,6], *D. lacteum*, *D. minutum* and *Polysphondylium violaceum* (Kakebeeke, personal communication), and by partly purified, active extracts, which specifically attract *D. lacteum* [7,8] and *Polysphondylium violaceum* [9,10]. Cyclic GMP may be either hydrolyzed by a cyclic nucleotide phosphodiesterase, or bound to intracellular proteins, thus transmitting the extracellular signal.

Cyclic GMP-binding proteins have been found in several organisms [11–16] and are often not associated with protein kinase activity [11–15]. Also, the cyclic GMP-binding activity in homogenates of *D. discoideum* cells [17–19] seems not to be associated with cyclic GMP-dependent protein kinase activity [18,19] and can be divided into at least 3 fractions of different molecular weights [19]. A cyclic nucleotide phosphodiesterase from a homogenate of *D. discoideum* is more specific for cyclic GMP than for cyclic AMP, and expresses positive co-operativity [6]. Cyclic GMP-dependent cyclic GMP-specific phosphodiesterases also occur in other organisms [20–23].

Unfortunately, the binding activity and the cyclic GMP-hydrolyzing activity of *D. discoideum* are unstable. (The half-life at optimal pH and ion concentration is not more than one day, and mostly less than 5 h.) To obtain more information on these 2 significant proteins, non-purified preparations have to be used. In this paper, we describe the cyclic nucleotide specificity of these proteins. The results show a high degree of specificity, especially in the purine moiety, indicating that intracellular cyclic AMP does not interact with these proteins.

## MATERIALS AND METHODS

### *Materials*

Cyclic GMP, cAMP, cIMP, cXMP, 5′-GMP and guanosine were purchased from Boehringer, dithiothreitol (DTT), 2′-H-cGMP, and snake venom (*Ophiophagus hannah*) were from Sigma, and [8-$^3$H]-cyclic GMP (0.55 TBq/mmole; 1 TBp = $10^{12}$ dps = 27 Ci) was from Amersham. 8-$NH_2$-cGMP, 8-OH-cGMP, 8-BA-cGMP and 8-M-cGMP (Fig. 1) were generously given by Dr. Mühlegger, Boehringer.

### *Culture conditions*

*Dictyostelium discoideum*, NC-4(H), was grown on SM-agar in association with *Escherichia coli* B/r [24]. Cells were harvested in 10 mM sodium–potassium phosphate buffer (pH 6.0), and freed from bacteria by repeated washing and centrifugation at $100 \times g$ for 4 min. Cells were starved by shaking in 10 mM phosphate buffer (pH 6.0) at a density of $10^7$ cells/ml.

### *Homogenate*

For the cyclic GMP-binding experiments, cells were starved for 2 h, washed

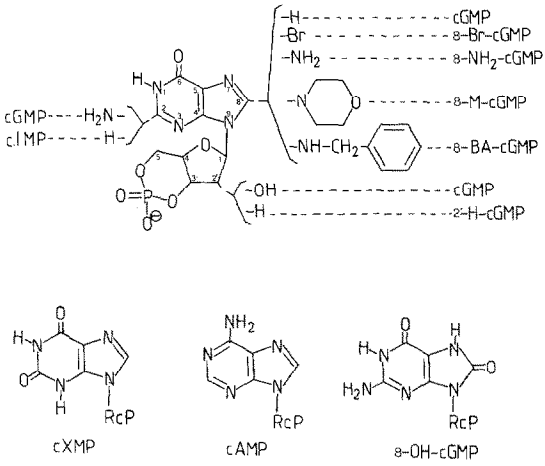


Fig. 1. The structure of cyclic GMP and cyclic GMP derivatives. All keto–enol equilibria are shown in the keto conformation. RcP, ribose 3′,5′-monophosphate.

twice in the homogenization buffer (5 mM Tris–HCl, pH 7.5) and suspended at a density of $2 \times 10^8$ cells/ml in the same buffer. Cells were homogenized at 0°C by sonic disruption, with a Branson B12 sonifier with microtip, twice for 5 sec at 50 W. The homogenate was centrifuged at 0°C for 10 min at $30\,000 \times g$, and the supernatant for 1 h at $48\,000 \times g$. The $48\,000 \times g$ supernatant was used for the experiments. For the phosphodiesterase experiments the same procedure was followed, except that the homogenization buffer was 10 mM phosphate buffer (pH 7.0), the cell density was $5 \times 10^7$/ml, and sonication was carried out 3 times for 5 sec.

*Cyclic GMP-binding assay*

The incubation mixture at 0°C contained 50 mM phosphate buffer (pH 6.5), 3 mM $MgSO_4$, 2 mM DTT (Sigma), $10^{-9}$ M [8-$^3$H]cyclic GMP (0.55 TBq/mmole) (Amersham), the $48\,000 \times g$ supernatant and various concentrations of unlabeled cyclic GMP and cyclic GMP derivatives in a total volume of 250 µl. The incubations (in duplicate) were started by the addition of 100 µl $48\,000 \times g$ supernatant, and terminated 10 min later by filtration of 200 µl over millipore filters (diameter 2.4 cm, pore size 0.45 µm). Filters were washed twice with 4 ml 50 mM phosphate buffer, and transferred to 4 ml Insta-Gel (Packard). The radioactivity was determined with an LKB Beta-rack liquid scintillation counter.

Hydrolysis of [8-$^3$H]cyclic GMP was negligible (<10% per h) owing to the temperature and pH at which binding experiments were performed.

The identity of bound radioactivity was determined by extraction of 7 filters with 10 ml $H_2O$, and chromatography of the extract by HPLC on Partisil 10-SAX with 50 mM $KH_2PO_4$, 15% propanol-1, 5% methanol (pH 4.9) as mobile-phase

liquid. More than 90% of the radioactivity co-chromatographed with cyclic GMP and not with guanosine, guanine or 5'-GMP.

*Phosphodiesterase assay*

Phosphodiesterase was assayed according to the method of Thompson et al. [26]. The 400-$\mu$l incubation mixture contained 10 mM phosphate buffer (pH 7.0), 2.5 mM DTT, 1.0 mM $MgSO_4$, 2 kBq [8-$^3$H]cyclic GMP, 48 000 $\times g$ supernatant, and unlabeled cyclic GMP or one of the cyclic GMP derivatives. DTT, an inhibitor of non-specific phosphodiesterases in *D. discoideum* [25], was added to the homogenate 15 min before the incubations [6]. The incubations (in duplicate) were started by the addition of 200 $\mu$l 48 000 $\times g$ supernatant at 22°C and terminated after 15 or 30 min by boiling for 2 min. The homogenate was cooled, 100 $\mu$l snake venom (100 $\mu$g) was added, and the mixture was incubated for 30 min at 22°C. The remaining [$^3$H]cyclic GMP was removed by the addition of 1 ml anion-exchanger (1 part AG-1-$X_2$ in water plus 2 parts ethanol); 15 min later, samples were centrifuged at 8000 $\times g$ for 2 min. The radioactivity of 500 $\mu$l of the supernatant was measured. The hydrolysis of cyclic GMP at concentrations between $10^{-8}$ and $10^{-4}$ M is linear with time, if hydrolysis remains below about 50%. The hydrolysis is linear with homogenate concentration as long as the enzyme concentration in the incubation mixture is no higher than the equivalent of $10^7$ cells/ml.

## RESULTS AND DISCUSSION

Hydrogen bonds contribute significantly to the specificity of the binding of a ligand to a protein, because these interactions are energy-rich and dependent on the correct orientation of the dipoles. By selecting cyclic GMP derivatives (Fig. 1) in which such interactions cannot take place, it is possible to determine the atoms or atom groups of cyclic GMP that are directly involved in an interaction with the pro-

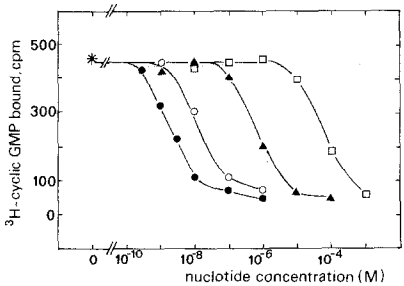


Fig. 2. Inhibition of the binding of [8-$^3$H]cyclic GMP by cyclic GMP and cyclic GMP derivatives. $10^{-9}$ M [8-$^3$H]cyclic GMP was incubated with a 48 000 $\times g$ supernatant and various concentrations of cyclic GMP or cyclic GMP derivatives for 10 min at 0°C; then the incubation mixture was filtered over millipore filters and the filter-associated radioactivity was determined. ●, cylic GMP; ○, 8-Br-cGMP; ▲, 2'-H-cGMP; □, cAMP.

tein [27–29]; the involved atoms or atom groups of the protein, however, are not determined.

*Specificity of the cyclic GMP-binding activity*

The inhibition of the binding of [$^3$H]cyclic GMP by cyclic GMP, 8-Br-cGMP, 2′-H-cGMP and cAMP is shown in Fig. 2. None of the derivatives revealed the existence of more than one binding protein with different specificity. The results are summarized in Table 1. In a few experiments (not included in Table 1) the binding of [$^3$H]cyclic GMP was reduced by 20–30% by the addition of $10^{-9}$ M cyclic nucleotides. Since all derivatives showed this at the same low concentration, we consider this as non-specific saturable binding with high affinity [30]. This component is probably identical with peak II in ref. [19].

Kinetic experiments, in which the dissociation constant and the rate constants of association and dissociation were measured, have not given support to the existence of 2 different binding proteins ([17] and unpublished observations).

We used the method of Jastorff et al. [27–29] to explain the results of Table 1. The inhibiting potency of cyclic GMP derivatives is standardized by [31]:

$$\delta\Delta G = -RT \ln \frac{K_{0.5}\ \text{cGMP}}{K_{0.5}\ \text{derivative}}$$

This change of free energy represents the reduction of binding energy between receptor and derivative as compared with cyclic GMP.

Table 1
Specificity of the cyclic GMP receptor

| Nucleotide | Binding activity [a] (nM) | $\delta\Delta G$(kJ/mole) |
|---|---|---|
| Cyclic GMP | 2.8 + 1.5 (14) | 0 |
| cIMP | 580 ± 350 (5) | 12.1 |
| cXMP | 2 800 ± 1 500 (6) | 15.7 |
| cAMP | 56 000 ± 16 000 (6) | 22.5 |
| 8-$NH_2$-cGMP | 18 ± 5 (6) | 4.2 |
| 8-Br-cGMP | 22 ± 7 (5) | 4.7 |
| 8-OH-cGMP | 470 ± 250 (5) | 11.6 |
| 8-M-cGMP | 6 900 ± 3 200 (6) | 17.7 |
| 8-BA-cGMP | 5 900 ± 3 610 (6) | 17.4 |
| 2′-H-cGMP | 450 ± 141 (4) | 11.5 |
| 5′-GMP | >1 000 000 [b] (3) | >29 |
| Guanosine | >1 000 000 [b] (3) | >20 |

[a] Cyclic GMP-binding protein was incubated with $10^{-9}$ M [8-$^3$H]cyclic GMP and different concentrations of various nucleotides. The concentration that results in 50% inhibition of the binding of [8-$^3$H]cyclic GMP to the specific component(s) is given as binding activity. Data in nM ± standard deviation and number of determinations in parentheses.

[b] No inhibition at $10^{-3}$ M.

According to Gabler [32], the energy of interaction of a hydrogen bond is between 8 and 40 kJ/mole (2–10 kcal/mole). Arbitrarily, we suggest that $\delta\Delta G$ values above 11 kJ/mole represent hydrogen bonds or charge–charge interactions, while $\delta\Delta G$ values below 5 kJ/mole are thought to be derived from general stereochemical and electronic features that have been changed by the modification of the ligand. Jastorff et al. [27–29] have placed the arbitrary division at about $\delta\Delta G$ = 15 kJ/mole.

Removal of the amino group at the 2 position (cGMP → cIMP) results in a change of binding energy of about 12.1 kJ/mole, indicating a hydrogen-bond interaction between $N^2H_2$ and a hydrogen-bond acceptor of the protein. Addition of a hydroxyl group at the 2 position (cIMP → cXMP), which is in keto–enol tautomerism with $N^3$, does not reduce the binding activity essentially further. Because the keto form is probably favored [33,34], a hydrogen-bond interaction at $N^3$ is unlikely. The $\delta\Delta G$ of cyclic AMP is about 22.5 as compared with cyclic GMP and about 11.4 as compared with cIMP, probably indicating the existence of another hydrogen-bond interaction at $N^1$–H and/or $C^6$ = O of cyclic GMP with the binding protein. 8-Br-cGMP and 8-$NH_2$-cGMP have binding energies comparable to cyclic GMP. Since bulky groups at the 8-position change the syn-anti equilibrium to syn [35], and since cyclic GMP itself exists predominantly in the syn conformation [36] we propose that cyclic GMP is bound in the syn conformation. Although 8-$NH_2$-cGMP and 8-OH-cGMP are comparable in size and polarity, the binding energies differ significantly. This could be explained by the assumption that, in 8-OH-cGMP, the keto–enol tautomerism of 8-OH with $N^7$ exists predominantly in the keto conformation [33,34]. This results in a change of $N^7$ from a hydrogen-bond acceptor ($N^7$) into a hydrogen-bond donator ($N^7H$). Therefore, we propose a hydrogen-bond interaction with the protein at $N^7$. The low binding energy of 8-BA-cGMP and 8-M-cGMP can be explained by the size of these bulky groups, and by the influence that these substitutents have on the electron distribution in the purine moiety. This may change the electron density at $N^1$, $N^2H_2$, $C^6$ = O and $N^7$ and therefore also the energies of interactions of the hydrogen bonds at these positions. 2′-H-cGMP shows a hydrogen-bond interaction at the 2′-OH position. 5′-GMP and guanosine are inactive, which may indicate a hydrogen-bond interaction at the 3′-O position and possibly a charge–charge interaction with phosphate. Information on the existence of a hydrogen bond at the 5′-O position is not available.

As far as these analogs have been tested for cyclic GMP-dependent protein kinases [16], it is clear that the binding protein of *D. discoideum* is more specific.

*Specificity of the cyclic GMP-hydrolyzing activity*

The fraction of substrate hydrolyzed during a certain time by an enzyme with normal Michaelis–Menten kinetics is given by

$$\text{fraction hydrolyzed} = A\,\frac{v}{[S]} = A\,\frac{V_{max}}{[S] + K_m} \qquad (1)$$

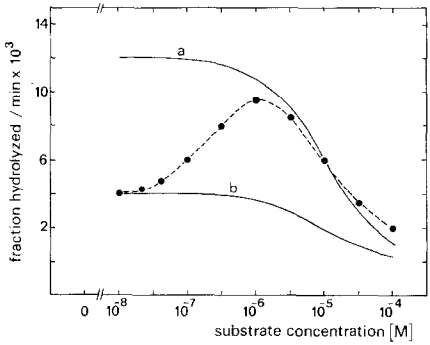


Fig. 3. Demonstration of activation and inhibition of hydrolysis of cyclic GMP by cyclic GMP. Curves a and b are calculated for 2 enzymes with Michaelis–Menten kinetics. a, $AV_{max}K_m^{-1} = 4 \times 10^{-3}\,min^{-1}$, $K_m = 10^{-5}$ M; b, $AV_{max}K_m^{-1} = 12 \times 10^{-3}\,min^{-1}$, $K_m = 10^{-5}$ M. (See text for explanation.) •, Various concentrations of cyclic GMP were incubated for 30 min with homogenate, after which the hydrolyzed fraction was determined.

where $A$ is the proportionality constant. In Fig. 3 the hydrolysis of cyclic GMP at different substrate concentrations is presented as substrate concentration versus fraction hydrolyzed/min, and compared with the activity of 2 enzymes with normal Michaelis–Menten kinetics. This figure shows that, at between $10^{-8}$ and $10^{-6}$ M cyclic GMP, the phosphodiesterase is activated by cyclic GMP. At about $3 \times 10^{-6}$ M, activation seems to be completed; at higher substrate concentrations the enzyme follows normal Michaelis–Menten kinetics with an apparent $K_m$ of about $10^{-5}$ M. We shall use this plot to visualize the specificity of the activation and inhibition of the hydrolysis of [$^3$H]cyclic GMP by cyclic GMP and cyclic GMP derivatives.

The cyclic GMP phosphodiesterase described by Dicou and Brachet [37] has several characteristics in common with our enzyme preparation, such as high substrate specificity, activation by magnesium ions, calcium independency, and an apparent Michaelis–Menten constant at high substrate concentrations of 3–10 μM (Mato et al. [6], this report and data not shown). However, in the experiment of Dicou and Brachet [37], phosphodiesterase is not activated by cyclic GMP. This discrepancy is not due to the use of different strains, and probably also not because of different assay conditions; more likely it is due to the instability of the enzyme complex.

*Inhibition of the hydrolysis of cyclic GMP*

Addition of different concentrations of cyclic nucleotides to an incubation mixture containing $10^{-6}$ M [$^3$H]cyclic GMP particularly shows the competition of these cyclic nucleotides with [$^3$H]cyclic GMP for binding to the activated hydrolysis site. Fig. 4 shows that activation of the phosphodiesterase is not yet complete at $10^{-6}$ M cyclic GMP (8-Br-cGMP, 8-$NH_2$-cGMP, 8-BA-cGMP, and 8-OH-cGMP).

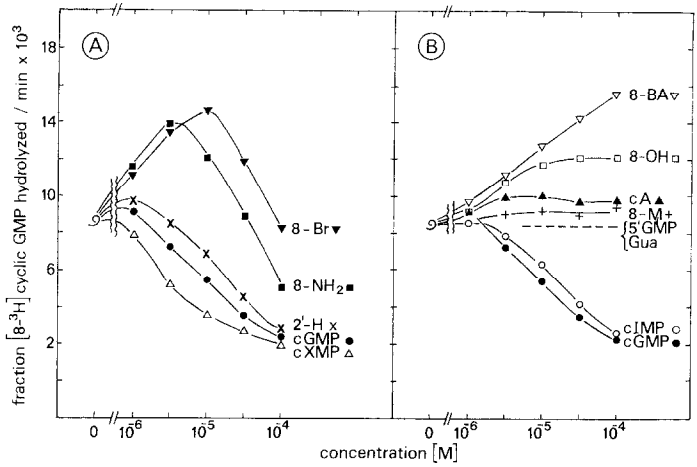


Fig. 4. Effect of cyclic GMP derivatives on the hydrolysis of $10^{-6}$ M [$^3$H]cyclic GMP. [$^3$H]-cyclic GMP ($10^{-6}$ M) was incubated with various concentrations (indicated on abscissa) of cyclic GMP, or cyclic GMP derivatives for 30 min, after which the fraction of hydrolyzed cyclic GMP was determined. All data are taken from 1 Expt.; duplicate experiments gave similar results.

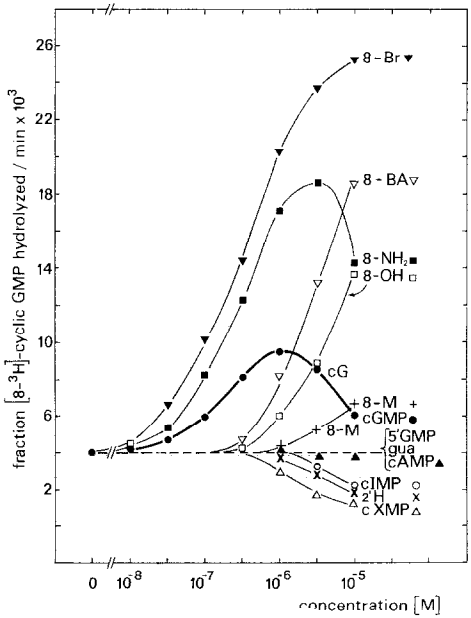


Fig. 5. Effect of cyclic GMP derivatives on the hydrolysis of $10^{-8}$ M [$^3$H]cyclic GMP. [$^3$H]-Cyclic GMP ($10^{-8}$ M) was incubated with various concentrations of cyclic GMP or cyclic GMP derivatives for 15 or 30 min, whereafter the fraction of cyclic GMP hydrolyzed was measured. 8-OH-cGMP, 8-$NH_2$-cGMP, 8-BA-cGMP, 8-Br-cGMP and cGMP were incubated for 15 min; cyclic GMP and other derivatives for 30 min. All data are taken from 1 Expt. which was repeated twice, with similar results.

8-Br-cGMP and 8-$NH_2$-cGMP inhibit the hydrolysis of [$^3$H]cyclic GMP at concentrations above $10^{-5}$ M, which is about 10 times higher than the concentration of cyclic GMP that causes inhibition. cIMP, cXMP and 2′-H-cGMP show strong inhibition of the hydrolysis of cyclic GMP, indicating that $N^2H_2$ and 2′-OH are probably not directly involved in the interaction of cyclic GMP with the hydrolysis site of the activated enzyme. Cyclic AMP only slightly affects the hydrolysis of [$^3$H]-cyclic GMP, which suggests that unspecific phosphodiesterases are blocked by DTT, and that $N^1$-H and/or $C^6$ = O are/is directly involved in binding to the hydrolytic site of the activated enzyme. 8-OH-cGMP is inactive as inhibitor. Assuming that the keto form is favored in purines [33,34] this may suggest a direct interaction at $N^7$. Guanosine and 5′-GMP are inactive, which probably indicates direct interaction at the phosphate and 3′-O.

*Activation of the hydrolysis of cyclic GMP*

Addition of cyclic nucleotides to incubation mixtures containing $10^{-8}$ M [$^3$H]-

Table 2
Binding characteristics of cyclic GMP to a binding protein and a cyclic GMP-dependent cyclic GMP phosphodiesterase of *D. discoideum*

| Atom or atom group | Binding to [a] binding protein | Activation [b] of PDE | Hydrolysis [c] at $10^{-8}$ M | Hydrolysis [d] at $10^{-6}$ M |
|---|---|---|---|---|
| $N^1$-H | + [e] | ? | + [e] | + [e] |
| $N^2H_2$ | + | + | – | – |
| $N^3$ | – | ? | – | – |
| $C^6$ = O | + [e] | ? | + [e] | + [e] |
| $N^7$ | + | + | ? | + |
| 2′-OH | + | + | – | – |
| 3′-O | + | + | + | + |
|  | + | + | + | + |
| syn–anti | syn | syn | ? | syn |
| space at 8 position | ample | ample | ? | limited |

[a] Derived from Table 1.

[b] Derived from the potency of cyclic GMP derivatives to stimulate the hydrolysis of cyclic GMP, shown in Fig. 5.

[c] Derived from the potency of cyclic GMP derivatives to inhibit the hydrolysis of cyclic GMP, shown in Fig. 5.

[d] Derived from the potency of cyclic GMP derivatives to inhibit the hydrolysis of cyclic GMP, shown in Fig. 4.

[e] $N^1$-H and/or $C^6$ = O are/is involved in a binding interaction. +, the atom or atom group is directly involved in binding, –, the atom or atom group is not directly involved in binding; ?, data from Figs. 4 and 5 are not sufficient to establish the involvement in binding.

cyclic GMP shows the capacity of these cyclic nucleotides to activate the phosphodisterase (Fig. 5). 8-Br-cGMP and 8-$NH_2$-cGMP activate the hydrolysis of [$^3$H]-cGMP even better than cyclic GMP itself; probably because there is less competition for hydrolysis. 8-BA-cGMP, 8-M-cGMP and 8-OH-cGMP also activate the hydrolysis of [$^3$H]cyclic GMP, but 10–100 times higher concentrations are required. The activation at high and not at low concentrations of 8-OH-cGMP may indicate a direct interaction at $N^7$. cIMP, cXMP, and 2′-H-cGMP cannot activate cyclic GMP hydrolysis, indicating that atomic interactions between cyclic GMP and the activator site occur at the $N^2H_2$ and 2′-OH positions. At high concentrations these com-

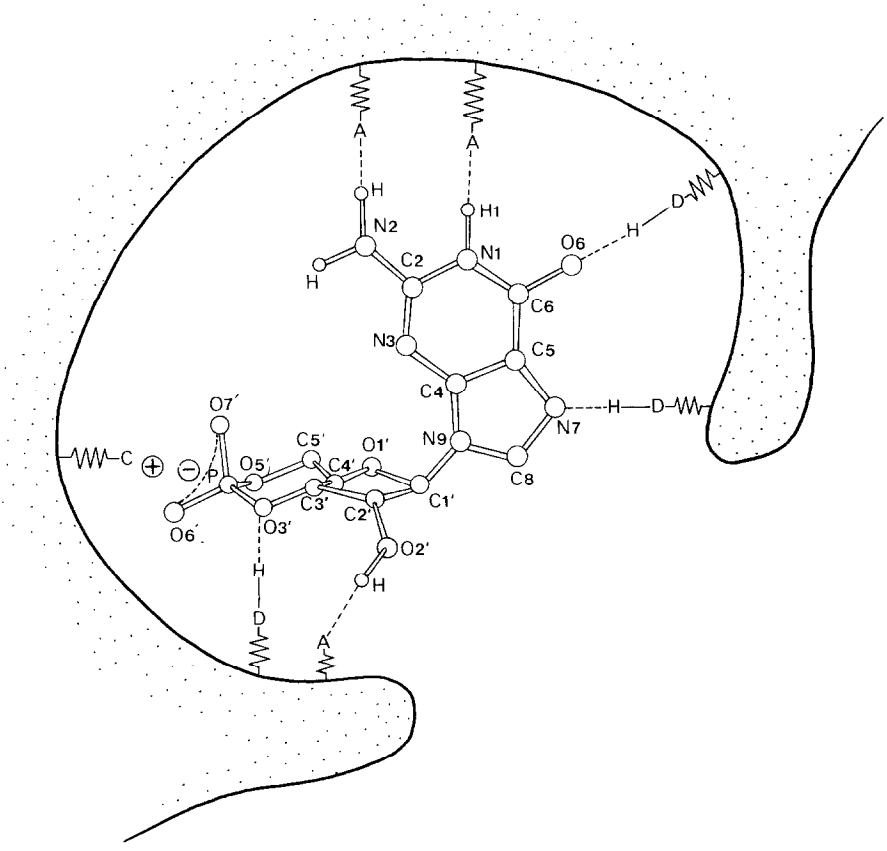


Fig. 6. Model of the binding site of the cyclic GMP binding protein. We propose that cyclic GMP is bound to the binding protein by hydrogen bonds at $N^1$-H and/or $C^6$ = O, at $N^2H_2$, $N^7$, 2′-OH and 3′-O, and possibly by a charge–charge interaction with the phosphate moiety. Interactions at 5′-O and 4′-O have not been established. The protein contains atoms or atom groups that accept a hydrogen bond (A), that donate a hydrogen bond (D), or that contain a positive charge ($C^+$).

pounds even start to inhibit hydrolysis of [$^3$H]cylic GMP. The hydrolysis site of the enzyme with low activity binds virtually no cyclic GMP at the $N^2H_2$ and 2′-OH positions. Cyclic AMP does not show activation or inhibition. Because cIMP did not show activation, we cannot establish the involvement of $N^1$-H or $C^6$ = O in binding to the activator site. 5′-GMP and guanosine neither activate nor inhibit hydrolysis.

These specificities are different from those for cyclic GMP-dependent cyclic GMP phosphodiesterase found in other organisms [22,23,38]. The enzyme of *D. discoideum* seems to be more specific, so that cyclic AMP has no effect on the activity of the enzyme, and the enzyme has no effect on the degradation of cyclic AMP in this system.

*Models*

In Table 2 the structural requirements of the cyclic GMP molecule are listed for binding to the binding protein, to the activator site of the phosphodiesterase, to the hydrolysis site of the phosphodiesterase with low activity and to the hydrolysis site of the phosphodiesterase with high activity.

The specificity of the activator site is similar to the specificity of a cyclic GMP-binding protein, except that half-maximal binding occurs at $10^{-9}$ M [17], and half-maximal activation occurs at about $10^{-7}$ M (Fig. 3). A provisional model of the interactions of the cyclic GMP-binding protein with cyclic GMP is shown in Fig. 6.

Binding of cyclic GMP to the hydrolysis site of the activated form of phosphodiesterase requires binding to $N^1$-H and/or $C^6$ = O, to $N^7$, 3′-O, and phosphate. Binding of cyclic GMP to the hydrolysis site of the phosphodiesterase with sub-maximal activity also requires binding to $N^1$-H and/or $C^6$ = O, 3′-O and phosphate, while binding to $N^7$ is unknown. This similarity of specificity may indicate that activation is due to an increase of $V_{max}$, rather than a decrease of $K_m$.

Activation of the enzyme by cyclic GMP and hydrolysis of cyclic GMP show different specificities (Table 2), indicating that these processes occur at different sites of the enzyme.

## ACKNOWLEDGEMENT

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