Aberrant cGMP-binding activity in non-chemotactic Dictyostelium discoideum mutants

Kuwayama, Hidekazu; Viel, Gerhard T.; Ishida, Shuji; Haastert, Peter J.M. van

Published in:
Biochimica et Biophysica Acta-Molecular Cell Research

DOI:
10.1016/0167-4889(95)00082-4

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1995

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Aberrant cGMP-binding activity in non-chemotactic Dictyostelium discoideum mutants

Hidekazu Kuwayama a, Gerhard T. Viel a, Shuji Ishida b, Peter J.M. Van Haastert a, *a

* Department of Biochemistry, University of Groningen, Nijenborgh 4, Groningen 9747 AG, The Netherlands
b Department of Botany, Faculty of Science, Kyoto University, Kyoto, 606-01, Japan

Received 17 March 1995; accepted 20 April 1995

Abstract

The kinetics of cGMP-binding to the major cGMP-binding activity in Dictyostelium were investigated in 10 non-chemotactic mutants (KI mutants; KI-1 ~ 10). A wild-type cell contains about 3000 binding sites with a K_d of 1.5 nM. cGMP may dissociate from these binding sites with fast (F-type) or slow (S-type) kinetics, and DNA has been shown to promote the conversion of F- to S-type of cGMP-binding. The 10 mutants were placed in 4 classes, based on equilibrium and non-equilibrium binding properties and the effect of DNA. Class I mutants (KI-1, 3 and 8) have normal cGMP-binding properties. Class II mutants (KI-2, 6 and 7) show increased K_d values but nearly normal B_max, normal F/S ratio and normal effects of DNA. Class III mutants (KI-4, 5 and 10) have a strongly decreased K_d and increased B_max; nearly all binding sites are of the S-type and DNA does not affect the binding; apparently these mutants have a cGMP-binding protein locked in the S-form. cGMP-binding in class IV mutant (KI-9) is normal except that the number of binding sites is increased about 3-fold. The finding of seven mutants with altered cGMP-binding in 10 non-chemotactic mutants suggests that the cGMP-binding activity plays an important role in the chemotactic signal transduction pathway.

Keywords: Cyclic adenosine 3',5'-monophosphate; cyclic guanosine 3',5'-monophosphate

1. Introduction

Two compounds are known as chemoattractants in the amoeboid micro-organism Dictyostelium discoideum (reviewed in Ref. [2]). Folic acid, a secreted compound from the food source bacteria is a chemoattractant which acts mainly in the vegetative stage of the life cycle [17]. After removal of bacteria, folic acid ceases to induce a chemotactic response. Instead, cAMP, secreted by D. discoideum cells themselves, becomes a chemoattractant functioning as the main trigger to induce a developmental program: cell aggregation, multicellular organization and the formation of fruiting bodies [7].

Folic acid and cAMP are detected by different surface receptors, which are coupled to different G-proteins [6]. Various responses such as a transient increase of intracellular cGMP and the association of actin and conventional myosin to the cytoskeleton are observed when cells are stimulated with either folic acid or cAMP (reviewed in Refs. [5,16]). This led to the assumption that the folic acid and cAMP chemotactic signal transduction pathways start at different receptors but combine into one common pathway [12,26].

Based on this assumption, non-chemotactic mutants were isolated which do not respond to both folic acid and cAMP with the aim to unravel the part of the signal transduction pathway that is shared by different chemoattractants (KI-mutants). Chemotaxis is a complex process involving movement and orientation of a cell which are both regulated by extracellular signals. In chemotactic mutants selected from a large pool of 10000 randomly mutated cells, it may be expected that proteins involved in different second messenger systems can be found to be mutated, for instance cGMP, cAMP, Ca^{2+}, inositol phosphates, and protein kinases. In this context it is surprising that several mutants have an altered cGMP response (see Table 1), suggesting that cGMP plays a central role in the regulation of chemotaxis; notably, KI-8 has strongly reduced levels of guanylyl cyclase activity, whereas this enzyme is present but cannot be activated by cAMP in...
Table 1

Properties of chemotaxis KI mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Responses</th>
<th>Biochemical properties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>chemotaxis</td>
<td>cGMP</td>
</tr>
<tr>
<td>XP55</td>
<td>++ +</td>
<td>+ + +</td>
</tr>
<tr>
<td>KI-1</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>KI-2</td>
<td>-</td>
<td>+ + /del*</td>
</tr>
<tr>
<td>KI-3</td>
<td>+ +</td>
<td>+ + +</td>
</tr>
<tr>
<td>KI-4</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>KI-5</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>KI-6</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>KI-7</td>
<td>-</td>
<td>+ /del*</td>
</tr>
<tr>
<td>KI-8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KI-9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KI-10</td>
<td>- /++ #</td>
<td>-</td>
</tr>
<tr>
<td>stmF</td>
<td>+ + +</td>
<td>-</td>
</tr>
</tbody>
</table>

XP55 is the parental wild-type strain. Data are from Kuwayama et al. [9].

The properties of mutant stmF are shown for comparison. + + +, strong response/activity as in wild-type XP-55; + +, strong, but significantly slightly reduced response/activity if compared with XP-55; +, small, but still significant response/activity; -, insignificant response/activity. #, mutant KI-10 responds to some but not to other chemotactants; *, delayed response with a maximum at 20 s, relative to 10 s in wild-type XP55.

This binding activity was shown to exist in two forms: a slow dissociating type (S-type), and a fast dissociating type (F-type) [19]. Oligonucleotides promote the conversion from the F-type to the S-type of the cGMP-binding activity.

So far, there is no evidence that this cGMP-binding activity is associated with chemotactic signal transduction. In order to investigate the possible relationship between cGMP-binding activity and chemotaxis, we analyzed the cGMP-binding activity of the 10 non-chemotactic mutants and present evidence that the non-chemotactic phenotype of some mutants may be due to a defect in the cGMP-binding activity.

2. Materials and methods

2.1. Materials

cAMP and cGMP were purchased from Boehringer. [8-3H]cGMP (910.2 GBq/mmol) was obtained from Amersham. Polycarbonate filters and nitrocellulose filters were from Nucleopore (Coster, Badhoevedorp, The Netherlands) and Schleicher and Schuell, respectively.

2.2. Strains and culture condition

KI mutants [9] and the parental strain XP-55 [15] were grown on 1/3 SM plates (0.3% glucose, 0.3% bactopeptone, 40 mM KH₂PO₄/Na₂HPO₄ buffer pH 6.0 and 1.5% agar) with Klebsiella aerogenes. Cells were harvested in the late logarithmic phase with 10 mM KH₂PO₄/Na₂HPO₄ buffer (pH 6.5) (PB). Bacteria were removed by repeated centrifugations at 300 × g for 3 min. Then cells were starved for 5 h by shaking in PB at a density of 10⁷ cells/ml at 21°C.

2.3. Preparation of supernatant

For the cGMP-binding assay, starved cells were washed 3 times in cGMP-binding assay buffer (40 mM Hepes/NaOH and 0.5 mM EDTA, pH 7.0), and resuspended at a density of 2 × 10⁸ cells/ml in the same buffer including 250 mM sucrose. Cells were then homogenized at 0°C by passed through a polycarbonate filter (pore size 3 μm). The homogenate was centrifuged at 4°C for 1 h at 48,000 × g. The 48,000 × g supernatant was used for the cGMP-binding and the cGMP-dissociation experiments.

2.4. cGMP-binding activity

cGMP-binding to proteins in the supernatant was measured as described previously [14,24,25]. Briefly, 100 μl of the supernatant was added to an equal volume of binding mixture (100 mM PB, 6 mM MgCl₂, 10 mM dithiothreitol and 0.5 mM, 1.5 mM, 3.0 mM or 6.0 mM

mutant KI-10 [9]. All mutants showed similar activity of cGMP-specific phosphodiesterase, and all mutants, except KI-8, possess nearly normal guanylyl cyclase activity in vitro.

The importance of cGMP is supported by other observations. First, all known chemoattractants induce a similar transient increase of cGMP levels, not only folic acid and cAMP in D. discoideum but also other chemoattractants in other cellular slime mould species [4,13,28]. Second, a mutant called Streamer F, shows a prolonged chemotactic movement and, at the same time, a prolonged transient increase of cAMP-induced cGMP levels, due to a mutation in the cGMP-specific phosphodiesterase [20,23,3].

One important aspect of chemotactic movement is the regulation of sliding locomotion of myosin along actin filament [22]. This interaction between myosin and actin is organized by the formation and breakdown of actin and myosin filaments [8]. Myosin filament formation is regulated by myosin phosphorylation [29]. cGMP appears to be important for this process, because a cGMP response-less mutant (KI-10) does not show a cAMP-induced increase of myosin phosphorylation [10], whereas myosin phosphorylation is prolonged in mutant Streamer F with a defective cGMP-phosphodiesterase [11].

Although the importance of cGMP for chemotaxis has been established, the target of cGMP leading to myosin phosphorylation is still unknown; it is likely to be a cGMP-binding protein. Previously, it was shown that the major cGMP-binding activity of Dictyostelium is detectable in the cytosol [14], and the binding activity is associated with neither cGMP-dependent protein kinase activity [18] nor cGMP-specific phosphodiesterase [24,18].
After incubation for 15 min at 0°C, the reactions were terminated by filtering the reaction mixture through a nitrocellulose filter (pore size 0.45 μm). The filters were washed twice with 4 ml of 50 mM PB (pH 6.0) and, after being dried, the radioactivity of the filters was measured in 4 ml of scintillant. Non-specific binding was measured by including 50 μM unlabelled cGMP in the binding reaction; it was not significantly different between KI mutants and wild-type. Non-specific binding was subtracted from all data shown.

2.5. Dissociation kinetics of cGMP-binding

The binding reaction was performed as described above with 3.75 nM labeled cGMP. The dissociation kinetics of the [3H]cGMP-protein complex was measured following addition of 50 μl of unlabelled cGMP (100 μM) to 200 μl of this equilibrium binding mixture. The samples were filtered through nitrocellulose filters at 0 min, 3 min or 15 min after the addition of unlabelled cGMP. These binding assays were performed with or without 100 μg/ml denatured herring sperm DNA (boiled for 3 min and immediately cooled down on ice).

3. Results

3.1. cGMP-binding activity

A cGMP-binding activity exists mainly in the soluble fraction of *D. discoideum* cell lysates [14]. Since cGMP is an important second messenger for chemotaxis in *Dictyostelium*, the cGMP-binding activity was analyzed in non-chemotactic mutants (KI mutants).

The soluble fraction of lysates from all KI mutants were assayed for [3H]cGMP-binding activity. Non-specific binding, measured in the presence on excess non-radioactive cGMP, was not different between lysates obtained from mutant and wild-type cells; non-specific binding is subtracted from all data shown. Using binding at different [3H]cGMP concentrations, Scatchard plots were obtained which show linear curves for all mutants as well as for the parental strain, XP55 (Fig. 1). This suggests that the cGMP-binding activity behaves in this assay as a single class of binding sites in every strain as was reported previously for other wild-type strains [14,25]. However, the slopes and/or intercepts with the X-axis are very different in several mutants if compared to in the parental strain XP55 (Fig. 1). To address the differences clearly, apparent dissociation constants (Kd) and putative maximum number of binding sites (Bmax) were calculated for each strain from the Scatchard plots (Table 2). Using these data the mutants were placed in 4 groups according to their altered Kd and Bmax values (Table 4). (I) KI-1, 3 and 8 show almost normal Kd values and Bmax. (II) KI-2, 6 and 7 show significantly higher Kd values but have relatively normal or a slightly decreased Bmax. (III) KI-4, and 5 have significantly lower Kd values as well as a strongly increased Bmax; KI-10 also shows this phenotype, although less strongly. (IV) KI-9 has a relatively normal Kd value but a much higher number of binding sites. These results indicate that 7 of the 10 KI mutants possess aberrant equilibrium cGMP-binding properties.

3.2. Dissociation kinetic of cGMP-binding

To determine if the aberrant Kd values of the cGMP-binding protein from the mutants are due to abnormal dissociation of cGMP from its binding protein, the dissociation rate of the cGMP-protein complex was examined in each KI mutant. Data are presented as the binding ratio at normal or a slightly decreased Bmax. Table 2 shows the dissociation rate of the cGMP-protein complex was examined in each KI mutant. Data are presented as the binding ratio at normal or a slightly decreased Bmax.

### Table 2

<table>
<thead>
<tr>
<th>Strain</th>
<th>Kd nM</th>
<th>Bmax sites/cell (% to XP55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XP55</td>
<td>1.56±0.27</td>
<td>2860±250 (100)</td>
</tr>
<tr>
<td>KI-1</td>
<td>1.40±0.23</td>
<td>2340±140 (82)</td>
</tr>
<tr>
<td>KI-2</td>
<td>2.40±0.16</td>
<td>2840±150 (99)</td>
</tr>
<tr>
<td>KI-3</td>
<td>1.41±0.39</td>
<td>2550±360 (89)</td>
</tr>
<tr>
<td>KI-4</td>
<td>0.30±0.03</td>
<td>4720±150 (165) *</td>
</tr>
<tr>
<td>KI-5</td>
<td>0.50±0.08</td>
<td>5360±300 (187) *</td>
</tr>
<tr>
<td>KI-6</td>
<td>2.93±0.70</td>
<td>1950±300 (68)</td>
</tr>
<tr>
<td>KI-7</td>
<td>3.28±0.46</td>
<td>2040±190 (71)</td>
</tr>
<tr>
<td>KI-8</td>
<td>2.06±0.34</td>
<td>2510±250 (88)</td>
</tr>
<tr>
<td>KI-9</td>
<td>1.12±0.31</td>
<td>7510±910 (263) *</td>
</tr>
<tr>
<td>KI-10</td>
<td>0.78±0.08</td>
<td>9700±350 (339) *</td>
</tr>
</tbody>
</table>

Kd and Bmax represent the dissociation constant and the putative maximum number of binding sites per cell, respectively. All data shown are means with S.D. from triplicate determinations. *, significantly increased; **, significantly decreased relative to XP55 with P < 0.05.
Fig. 2. Dissociation kinetics. Cytosolic fractions prepared from XP55 (A) and KI-4 (B) were incubated with 3.75 nM [3H]cGMP. The reactions were performed with (open symbols) or without (filled symbols) denatured herring sperm DNA (100 μg/ml). At time 0 excess unlabelled cGMP was added and the residual binding of [3H]cGMP was measured at the times indicated. (C) Residual [3H]cGMP-binding of XP55 and all the KI mutants at 3 min after addition of the unlabelled cGMP without DNA. The results shown here are the mean ± S.D. of two independent experiments with triplicate determinations. The data for all mutants are presented in Table 3.

3 min or 15 min after addition of unlabelled cGMP compared to the binding before addition of cGMP. Fig. 2b shows that in a mutant, KI-4, the [3H]cGMP-protein complex dissociates much slower than in the parental strain (Fig. 2a). The data for all mutants are summarized in Fig. 2c. In all mutants except KI-4, 5 and 10, more than 70% of complex.

Table 3
Dissociation activities of cGMP-binding

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relative number of binding sites with DNA</th>
<th>Relative binding after dissociation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% to binding without DNA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>− DNA</td>
</tr>
<tr>
<td>XP55</td>
<td>161 ± 14 *</td>
<td>19 ± 7</td>
</tr>
<tr>
<td>KI-1</td>
<td>146 ± 16 *</td>
<td>29 ± 8</td>
</tr>
<tr>
<td>KI-2</td>
<td>125 ± 14 *</td>
<td>26 ± 5</td>
</tr>
<tr>
<td>KI-3</td>
<td>180 ± 10 *</td>
<td>9 ± 4</td>
</tr>
<tr>
<td>KI-4</td>
<td>107 ± 21 *</td>
<td>75 ± 5</td>
</tr>
<tr>
<td>KI-5</td>
<td>89 ± 5 *</td>
<td>89 ± 8</td>
</tr>
<tr>
<td>KI-6</td>
<td>126 ± 4 *</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>KI-7</td>
<td>262 ± 5 *</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>KI-8</td>
<td>113 ± 2 *</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>KI-9</td>
<td>229 ± 28 *</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>KI-10</td>
<td>129 ± 6 *</td>
<td>43 ± 4</td>
</tr>
</tbody>
</table>

The relative number of binding sites was determined at equilibrium with and without 100 μg/ml DNA; the binding ratio +DNA/-DNA is shown for each strain. Dissociation of the cGMP-protein complex is presented as the % of residual [3H]cGMP-binding activity at 3 min or 15 min after addition of excess unlabelled cGMP, relative to before addition of cGMP (+ or −DNA for each strain). All data shown are means with S.D. from triplicate determinations. *: significantly increased with \( P < 0.05 \).
the [³H]cGMP-protein complexes has dissociated within 3 min. Comparison with equilibrium binding reveals that all mutants with a very slow dissociation of the cGMP-protein complex belong to the class III mutants. None of the other mutants showed a rate of dissociation significantly different from wild-type cells.

### 3.3. Effect of DNA on the dissociation rate and number of binding sites

As addition of oligonucleotides or long single stranded DNA to the cytosolic fraction of wild-type cells decreases the dissociation rate of the cGMP-binding protein and increase the number of binding sites [19], we examined this effect of single stranded DNA on cGMP-binding activity in all KI mutants (Fig. 2 and Table 3). In all mutants, except KI-4 and KI-5, DNA induced a significant increase of the number of binding sites. In addition, in all mutants except the class III mutants, KI-4, 5 and 10, DNA retarded the rate of dissociation of the cGMP-protein complex (Table 3); in these type III mutants, DNA was found to have no effect on the dissociation. In summary, DNA retards dissociation of cGMP in the parental strain, and dissociation of cGMP is already slow in class III mutants in the absence of DNA. This suggests that the cGMP-binding protein of class III mutants is locked in the slow dissociating state.

### 4. Discussion

The chemoattractants folate acid and cAMP are detected by *Dictyostelium* cells using different G-protein coupled surface receptors. Somewhere in the signal transduction pathway, the signals from different receptors merge into one pathway leading to directed cell locomotion. Several chemotactic mutants with a defect in the common signal transduction pathway show an altered cAMP and folate acid induced cGMP response [9]. Further analysis revealed that cGMP may function through the phosphorylation of conventional myosin, thus regulating the assembly and disassembly of myosin filaments [10]. The target of cGMP is most likely a cGMP-binding protein. Previously, the cGMP-binding activity in *D. discoideum* has been studied extensively and characterized as follows. (1) Most of the activity is found in a cytosolic fraction obtained by centrifugation of a homogenate [14]. (2) cGMP-stimulated phosphodiesterase and cGMP-dependent protein kinase have been investigated as potential cGMP-binding proteins. Although the allosteric site of cGMP-stimulated phosphodiesterase and the cGMP-binding activity have nearly identical cGMP specificity [24], they belong to different proteins as the cGMP-binding protein and the cGMP-phosphodiesterase have been separated by column chromatography [18]. It should be mentioned that none of the KI mutants has an altered cGMP-phosphodiesterase activity. It is not known yet whether the cGMP-stimulated protein kinase represents the major cGMP-binding activity in *Dictyostelium* [27]. (3) Parisiensi and Coukell reported that the cGMP-binding activity can be separated into two kinetic forms; one form shows fast association/dissociation of cGMP (F-type), whereas association/dissociation of the other form is slow (S-type) [19]. Moreover, oligonucleotides like single stranded DNA convert the F-type of cGMP-binding to the S-type and also result in an increase of the total number of detected cGMP-binding sites. Recently, it was reported that 50% of the S-type activity is associated with nuclei, suggesting that the cGMP-binding protein may also function in nuclei [1].

Analysis of the cGMP-binding activity of the ten chemotactic mutants and their parental strain reveal altered cGMP-binding activity in 7 mutants; the binding activity of the mutants was classified into 4 groups (see Table 4). In class I mutants the cGMP-binding activity was essentially identical to wild-type cells with respect to the number of binding sites, the affinity, the ratio of F-and S-type of binding forms and the effect of DNA on these forms. The strains include mutants KI-1, KI-3 and KI-8. Interestingly, all these mutants show different patterns of receptor-stimulated cGMP response. Mutant KI-1 shows a small cGMP response to chemoattractants, mutant KI-3 has a normal cGMP response, whereas mutant KI-8 has no cGMP response due to the absence of nearly all guanylyl cyclase activity. Class II mutants show a cGMP-binding
activity with reduced affinity (from 1.5 nM in the parental strain to 2.4–3.3 nM in these mutants); the number of binding sites, the dissociation rate and the effect of DNA are not very different from those of the parental strain. Interestingly, the two mutants KI-2 and KI-7 with a relatively large but delayed cGMP response both belong to this class; the third mutant in this class is KI-6 which has a very weak cGMP response. The phenotype of class III mutants is very pronounced: the affinity of the cGMP-binding protein is increased from 1.5 nM to 0.3–0.8 nM, the number of detectable binding sites has increased about 2-fold, dissociation of the cGMP-protein complex is very slow and addition of DNA does not alter cGMP-binding activity. These data are consistent with the binding activity being locked in the S-type. Mutants KI-4 and KI-5 show the strongest phenotype, whereas mutant KI-10 is weaker. In this respect it is worth mentioning that mutant KI-10 is the only dominant mutant. Mutants KI-4 and 5 both show a weak cGMP response whereas mutant KI-10 does not respond to cAMP or folic acid with an increase of cGMP levels. The last class contains one mutant KI-9, which shows normal characteristics in respect to affinity and dissociation rate, except that the number of cGMP-binding sites is increased about 2.5-fold. It is possible that in this mutant there is just more of an otherwise normal cGMP-binding protein.

Upon starvation of *Dictyostelium* cells the number of cGMP-binding sites remains relatively constant during the first 4 h of development and then increases about 3-fold during the next 8 h of development [18]. Therefore, it could be possible that the increased number of cGMP-binding sites in mutants KI-4, 5, 9 and 10 is due to an enhanced developmental program. However, from the observations that all KI mutants have little expression of developmental markers such as cAMP receptors and contact sites A (data not shown), it is unlikely that these mutants develop very fast. Therefore, the increased number of binding sites is probably due to qualitative (KI-4, 5 and 10) or quantitative (KI-9) changes of cGMP-binding activity.

In class III mutants KI-4 and KI-5 the dissociating rate of bound cGMP is very slow and DNA has no effect on this rate; apparently the cGMP-binding protein is locked in the S-type. One explanation for these observations could be that crude cytosolic fractions from these mutants contained large amounts of oligonucleotide fragments, RNA and/or DNA. This is unlikely, because after partial purification of the crude cytosolic fraction of the mutants by DEAE-cellulose, which should remove most of the oligonucleotides, the cGMP-binding activity still has S-type dissociating properties (data not shown).

The observation that seven out of ten chemotactic mutants show altered cGMP-binding activity strongly suggests that this binding protein is involved in the chemotactic signal transduction pathway leading to myosin phosphorylation and finally directed cell locomotion. On the other hand, it may be proposed that the cGMP-binding activity is not involved in this chemotactic signal transduction pathway and that the abnormalities of cGMP-binding activity are due to secondary effects from the mutations in all these seven mutants, i.e. they are due to the altered cGMP response, altered development or altered chemotaxis. This seems contradicted by the observation that mutant KI-8 has normal cGMP-binding activity, but lacks guanylyl cyclase activity, basal and receptor-stimulated cGMP levels, and shows no cAMP-induced development and chemotaxis. Thus the absence of cGMP has no effect on the properties of the isolated cGMP-binding protein.

As all KI mutants belong to different complementation groups [9], the finding of 7 'cGMP-binding' mutants suggests that multiple genes are associated with cGMP-binding activity. The number of genes may be less than the number of complementation groups. For instance, if the cGMP-binding protein functions as a dimer, different mutations in the same protein may complement each other in the diploid. In this respect it is interesting to note that mutants KI-4 and KI-5 are nearly identical to each other as are mutants KI-2 and KI-7. On the other hand, associated proteins may alter the kinetics and activity of the cGMP-binding protein. Isolation of the mutated genes from the KI mutants and subsequently identification of the mutated proteins should give a better understanding of the cGMP-binding protein unit.

Interestingly, all 7 KI mutants with altered cGMP-binding activity also show a diminished production of cGMP in response to the chemotacticants cAMP and folic acid (Table 4). This could mean that either (1) the cGMP-binding protein is an unusual guanylyl cyclase, (2) the cGMP-binding protein is involved in guanylyl cyclase activation, or (3) that mutants have multiple mutations, one affecting cGMP-binding and one affecting guanylyl cyclase activation. The first hypothesis is unlikely, because mutant KI-8 with nearly no guanylyl cyclase activity has normal cGMP-binding activity, and many other mutants with different cGMP-binding properties have normal basal guanylyl cyclase activity (including mutants KI-4, 5 and 10); moreover, guanylyl cyclase is membrane-bound but the cGMP-binding activity is present in the cytosol. Concerning the second hypothesis, previous experiments by Schulkes et al. [21] demonstrate the necessity of a soluble protein for optimal magnesium-dependent guanylyl cyclase activity. Recently we observed that mutants KI-4 and 5 do not possess this soluble protein that activates guanylyl cyclase in vitro (Kuwayama and Van Haastert, unpublished observations). This strongly suggests that the cGMP-binding protein regulates guanylyl cyclase activity. In this respect, the observation of a soluble cGMP-binding protein that is required for optimal magnesium-dependent guanylyl cyclase activity in *Dictyostelium* lysates could provide a mechanism how several mutations may affect both cGMP-binding properties as well as receptor-stimulated guanylyl cyclase activity.
The detailed analysis of the 10 chemotactic mutants has established that only two mutants are not altered in either the production of cGMP by guanylyl cyclase or the detection of cGMP by the intracellular cGMP-binding protein: mutant KI-1 is relatively normal in all these properties, but still shows no chemotaxis to different chemoattractants, whereas mutant KI-3 shows significant chemotactic responses to all chemoattractants. The observation that 8 mutants have altered cGMP functioning places this second messenger in the center of chemotactic signal transduction in Dictyostelium.

Acknowledgements

We thank Lyndsay Drayer for reading the manuscript.

References