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Published in:
FEBS Letters

DOI:
10.1016/0014-5793(82)81029-6

Publication date:
1982

Altered cGMP-phosphodiesterase activity in chemotactic mutants of *Dictyostelium discoideum*

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Received 23 August 1982

Chemotaxis *Signal transduction* *Cyclic GMP* (Dictyostelium discoideum) *Cyclic GMP phosphodiesterase* *Mutant*

1. INTRODUCTION

Chemotaxis is very important during the whole life cycle of the cellular slime molds. In the vegetative stage the amoebae have to find their bacterial food in the soil which they inhabit. At this time, the amoebae are chemotactic to folic acid and pterin [1,2], both of which are excreted by bacteria; therefore it seems probable that this mechanism is used to find food [1]. When the food source is exhausted the amoebae aggregate to form a multicellular slug which then differentiates into a fruiting body. Different species of slime mold use different compounds as the chemoattractant for this aggregation. The best studied system is *Dictyostelium discoideum* which utilizes pulsatile signals of cAMP [3]; however, chemoattractants from other species have been partially purified [4,5].

Since 1977 evidence has been accumulating for the involvement of cGMP in the transduction of the chemotactic signal [6–12], particularly as all chemoattractants induce a similar accumulation of cGMP in sensitive cells. This increase in cGMP is transitory, peaking at 10 s and returning to the basal level by 30 s after stimulation. In addition to the well-documented non-specific phosphodiesterase, *D.discoideum* has an intracellular PDE specific for cGMP [8,13–15]. This enzyme is activated 4-fold by concentrations of cGMP within the range of the change during chemotactic stimulation [14,15] which suggests that it may play an important role in the transduction of the signal.

A group of chemotactic mutants of *D.discoideum* (*stmF* 'streamer' mutants) have been isolated which show an alteration in the cGMP response to cAMP [12]. In these strains the increase in cGMP reaches a higher level than in the wild type and attainment of the peak is delayed until ~20 s. Prestimulation levels are not recovered within 2 min. Here we show that the chemotactic stimulation of cGMP by folic acid is also altered in *stmF* mutants, and that the change in cGMP response can be accounted for by a defect in the specific cGMP PDE: one of the mutants tested does not have measurable levels of the enzyme (<0.4% that of wild type), the other has low levels (~2% wild type) and stimulation of this enzyme requires 10-times the cGMP concentration of the wild type.

2. MATERIALS AND METHODS

cGMP, 8-bromoguanosine 3′,5′-monophosphate (8-bromo-cGMP), and cAMP were purchased from Boehringer; folic acid from Sigma; c[8-^{3}H]-GMP (0.55 TBq/mmol, c[8-^{3}H]AMP (0.9 TBq/mmol) and the cGMP radioimmunoassay kit were from Amersham. Concanavalin (con) A–Sepharose was obtained from Pharmacia.

2.1. *Culture conditions*

Dictyostelium discoideum XP55 (wild type) and NP 368 and NP 377 (*stmF* mutant strains) were

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Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

grown in association with *Escherichia coli* B/r on 0.1% lactose–peptone. Cells were harvested in 10 mM phosphate buffer (pH 6.5) and freed from bacteria by repeated centrifugations at 100 × *g* for 4 min. The isolation and characterization of the mutants was described in [12,16]. Growth conditions for folate stimulation experiments were as in [16].

2.2. *Isolation of the cGMP-phosphodiesterase*

The enzyme was isolated as in [15]. Briefly, cells were starved in suspension for 2 h, collected in 10 mM phosphate buffer (pH 7.2) at 10^8 cells/ml and homogenized at 0°C by sonication (Branson B12 with microtip; 2 × 5 s at 50 W). The homogenate was centrifuged at 48 000 × *g* for 10 min, and the supernatant at 48 000 × *g* for 60 min. Non-specific phosphodiesterase was removed by chromatography of 2 ml of the newly obtained supernatant through con A–Sepharose (column dimensions 120 × 9 mm i.d.). The non-retarded fractions were diluted to 10 ml (wild type) or concentrated by Minicon B15 to 1 ml (mutants). Phosphodiesterase activity was measured as in [15].

3. RESULTS

Dictyostelium discoideum cells contain 2 classes of PDE. One class hydrolyzes cAMP and cGMP at approximately the same rate. These enzymes are located extracellularly, on the cell surface and intracellularly. They are inhibited by DTT and bind to con A–Sepharose columns [15,17,18]. The second class apparently comprises only one enzyme which is specific for cGMP and is located intracellularly. This enzyme is not inhibited by DTT and does not bind to con A–Sepharose [8,13–15]. The isolation of the cGMP-specific PDE used in this study is based on the differential lectin-binding properties of the 2 types of enzyme.

The cGMP PDE of the wild type XP55 (from which the mutant strains were derived) is essentially identical to the enzyme of other wild type strains of *D.discoideum* [15]. It is activated by low cGMP concentrations (10^{-8}–10^{-6} M) and activation is complete at ~ 10^{-6} M cGMP. The K_M of the activated enzyme is ~ 5.5 μM and the V_{max} is ~ 90 pmol cGMP hydrolyzed · min^{-1} · (10^7 cells)$^{-1}$ (fig.1).

In contrast, mutant strain NP 368 has no mea-

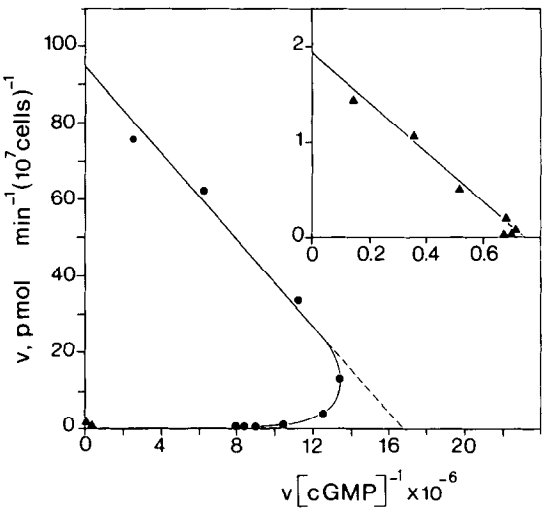

Fig.1. Kinetics of cGMP-phosphodiesterase activity from wild type and mutant *D.discoideum* cells. cGMP-specific phosphodiesterase was isolated from wild type XP 55 (•), and mutant NP 377 (▲) as in section 2. Enzyme activity was measured over 10^{-8}–10^{-4} M. Mutant NP 368 does not have measurable enzyme activity.

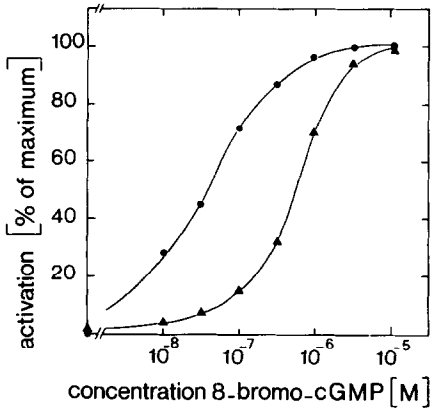

Fig.2. Activation of the hydrolysis of c[^{3}H]GMP by 8-bromo-cGMP. The isolated cGMP-phosphodiesterase from XP 55 (•) and NP 377 (▲) were incubated with 10^{-8} M c[^{3}H]GMP and different concentrations of 8-bromo-cGMP. The hydrolysis of c[^{3}H]GMP in the presence of 10^{-5} M 8-bromo-cGMP was set at 100% for both strains.

Table 1

Kinetic constants of cGMP-phosphodiesterase from wild type and mutant *D.discoideum* cells

Strain	App. V_{max} (pmol . min^{-1} . 10^7 cells^{-1})	App. K_M (μM)	K_a[a] (nM)
Wild types			
NC_4(H)	70	4.5	50
XP 55	90	5.5	40
Mutants			
NP 368	<0.4	n.d.	n.d.
NP 377	2	3	500

[a] K_a is the concentration of 8-bromo-cGMP which induces half-maximal activation of the hydrolysis of c[^{3}H]GMP.
n.d., not detectable

surable cGMP-specific PDE activity. The sensitivity of the assay is such that the maximum possible activity in this strain is 0.4% that of wild type cells. In some experiments with purified preparations of the mutant strain very low levels of cAMP hydrolyzing activity were seen (~1% of the specific cGMP hydrolyzing activity of the wild type and 0.01% of the cAMP hydrolyzing activity of the original homogenates). In these experiments, small amounts of cGMP hydrolyzing activity were also detected, but all activity disappeared on addition of 2 mM DTT. This suggests that the activity was due to small impurities of non-specific cyclic nucleotide PDE which had not been completely removed by con A–Sepharose column chromatography.

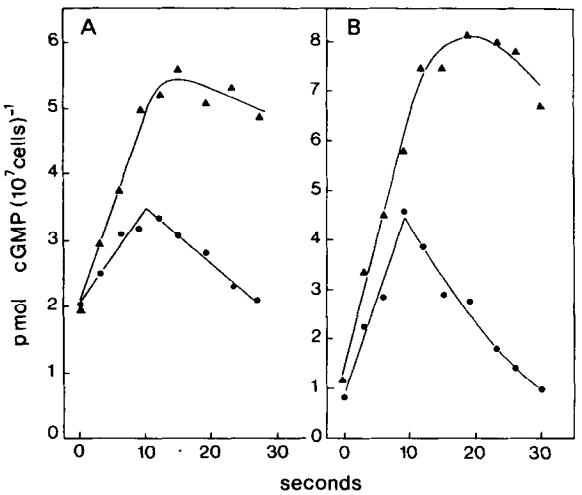

Fig.3. cAMP and folic acid mediated cGMP accumulation in wild type and mutant cells: (A) cells starved for 4 h were stimulated at 0 s with 16 μM folic acid; (B) cells starved for 8 h were stimulated at 0 s with 50 nM cAMP. The experiment was done as in [12]. (•) wild type XP 55; (▲) mutant NP 377.

Mutant strain NP 377 consistently shows levels of cGMP-specific PDE which are only 2% of those of wild type cells. Another unusual feature is that the Eady-Hofstee plot (fig.1) shows almost normal Michaelis-Menten kinetics for this enzyme. This implies either that cGMP does not activate the enzyme, or that activation occurs at higher cGMP concentrations than in the wild type. 8-Bromo-cGMP was used to determine which of these possibilities is correct. Low concentrations of 8-bromo-cGMP will activate the enzyme but this molecule does not bind effectively to the catalytic site of the wild type enzyme [14,15]. Half-maximal activation of the hydrolysis of cGMP by 8-bromo-cGMP occurs at $\sim 4 \times 10^{-8}$ M for the wild type enzyme but at about 5×10^{-7} M for the NP 377 enzyme (fig.2), showing that activation does occur but with abnormal kinetics.

Clearly the *stmF* mutants have altered cGMP-specific PDE activity (table 1). If these abnormalities are the principal cause of the altered cAMP-stimulated cGMP response seen in the mutants [12] then it would be expected that all chemotactic stimuli should give rise to excessive levels of cGMP. Fig.3 shows that this is true for the other major chemoattractant for *D.discoideum*, folic acid.

4. DISCUSSION

Chemotactic stimulation of *D.discoideum* cells induces a fast round-up of the cell, which is followed by the protrusion of a pseudopod [10,19]. During cell aggregation the period of directed locomotion lasts ~100 s in wild type cells [20]. The *stmF* mutants used in this study are probably fully able to detect chemotactic signals [12]. However, directed cell locomotion does not stop after 100 s but can last as long as 500 s. These mutants have an altered chemoattractant-mediated cGMP response; cGMP levels increase to higher values, and

basal levels are recovered only after 2–3 min, which is 5–10-times more slowly than in wild type cells [12].

PDE specific for cGMP is activated by low levels of cGMP within 10^{-8}–10^{-6} M. Because intracellular levels of cGMP vary within this range after chemotactic stimulation it seemed possible that this enzyme might play a crucial role in the transduction of chemotactic signals in *D.discoideum*. This report considerably strengthens that hypothesis by showing that the altered cGMP accumulation patterns, and therefore, presumably, the altered chemotactic behaviour, seen in mutants of the *stmF* complementation group can be accounted for by defects in the cGMP-specific phosphodiesterase.

ACKNOWLEDGEMENTS

We wish to thank Theo M. Konijn and Peter C. Newell for helpful discussions. The work of P.J.M.v.H. was supported by a grant to T.M.K. from the Foundation for Fundamental Biological Research (BION), which is subsidized by the Netherlands Organization for the Advancement of Pure Research, and that of F.M.R. by a grant to P.C.N. from the Science and Engineering Research Council (UK).

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