GUANYLATE CYCLASE ACTIVATION IN RESPONSE TO CHEMOTACTIC STIMULATION IN *DICTYOSTELIUM DISCOIDEUM*

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Received 3 April 1978

1. Introduction

In aggregating cells of *Dictyostelium discoideum* cyclic AMP induces chemotaxis [1] and the secretion of cyclic AMP after a brief activation of adenylate cyclase [2]. Cyclic AMP binds to cell surface receptors [3] and subsequently causes a fast transient increase in the cellular cyclic GMP content which accompanies pseudopod formation [4,5] and precedes cyclic AMP synthesis [5]. This increase in the level of cyclic GMP can occur as a result of an increased synthesis and/or inhibited degradation of the cyclic nucleotide. We show here that cyclic AMP induces a rapid 3–6-fold transient stimulation of guanylate cyclase in aggregation competent cells of *D. discoideum*. Also we found that physiological concentrations of ATP enhance the basal enzyme activity.

2. Methods

2.1. *Organism*

Cells of *D. discoideum*, Ax-2, were grown on nutrient medium [6] supplemented with 1.8% maltose and harvested in the logarithmic growth phase [7].

2.2. *Cyclic AMP stimulation and guanylate cyclase assay*

4–5 h after harvesting, cells were washed 4 times in 10 mM KCl and 10 mM NaCl, resuspended at a density of 3×10^7 cells/ml and 10 ml were oxygenated for 1 h at 23°C to allow the pH to rise to about 7 [7]. Cells were then equilibrated at 12°C under oxygenation. pH changes in response to cyclic AMP were measured as in [7]. Before and after cAMP stimulation a 500 μl sample was sonicated for 3 s using a Branson B-12 sonifier equipped with a microtip (at least 95% cell breakage). About 10 s after removal of the sample guanylate cyclase assays were carried out at 22°C essentially as in [8] in 450 μl reaction volume containing 50 mM Tris–HCl, pH 7.5, 10 mM cyclic GMP (Boehringer), 0.2 mM GTP (Boehringer), 5 mM $MgCl_2$, 1 mM $MnCl_2$, about 9×10^6 cpm [α-^{32}P] GTP (Amersham, spec. act. 10.8 Ci/mmol), 20 mM creatine phosphate (Boehringer), 100 units creatinekinase (Boehringer) and 135 μl homogenate (about 300 μg protein). Aliquots, 100 μl, were added to 100 μl terminating solution containing 10% SDS, 1.4 mM cyclic GMP and 20 mM GTP. Cyclic [^{32}P]GMP formed was purified as in [8] by sequential chromatography on Dowex AG 50Wx8 (Serva) and neutral alumina (Sigma). Cyclic GMP recovery, as calculated from the cyclic [8-^{3}H] GMP (Amersham, spec. act. 21 Ci/mmol) recovered, ranged from 45–55%.

Protein was determined by the method in [9]. GTP hydrolysis was measured by sequential chromatography on polyethyleneimine-cellulose sheets (PEI-cellulose, Macherey-Nagel) with 0.1 M acetic acid and

1.0 M LiCl as solvents [10]. For cyclic GMP hydrolysis about 10^6 cpm cyclic [8-^{3}H]GMP/100 µl assay mixture was incubated at 22°C. Cyclic GMP was isolated by 3 successive runs with 0.05 M acetic acid, distilled water, and 0.1 M LiCl as solvents on PEI-cellulose layers [10].

3. Results

Guanylate cyclase activity was proportional to the amount of protein up to ~150 µg/100 µl assay mixture (fig.1). In the presence of the regenerating system the GTP concentration was constant at all protein concentrations, whereas in its absence up to 60% GTP hydrolysis was found after a 5 min incubation. Therefore the GTP regenerating system was used throughout and the protein content was kept from 50–100 µg/100 µl assay mixture. Non-radioactive cyclic GMP (10 mM) was present in all assay mixtures to protect the cyclic [^{32}P]GMP formed. Under these conditions less than 5% of cyclic [^{3}H]GMP was hydrolysed within 8 min.

Since oscillations in the cGMP level do occur [5] it is important to know the dynamic state of the cells prior to chemotactic stimulation. Autonomous oscillations and chemotactic stimulation both are accompanied by a change in the extracellular pH (11.7). We therefore used extracellular pH-recording to monitor cellular activities. Figure 2a shows the pH-change in response to a cyclic AMP pulse of 3×10^{-8} M magnitude, and fig.2b shows the guanylate cyclase activity of samples taken 2 min before and immediately after stimulation. Cyclic AMP induced a rapid 3–6-fold increase in the activity of guanylate cyclase. The stimulated activity decayed to almost the basal activity within 1–2 min. Similar enhanced activity was observed in the presence of 2 mM dithiothreitol, a phosphodiesterase inhibitor in *D. discoideum* [12]. The same effect was observed when cells were sonicated in the presence of 8 mM EGTA and assayed at final conc. 2 mM EGTA. Stimulation of an homogenate with 1×10^{-7} M cyclic AMP did not enhance guanylate cyclase activity.

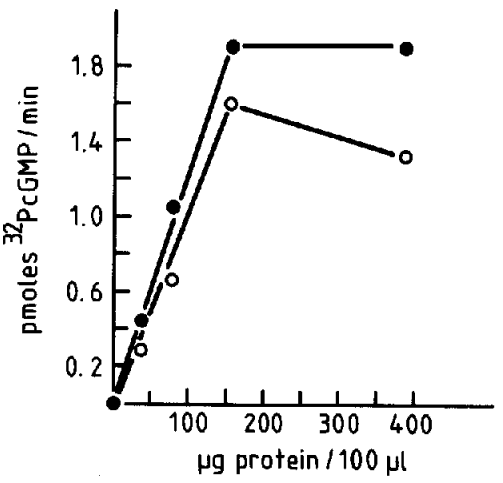

Fig.1. Guanylate cyclase activity as a function of the protein concentration in the assay mixture. Reactions were carried out in the presence (•) or absence (○) of GTP regenerating system and were terminated at 2.5 min and 5.0 min (for 135 µg and 390 µg protein) or 5.0 min and 10.0 min (for 35 µg and 80 µg protein). The reactions were linear with time.

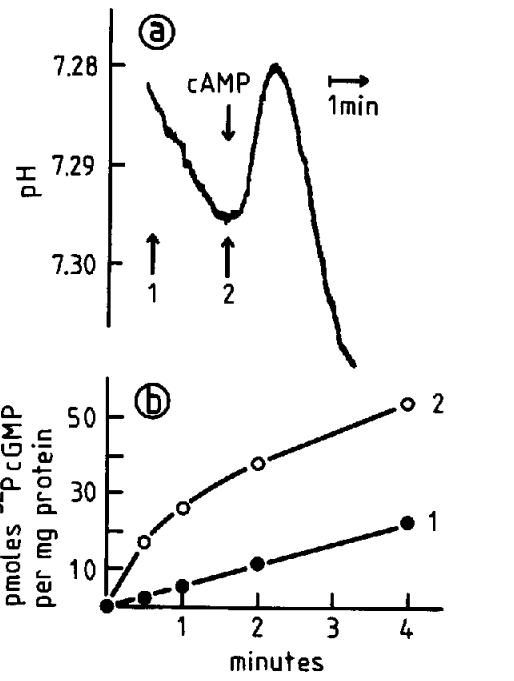

Fig.2. (a) pH recording of a cell suspension before and after stimulation with 3×10^{-8} M cAMP. At arrows 1 and 2 samples were removed for guanylate cyclase assay. (b) Guanylate cyclase activity of the samples taken from the cell suspension shown in fig.2a 2 min before (•, 1) and immediately after (○, 2) cyclic AMP addition. The cells were sonicated and assayed immediately.

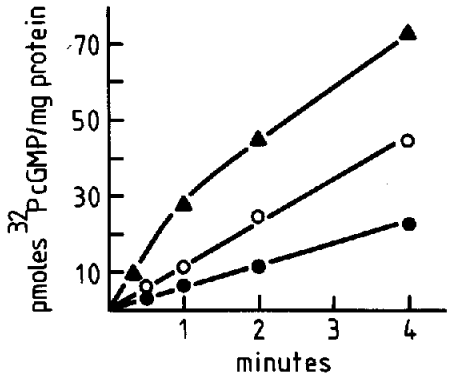

Fig.3. Guanylate cyclase activity in the absence (●) or presence (●, ▲) of 0.2 mM ATP. Samples were taken before (●, ○) and immediately after stimulation (▲) with 3×10^{-8} M cyclic AMP. The cells were immediately sonicated and assayed.

Addition of 0.2 mM ATP to the reaction mixture caused a 2–3-fold activation of guanylate cyclase. The ATP effect seemed to be additive to that induced by chemotactic stimulation of the cells with cAMP (fig.3). ATP concentrations ranging from 0.05–1.0 mM enhanced enzyme activity while higher concentrations were inhibitory. Adenylylimidodiphosphate (AMP-PNP) even at 1 mM did not activate the enzyme.

Most of the guanylate cyclase activity remained in the pellet after centrifugation for 1 min at 9000 × *g* and the specific activity of the pellet was 2–3-fold higher than in the supernatant (table 1). About 30% activity was lost during handling. Guanylate cyclase activity of both, pellet and supernatant, was activated 2–3-fold by 0.3 mM ATP and was inhibited by 0.2% Triton X-100 by about 30% (table 1).

4. Discussion

These results demonstrate that cGMP levels are regulated, at least in part, by changes of guanylate cyclase activity similar to what has been found for adenylate cyclase activity in *D. discoideum* [2].

No effect on guanylate cyclase activity was observed [8] after chemotactic stimulation of a cell suspension with cyclic AMP. Probably because the enzyme was assayed under conditions where the activity was no longer proportional of the protein concentration. A plateau of the enzyme activity with higher protein concentrations has been described [13]. We found a plateau at similar protein concentrations, however, in contrast to their findings, the GTP regenerating system did not cause any inhibition of enzyme activity.

Most of the guanylate cyclase activity was present in a particulate form. It seems, however, that the enzyme is only loosely attached to the membrane and that the amount of enzyme associated with the pellet varies depending on the procedure used to break the cells [13].

Receptor-mediated stimulation of guanylate cyclase activity by cyclic AMP is sufficient to explain the increased cyclic GMP levels in response to this nucleotide [4,5]. Yet, due to the 10 s delay between stimu-

Table 1

	%Activity	pmol cyclic [^{32}P]GMP/ min/mg protein	%Inhibition by Triton X-100	%Activation by ATP
Homogenate	100	8.3	–	–
Pellet	46	5.3	30	–
Supernatant	20	2.3	39	–
Homogenate	100	4.8	–	240
Pellet	44	5.0	–	200
Supernatant	28	1.7	–	350

Effect of ATP and Triton X-100 on subcellular fractions of guanylate cyclase. Fractions were prepared by freezing and thawing and subsequent centrifugation at 9000 × *g* for 1 min in the cold (Beckman microfuge). The pellet was resuspended in a buffer consisting of 50 mM Tris–HCl, pH 7.5, 10% sucrose and 1 mM EDTA. The concentration of Triton X-100 was 0.2% and of ATP 0.3 mM. Values represent the mean of 2 determinations

lation and the beginning of the assay, the actual activation may be even higher.

ATP enhanced the basal activity of the enzyme, possibly by phosphorylation since a non-hydrolyzable analogue of ATP, AMP-PNP did not substitute for ATP. Transient activation of guanylate cyclase following chemotactic stimulation occurred even in presence of saturating ATP-concentrations suggesting 2 different mechanisms of enzyme regulation.

Acknowledgements

We thank Drs G. Gerisch and Th. M. Konijn for helpful discussions and G. Waser for technical assistance. José M. Mato was a recipient of a short term EMBO fellowship during part of his stay at the Biozentrum, Basel. We are grateful for financial support by the Schweizerischer Nationalfonds and the Deutsche Forschungsgemeinschaft.

References

[1] Konijn, T. M., Van de Meene, J. G. C., Bonner, J. T. and Barkley, D. S. (1967) Proc. Natl. Acad. Sci. USA 58, 1152–1154.
[2] Roos, W. and Gerisch, G. (1976) FEBS Lett. 68, 170–172.
[3] Gerisch, G. and Malchow, D. (1976) Adv. Cyclic Nucleotide Res. 7, 49–68.
[4] Mato, J. M., Krens, F. A., Van Haastert, P. J. M. and Konijn, T. M. (1977) Proc. Natl. Acad. Sci. USA 74, 2348–2351.
[5] Wurster, B., Schubiger, K., Wick, U. and Gerisch, G. (1977) FEBS Lett. 76, 141–144.
[6] Watts, D. J. and Ashworth, J. M. (1970) Biochem. J. 119, 171–174.
[7] Malchow, D., Nanjundiah, V., Wurster, B., Eckstein, F. and Gerisch, G. (1978) Biochim. Biophys. Acta 538, 473–480.
[8] Mato, J. M., Roos, W. and Wurster, B. (1978) Differentiation, in press.
[9] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[10] Böhme, E. and Schultz, G. (1974) Methods Enzymol. 38, 27–41.
[11] Malchow, D., Nanjundiah, V. and Gerisch, G. (1978) J. Cell Sci. in press.
[12] Chassy, B. M. (1972) Science 175, 1016–1018.
[13] Ward, A. and Brenner, M. (1972) Life Sci. 21, 997–1008.