CYCLIC AMP BINDING TO LIVING CELLS OF DICTYOSTELIUM DISCOIDEUM IN PRESENCE OF EXCESS CYCLIC GMP

D. Malchow and G. Gerisch

Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft

74 Tübingen, Germany

Received September 18,1973

SUMMARY

[^{3}H]-cyclic AMP binding to living, aggregating cells of Dictyostelium discoideum can be detected if, simultaneously, excess cyclic GMP is added. The binding reaches a peak within less than half a minute after the cAMP pulse and the label is subsequently released into the intercellular space. It is argued that cGMP blocks cAMP-hydrolysis by cell-bound phosphodiesterase and interferes only to a minor extent with binding. Sensitivity, selectivity and developmental regulation are in accord with the function of the binding sites in the receptor system for the chemotactic response to cAMP.

Cyclic AMP acts as a chemotactic factor in the cellular slime mold, Dictyostelium discoideum (1, 2). The sensitivity to cAMP is developmentally regulated, growth-phase cells being about 100-fold less sensitive than aggregating cells (3). This raises the question as to the nature and function of the receptors which enable the cells to orientate in a concentration gradient of cAMP. We have suggested that a membrane-bound phosphodiesterase hydrolyzing extracellular cAMP may be part of the receptor system, responsible for rapid turnover of receptor-bound cAMP (4). The observation that cyclic GMP is a better substrate for D.discoideum phosphodiesterases (5, 6) than expected from its chemotactic activity (2), led to the assumption that the membrane-bound enzyme is functionally associated rather than identical with the receptor. In this

case it should be possible to detect receptor-bound cAMP by applying cAMP in combination with cGMP, which because of the postulated higher selectivity of the receptor should preferentially block the phosphodiesterase. In this paper we report transient cAMP-binding to intact D.discoideum cells in presence of excess cGMP.

METHODS

Cells of the axenic Dictyostelium discoideum strain Ax-2 (7) were cultivated on a gyratory shaker in growth-medium and washed in phosphate buffer to induce differentiation to aggregation-competence (4). Cells harvested within 2 hours after washing were referred to as growth-phase cells, those harvested after 8-12 hours represented aggregation-competent cells. At the time of harvest cells were washed 3 times in imidazol-HCl puffer pH 6.0 and after adjustment to 2 x 10^8 cells/ml immediately shaken at 23°C with either [^{3}H]-cAMP (27 Ci/mmol, Amersham) or a mixture of [^{3}H]-cAMP and unlabeled cGMP. After the times indicated in fig. 1 the incubation mixture was centrifuged for 30 seconds in an Eppendorf microcentrifuge and the supernatant promptly removed for scintillation counting in a triton-toluene scintillator. cAMP-binding to cells was calculated as per cent label removed from the supernatant. These data were calculated on the basis of controls in which the cyclic nucleotides were added to the cell-free supernatant, and corrected for the volume of the cell sediment which was approximately 15 per cent of the total volume of the experimental samples, as determined by centrifugation in hematocrit tubes.

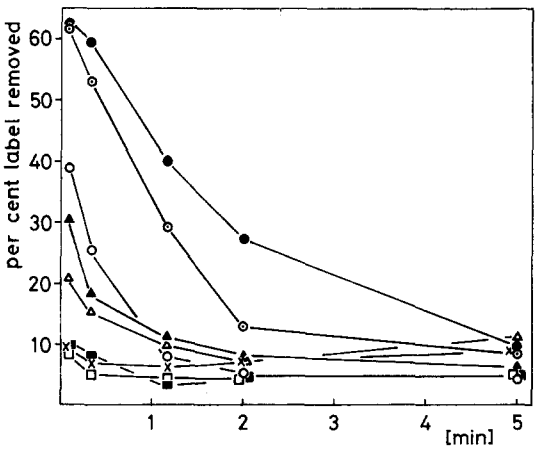

Figure 1. Binding of [^{3}H]-cAMP to living cells of Dictyostelium discoideum, depending on cGMP-concentration and developmental stage.
Ordinate: Label removed from the intercellular medium in per cent of cAMP added; the initial cAMP-concentration was always 1 x 10^{-8}M.
Abscissa: Time of sampling in minutes after cAMP addition.
Concentrations of cGMP, added simultaneously with cAMP:
□ zero; ■ 2 x 10^{-6}M; △ 5 x 10^{-6}M; ▲ 1 x 10^{-5}M; ○ 2 x 10^{-5}M; ⊙ 5 x 10^{-5}M; ● , × 5 x 10^{-4}M.
× growth-phase cells, the other symbols refer to aggregation-competent cells. ⊙ and ● represent means from 3 experiments, × from 4 experiments, the other symbols single determinations.

RESULTS AND DISCUSSION

Virtually no binding was observed when aggregation-competent cells were incubated with 10^{-8}M [^{3}H]-cAMP in the absence of cGMP. When, however, unlabeled cGMP was added simultaneously with cAMP, cAMP-binding became detectable and increased with rising cGMP concentrations (fig. 1). This indicates that the binding is due to the action of receptor sites sharply discriminating between cAMP and cGMP, rather than to unspecific nucleotide binding. The amount of cell-bound cAMP reached a peak within seconds after cAMP application. Subsequently the label was released into the extracellular medium at a rate declining with in-

creasing cGMP concentrations. The biological relevance of the cAMP binding sites detectable under cGMP excess is indicated by the inability of growth-phase cells to bind significant quantities of cAMP under the same conditions (fig. 1).

cAMP-binding protein(s) (8) as well as a cAMP-activated ATP-pyrophosphohydrolase (9) have been found in homogenates of D.discoideum cells. Conceivably these cells contain a number of different cAMP-binding proteins, e.g. cAMP-dependent proteinkinases, not all of them necessarily receptors for the chemotactic response. Criteria for a receptor would be (1) interaction in living cells with external cAMP, (2) developmental regulation, (3) specificity and (4) sensitivity. The sites responsible for cAMP-binding in presence of cGMP appear to fulfil these requirements. Experiments are in progress to establish a correlation between the release of cAMP bound to the cells, and its hydrolysis by membrane-bound phosphodiesterase.

ACKNOWLEDGEMENTS

We thank Misses Janet Fuchila, Karin Reinhardt and Mrs. Maria Bürk for valuable cooperation. The work was supported by the Deutsche Forschungsgemeinschaft and Stiftung Volkswagenwerk.

REFERENCES

1. Konijn, T.M., van de Meene, J.G.C., Bonner, J.T. and Barkley, D.S., Proc. Natl. Acad. Sci. U.S. 58, 1152 (1967).
2. Konijn, T.M. in: Advances in Cyclic Nucleotide Research, (Greengard, P., Robison, G.A. and Paoletti, R., eds.) Raven Press, New York, vol. 1, p. 17 (1972).

3. Bonner, J.T., Barkley, D.S., Hall, E.M., Konijn, T.M., Mason, J.W., O'Keefe, G. and Wolfe, P.B., Developm. Biology 20, 72 (1969).
4. Malchow, D., Nägele, B., Schwarz, H. and Gerisch, G., European J. Biochem. 28, 136 (1972).
5. Riedel, V., Malchow, D., Gerisch, G. and Nägele, B., Biochem. Biophys. Res. Commun. 46, 279 (1972).
6. Malchow, D., Fuchila, J. and Jastorff, B., FEBS letters 34, 5 (1973).
7. Watts, D.J. and Ashworth, J.M., Biochem. J. 119, 171 (1970).
8. Malkinson, A.M., Kwasniak, J. and Ashworth, J.M., Biochem. J. 133, 601 (1973).
9. Rossomando, E.F. and Sussman, M., Proc. Natl. Acad. Sci. U.S.A. 70, 1254 (1973).