DETERGENT SOLUBILIZES THE DISSOCIATED CATALYTIC UNIT OF TESTICULAR ADENYLATE CYCLASE

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Received 8 January 1981

1. Introduction

Membranes prepared from mature rat testes contain adenylate cyclase which is similar to that of other eukaryotic cells. It is stimulated by guanine nucleotide, fluoride and hormones [1–3]. The enzyme uses Mg · ATP as the substrate and is activated 2–3-fold by addition of Mn^{2+} to the assay [2]. Upon solubilization with the non-ionic detergents Triton X-100 or Lubrol 12A9 the properties of testicular adenylate cyclase become different not only from the membrane-bound form but from the properties of detergent-solubilized enzyme from most other cells*. The detergent-solubilized testicular enzyme can no longer effectively use Mg · ATP as the substrate but is activated 11-fold by 5 mM Mn^{2+} [2]. It is unresponsive to guanosine 5′(β,γ-imino)-triphosphate (Gpp(NH)p)[2] or fluoride [2]. These properties are very similar to those reported for the isolated catalytic unit of adenylate cyclase [4–7]. Activation of adenylate cyclase by guanine nucleotides and fluoride is mediated by a protein, the guanine nucleotide regulatory unit (G/F) which is separate form the catalytic unit [4,8]. Addition of G/F unit from bovine brain can reconstitute the testicular enzyme into a form which uses Mg · ATP as substrate and is activated by Gpp(NH)p. Therefore, detergent treatment seems physically or functionally to dissociate the adenylate cyclase catalytic unit from the endogenous G/F unit.

Preparation of the adenylate cyclase catalytic units had required the availability of mutant cell lines [4], affinity chromatography [8] or gel filtration in cholate at high ionic strength [5,6]. These experiments show that a detergent extract of testicular membranes is a convenient new source of adenylate cyclase catalytic units for use in reconstitution experiments.

Abbreviations: G/F, the component of the adenylate cyclase system which mediates activation of the catalytic unit by guanine nucleotides and fluoride; Gpp(NH)p, guanosine 5′(β,γ-imino)-triphosphate; EDTA, ethylenediaminetetraacetic acid

* In addition to membrane-bound adenylate cyclase, the rat testis also contains a naturally soluble form of the enzyme. This unique adenylate cyclase is a globular protein of 56 000 M_r [2]. It is inactive with Mg^{2+} as the only divalent cation and requires Mn^{2+} for expression of activity; it cannot be stimulated by guanine nucleotides or by fluoride [1,2]. Attempts to reconstitute the responsiveness of the naturally soluble enzyme with G/F units from S49 lymphoma cells were not successful [3]

2. Methods

Adenylate cyclase was solubilized from mature rat testes as in [2] except that Lubrol 12A9 was used instead of Triton X-100. G/F unit, separated from the adenylate cyclase catalytic unit, was prepared from bovine cerebral cortex as in [5]. Adenylate cyclase activity was measured by a modification of the method in [9] as in [2]. The assay mixture contained 0.8 mM [^{3}H]ATP, 80–95 cpm/pmol, 0.05–0.1 M Tris–HCl (pH 7.6), 0.1% bovine serum albumin, 1.6 mM cyclic AMP, 0.4 mg creatine kinase/ml (35 units/mg), 12 mM creatine phosphate, 12 mM Mg^{2+} and where indicated, 5 mM Mn^{2+}. Protein was determined by the method in [10] as modified [11] with bovine serum albumin as the standard.

3. Results and discussion

Fig.1 shows that addition of G/F unit from bovine brain can restore Gpp(NH)p responsiveness to detergent-solubilized adenylate cyclase from rat testis.

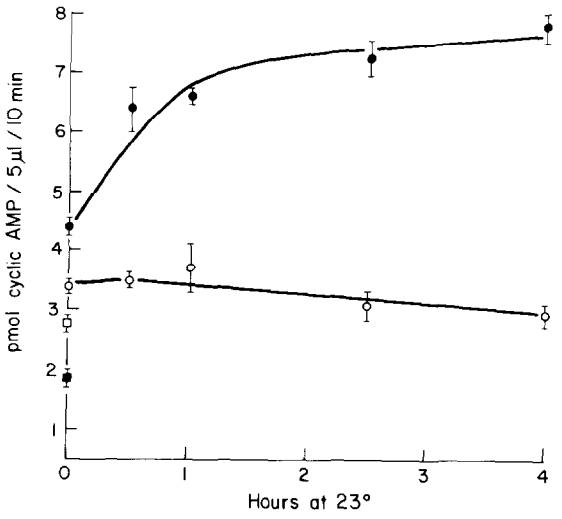

Fig.1. Time course of activation by Gpp(NH)p of reconstituted testicular adenylate cyclase. Adenylate cyclase was solubilized from mature rat testes with Lubrol 12A9 in 0.1 M Tris–HCl (pH 7.6), 0.075 M sucrose, 10 mM $MgCl_2$, 1 mM EDTA[2], 1 mM dithiothreitol as in [2]. The incubation mixture contained 5 µl soluble enzyme (0.03 mg protein), 20 µl bovine brain G/F unit in phospholipid buffer prepared as in [5] or 20 µl phospholipid buffer alone. Phospholipid buffer contained: 7 mg soy bean phosphatidylcholine/ml (Sigma, P5638); 0.1 M Tris–HCl, (pH 7.6), 0.075 M sucrose, 15 mM $MgCl_2$, 1 mM dithiothreitol, 0.1% Lubrol 12A9. The samples were made 1.6×10^{-5} M in Gpp(NH)p and incubated at 23°C for the time indicated. Control samples were also prepared without Gpp(NH)p. A mixture (20 µl) containing the adenylate cyclase assay reagents (see section 2) was then added and enzyme activity measured at 30°C for 10 min. Testicular adenylate cyclase and brain G/F unit: with Gpp(NH)p (•); without Gpp(NH)p (▪). Testicular adenylate cyclase and phospholipid buffer: with Gpp(NH)p (○); without Gpp(NH)p (□). The basal acitivity of testicular adenylate cyclase was lower with added G/F than with phospholipid buffer, probably because of carryover of a small amount of cholate with G/F. The data are given as the mean and range of duplicate assays. The experiment is representative of 3 similar ones.

Activation of the reconstituted enzyme occurs slowly, requiring several hours of incubation at 23°C for full activation. Similar kinetics were observed in this laboratory for membrane-bound testicular adenylate cyclase [2] as well as for membrane-bound and detergent-soluble adenylate cyclase from bovine brain [12].

The Gpp(NH)p-activated enzyme is able to use Mg · ATP as the substrate. The ratio of activity with 5 mM Mn^{2+} to activity with 12 mM Mg^{2+} falls from 11 in the soluble enzyme to 5 in the reconstituted system (table 1). The ratio before reconstitution is similar to that reported for the catalytic unit from functionally G/F-deficient S49 lymphoma cells [4] and for the isolated catalytic unit from bovine cerebral cortex [5].

Table 1
Effect of Mn^{2+} on the activity of testicular adenylate cyclase (pmol cyclic AMP . mg^{-1} . 10 min^{-1}) before and after reconstitution with G/F unit from bovine brain

Preparation	Adenylate cyclase activity		Activity ratio
	12 mM $MgCl_2$	12 mM $MgCl_2$ +5 mM $MnCl_2$	$\frac{(Mn^{2+} + Mg^{2+})}{(Mg^{2+})}$
A. Detergent-solubilized testicular adenylate cyclase	70 ± 20 (6)	770 ± 60 (6)	11
B. Detergent-solubilized testicular adenylate cyclase and G/F unit from bovine brain	200 ± 30 (4)	980 ± 20 (3)	5

Mixtures of soluble adenylate cyclase and G/F unit from bovine brain or phospholipid buffer were made up exactly as in fig.1. The reconstituted samples (B) were incubated with 1.6×10^{-5} M Gpp(NH)p for 4 h and then assayed for 10 min at 30°C with or without the addition of 5 mM $MnCl_2$ to the adenylate cyclase assay mixture. The ratio of activity with Mn^{2+} and Mg^{2+}/Mg^{2+} remained 10–11 if the solubilized, unreconstituted enzyme (A) was incubated with Gpp(NH)p or if the reconstitution mixture (B) was incubated without Gpp(NH)p

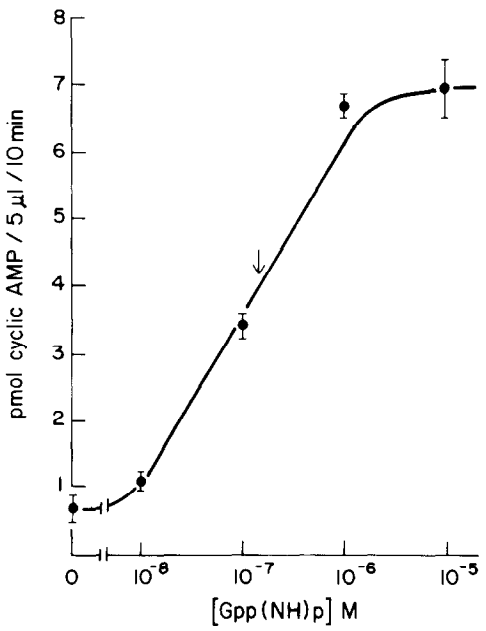

Fig.2. Gpp(NH)p concentration dependence of reconstituted adenylate cyclase. Mixtures of soluble testicular adenylate cyclase and G/F unit from bovine were made up exactly as in fig.1. Gpp(NH)p was added at the concentrations indicated in the figure. The mixtures were incubated at 23°C for 4 h, adenylate cyclase assay reagents added and enzyme activity measured for 10 min at 30°C. The soluble testicular enzyme contributed 0.03 mg protein/assay tube. The data is given as the mean and range of duplicate determinations. This experiment is representative of 3 similar ones.

Fig.2 shows the Gpp(NH)p concentration dependence of the reconstituted enzyme. Half-maximal activation occurs at $1.1 \pm 0.2 \times 10^{-7}$ M Gpp(NH)p (n=3). This is similar to values reported in other systems and is similar to that which we have found for the reconstituted bovine brain enzyme [5].

The observation that guanine nucleotide responsiveness can be restored to the detergent-solubilized testicular enzyme by addition of exogenous G/F component from bovine brain suggests that detergent treatment either dissociates adenylate cyclase catalytic unit from the endogenous G/F unit or inactivates the testicular regulatory protein. The observation is consistent with the idea that a high ratio of activity with Mn^{2+} to activity with Mg^{2+} is an indication of physical or functional dissociation of the catalytic and guanine nucleotide regulatory units.

The fact that bovine brain can provide G/F units which effectively interact with the adenylate cyclase catalytic unit from rat testis emphasize the fact that the interfaces between these components must be highly conserved in the course of evolution. Thus, the present studies show that the mature rat testis is a convenient source of adenylate cyclase catalytic unit which can be solubilized and used as a probe for G/F units from other tissues.

References

[1] Braun, T. and Dods, R. F. (1975) Proc. Natl. Acad. Sci. USA 72, 1097–1101.
[2] Neer, E. J. (1978) J. Biol. Chem. 253, 5808–5812.
[3] Catt, K. J. and Dufau, M. L. (1976) in: Hormone–Receptor Interactions: Molecular Aspects (Levey, G. S. ed) p. 171, Marcell Dekker, New York.
[4] Ross, E. M., Howlett, A. C., Ferguson, K. M. and Gilman, A. G. (1978) J. Biol. Chem. 253, 6401–6412.
[5] Strittmatter, S. and Neer, E. J. (1981) Proc. Natl. Acad. Sci. USA in press.
[6] Ross, E. M. (1981) J. Biol. Chem. in press.
[7] Pfeuffer, T. (1979) FEBS Lett. 101, 85–89.
[8] Pfeuffer, T. and Helmreich, E. J. M. (1975) J. Biol. Chem. 250, 867–876.
[9] Krishna, G., Weiss, B. and Brodie, B. B. (1968) J. Pharmacol. Exp. Ther. 163, 379–385.
[10] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[11] Bailey, J. L. (1967) in: Techniques in Protein Chemistry, p. 340, Elsevier/North-Holland, Amsterdam, New York.
[12] Neer, E. J. (1978) J. Biol. Chem. 253, 1498–1502.