Biochimica et Biophysica Acta, 391 (1975) 513—516

BBA Report

BBA 61302

DISPERSION OF EPINEPHRINE SENSITIVE AND INSENSITIVE ADENYLATE CYCLASE FROM THE CILIATE *TETRAHYMENA PYRIFORMIS*

SHOUKI KASSIS and SHMUEL H. KINDLER

Department of Microbiology, Tel-Aviv University, Tel-Aviv (Israel)

(Received March 24th, 1975)

Summary

The membrane bound adenylate cyclase (ATP pyrophosphate-lyase (cyclizing) EC 4.6.1.1) of the ciliate *Tetrahymena pyriformis* could be extracted by washing the membrane fraction with 0.25 M sucrose. The enzyme dissociated in this way did not sediment after centrifugation at 105 000 × *g* for 2 h and was still responsive to stimulation by epinephrine. Dispersion of the membranes with Triton-X 100 led to purified preparation of the cyclase, which was no longer stimulated by epinephrine but retained fully the activation by fluoride and serotonin.

In a previous communication from this laboratory [1] some properties of adenylate cyclase from the ciliate *Tetrahymena pyriformis* were reported. The adenylate cyclase activity was associated with the membrane fraction obtained after low speed centrifugation of the sonicated cells and could be activated by epinephrine, serotonin and NaF. Furthermore the β-adrenergic blocking agent propanolol abolished the stimulation produced by epinephrine but not by serotonin or NaF. This indicated strongly that the specific stimulation by the neurohormone epinephrine is mediated through a β-type adrenergic receptor. Adenylate cyclase from mammalian sources is usually firmly bound to membranes and has been solubilized by means of non-ionic detergents in only a few cases [2,3,4]. These solubilized cyclases were either unresponsive or less responsive to stimulation by the hormones which activated these enzymes when bound to membranes. It was thus of interest to see if the adenylate cyclase from *T. pyriformis* could be dissociated from the membrane fraction and then to examine the response to stimulatory substances.

Crude adenylate cyclase from *T. pyriformis* was prepared as described previously [1] except that the membrane fraction was suspended in neutralized sucrose 0.25 M. Adenylate cyclase activity was assayed by measuring the conversion of [^{3}H]ATP to cyclic [^{3}H]AMP [5]. Protein was estimated by the procedure of Lowry et al. [6] using bovine serum albumin as standard.

About 75% of the total adenylate cyclase activity of *T. pyriformis* is found in the crude membrane fraction of the sonicate, sedimenting at 610 × *g*. The remaining 25% of the total activity can be detected in the supernatant fraction. The activity found in the 610 × *g* supernatant could also be stimulated by epinephrine as well as by NaF and might have arisen from small membrane fractions detached during sonication or a soluble cytosol enzyme. In order to see if the cyclase can be easily detached from the heavy membrane fraction, the 610 × *g* pellet was washed four times either with 0.25 M sucrose or 40 mM Tris buffer, pH 7.3. In either case considerable activity was found in the washings. Differential centrifugation showed that little of the cyclase activity sedimented after 20 000 × *g* for 15 min or even 105 000 × *g* for 2 h centrifugation (Table I).

TABLE I

PURIFICATION OF ADENYLATE CYCLASE FROM *T. PYRIFORMIS*

T. pyriformis cells from 400 ml of culture were washed in 0.15 M KCl, resuspended in 20 ml of 0.25 M sucrose and sonicated. The heavy membrane fraction, the 610 × *g* pellet, was washed four times with 0.25 M sucrose, total volume 14 ml. These washings were then subjected to differential centrifugation.

Enzyme preparation	Addition to incubation mixture	Total activity cyclic AMP formed (nmol/30 min)	Adenylate cyclase specific activity (cyclic AMP formed) (nmol/mg protein/30 min)
Membranes	None	141.3	2.15
Sedimenting at	10 mM NaF	573.8	8.7
610 × *g*	10 μM epinephrine	530.6	8.1
610 × *g* supernatant	None	44.9	0.3
	10 mM NaF	195.5	1.2
	10 μM epinephrine	161.5	1.0
20 000 × *g* supernatant	None	120.4	17.2
of washings	10 mM NaF	406.3	58.0
	10 μM epinephrine	312.9	44.7
105 000 × *g* supernatant	None	104.7	16.6
of washings	10 mM NaF	349.6	55.4
	10 μM epinephrine	269.1	42.8

Since in all of these fractions the cyclase activity was stimulated by 10 mM fluoride and 10 μM epinephrine and the ratio of epinephrine to NaF stimulation remained constant, the postulated β-receptor appears to be an integral part of this cyclase preparation (see Table I).

Since not all of the cyclase found in the membranes could be detached by washing, solubilization with detergent was attempted. After treatment of the heavy membrane fraction with 1% Triton X 100 and centrifugation at 165 000 × *g* for 2 h about 90% of the recovered activity could be found in the soluble fraction.

The Triton X 100 dispersed enzyme does not respond any longer to epinephrine (Table II). It would thus appear that the action of the detergent

TABLE II

TRITON DISPERSED ADENYLATE CYCLASE FROM *T. PYRIFORMIS*

T. pyriformis cells from 400 ml culture were washed in 0.15 M KCl, resuspended in 20 ml of 0.25 M sucrose and sonicated. The heavy membrane fraction, the 610 × *g* pellet, was suspended in 6 ml 1 mM $NaHCO_3$ and 6 ml of 1% Triton X 100 were added. The suspension was homogenized in a glass-teflon homogenizer for 10 min in ice and centrifuged at 165 000 × *g* for 2 h. The supernatant was further fractionated on a DEAE cellulose column as described in Fig.1.

Enzyme preparation	Addition to incubation mixture	Adenylate cyclase specific activity (cyclic AMP formed) (nmol/mg protein/30 min)
165 000 × *g* supernatant	None	11.1
	NaF 10 mM	32.7
	Epinephrine 10 μM	10.6
	Serotonin 100 μM	27.9
DEAE cellulose Fraction 13	None	140.0
	NaF 10 mM	447.0
	Epinephrine 10 μM	129.2

removed or inactivated the β-receptor. Interestingly enough the Triton X 100 solubilized enzyme can still be stimulated by serotonin. This is in agreement with the observation that stimulation by serotonin is not exerted via the β-receptor since it has been shown previously that the β-adrenergic blocker propanolol does not inhibit activation by this neurohormone [1]. The enzyme was further purified on DEAE cellulose column (Fig. 1). The fraction containing the highest activity had a 54 fold higher specific activity than the crude membrane fraction with respect to fluoride stimulation (Table II).

Mammalian adenylate cyclase can not be easily dissociated from membranes [7]. In contrast the enzyme from bacteria is either soluble or associated with particles, possibly membranes, from which it can be easily dissociated by washing [8]. In this respect the cyclase from *T. pyriformis* is more similar to the bacterial enzyme. It seems to be associated with membranes but the binding is not very tight. Since in bacteria and in the ciliate the

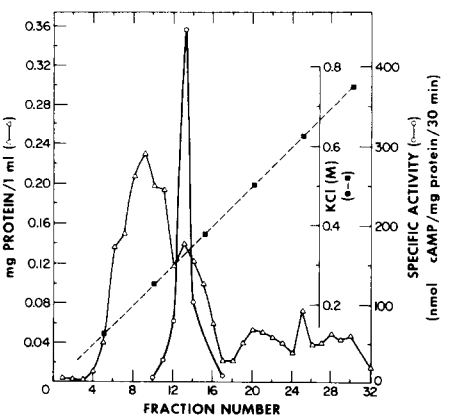

Fig.1. Detergent-dispersed enzyme. Chromatography of adenylate cyclase from *Tetrahymna pyriformis* on DEAE cellulose. The column 1.5 cm × 10 cm was equilibrated with 50 mM Tris·HCl buffer. The 165 000 × *g* supernatant of Triton X-100 membranes extract (10 mg protein/ml) was applied to the column. The column was then developed with 80 ml of a linear gradient from 0—0.8 M KCl in the above mentioned buffer. 2.5 ml fractions were collected and assayed for protein and cyclase activity.

cyclase plays a role only in internal regulation and need not be accessible to external hormones, the tight binding due to location inside the outer membrane, found in higher organisms, is not required here.

On the other hand the *T. pyriformis* enzyme appears to have a regulatory subunit like that of higher organisms [9]. This subunit has the properties of a β-receptor and does not easily dissociate from the catalytic component.

Experiments are now in progress to see if the epinephrine sensitivity of the Triton solubilized enzyme can be restored by addition of phospholipids as was demonstrated by Levey for the solubilized adenylate cyclase from cat myocardium [2].

References

1 Rosenzweig, Z. and Kindler, S.H. (1972) FEBS Lett. 25, 221—223.
2 Levey, G.S. (1971) J. Biol. Chem. 246, 7405—7407.
3 Neer, E.J. (1973) J. Biol. Chem. 248, 3742—3744.
4 Swislocki, N.I. and Tierney, J. (1973) Biochemistry 12, 1862—1866.
5 Krishna, G., Weiss, B. and Brodie, B.B. (1968) J. Pharmacol. Exp. Ther. 163, 379—389.
6 Lowry, O.H., Rosenbrough, A.L., Farr, A.L. and Randall, R.L. (1951) J. Biol. Chem. 193, 265—275.
7 Sutherland, E.W., Rall, T.W. and Menon, T.J. (1962) J. Biol. Chem. 237, 1220—1227.
8 Tao, M. and Lipmann, F. (1969) Proc. Natl. Acad. Sci. U.S. 63, 86—92.
9 Perkins, J.P. (1973) in Advances in Cyclic Nucleotide Research (Greengard, P. and Robinson, G.A., eds), Vol. 3, pp. 1—64, Raven Press, New York.