Biochimica et Biophysica Acta, 658 (1981) 64—75

BBA 69222

RAT BRAIN GUANYLATE CYCLASE

PURIFICATION, AMPHIPHILIC PROPERTIES AND IMMUNOLOGICAL CHARACTERIZATION

J. ZWILLER, P. BASSET and P. MANDEL

Centre de Neurochimie du CNRS, 5 rue Pascal, 67084 Strasbourg Cedex (France)

(Received July 4th, 1980)

Key words: Guanylate cyclase; Amphiphilic property; (Rat brain)

Summary

Soluble guanylate cyclase (GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2) has been purified to apparent homogeneity from rat brain by chromatography on Blue-Sepharose CL-6B, precipitation with $(NH_4)_2SO_4$, preparative isoelectric focusing and gel-filtration on Ultrogel AcA-34. On sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis the purified enzyme showed a single band with an apparent molecular weight 59 000, when stored in buffer without glycerol and 2-mercaptoethanol. Purified enzyme has been found to be very unstable; inactivation can however be partially reversed by an endogenous heat-stable activator fraction. A monospecific antiserum obtained by immunization of rabbits was found to precipitate guanylate cyclase. This antibody also reacted with membrane-bound enzyme, indicating a close similarity to the soluble enzyme.

Metal divalent cations were in general found to be strong inhibitors of the enzyme activity, though Ca^{2+} had no effect. ATP, CTP or UTP were shown to be competitive inhibitors of purified guanylate cyclase: Sodium nitroprusside increased cyclic GMP formation by the purified enzyme. Lysophosphatidylcholine and oleic acid, at low concentration, activated guanylate cyclase. Other unsaturated fatty acids, particularly arachidonic acid, dramatically inhibited the enzyme activity. Lipids may regulate the enzyme activity by binding to an apolar domain, as suggested by charge-shift electrophoresis.

The mechanism by which guanylate cyclase is regulated in the cell appears to be a complex phenomenon. It may occur through oxidative reductive processes, and/or depend on other effectors, such as triphospho-nucleotides, divalent cations and lipid microenvironment.

A number of hormones and biologically active substances are known to promote cellular accumulation of cyclic GMP (for review, see Ref. 1) and there is evidence that this accumulation may result chiefly from an increased activity of guanylate cyclase (GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2). In the homogenates of most animal tissues, guanylate cyclase is recovered in both particulate and soluble fractions [2,3]. A number of agents stimulate both particulate and soluble forms of the enzyme: azide, nitroprusside, nitroglycerin and nitrosamines [4—6]. However, only a few physiological agents have been shown to produce an activation of guanylate cyclase in disrupted cell preparations. Among them, phospholipase A_2, which degrades phosphatidylcholine to lysophosphatidylcholine and fatty acids, has been reported to stimulate particulate guanylate cyclase [7]. Crude guanylate cyclase from several tissues is also stimulated by lysophosphatidylcholine [8—10] and by various unsaturated fatty acids [11—15].

The preparation of the pure enzyme is a prerequisite to the elucidation of the precise regulation mechanism of these agents, and thus of the particular role of cyclic GMP in mammalian central nervous system [16]. We report here a relatively simple procedure for the purification to apparent homogeneity of soluble rat brain guanylate cyclase. The effects of triphosphonucleotides, nitroprusside, lysophosphatidylcholine and fatty acids were tested. It is demonstrated that these agents act directly on the purified enzyme. Immunological studies suggest that soluble guanylate cyclase derives from the membrane-bound enzyme. Some of these results have been reported briefly elsewhere [17].

Experimental procedures

Materials

Unlabelled nucleotides, phosphocreatine and creatine kinase were purchased from Boehringer, all labelled nucleotides from New England Nuclear Corp., alumina (W 200-neutral) from Woelm, phospholipids and fatty acids from Sigma, complete Freund's adjuvant from Merieux, agarose, Blue-Sepharose CL-6B, activated Thiol-Sepharose 4B, Octyl-Sepharose from Pharmacia, Ultrogel AcA-34, Ampholines and Ultrodex from LKB, Dowex from Roth and Zwittergent 3—14 from Calbiochem.

Methods

Assay of guanylate cyclase. Guanylate cyclase was determined as reported previously [18]. Reaction mixtures (130 μl) contained: 15 mM phosphocreatine/75 · μg creatine kinase/cyclic [^{3}H]GMP (3 mM, 2.1 · 10^4 cpm)/7 mM $MnCl_2$/[α-^{32}P]GTP (0.185 mM, 0.5 · 10^6—1 · 10^6 cpm)/60 mM Tris-HCl, pH 7.5 in addition to the enzyme preparation and were incubated at 37°C for 10 min. The reaction was terminated by adding unlabelled GTP (0.5 μmol) in 4 mM EDTA and immersion in a boiling-water bath for 3 min. No measurable hydrolysis of added cyclic GMP occurred. Cyclic GMP was separated from other labelled compounds by successive chromatography on alumina and Dowex 1 X2 columns. All results were corrected for recovery of cyclic [^{3}H]GMP (50—65%). Protein was determined by the method of Lowry et al. [19] with bovine serum albumin as standard.

For lipid treatment, the enzyme was preincubated with the indicated amount of lipid. An alcoholic solution of the lipid was dried in a stream of N_2 before adding guanylate cyclase. After 10 min preincubation at 0°C, the mixture was diluted 3-fold with 10 mM Tris-HCl, pH 7.5. An aliquot was taken for immediate determination of enzyme activity.

Purification of guanylate cyclase. Female Wistar rats were decapitated and the brains removed, weighed and homogenized with 8 vol. cold buffer A (10 mM Tris-HCl, pH 7.5 buffer/10 mM 2-mercaptoethanol/1 mM $MnCl_2$/5 mM $MgCl_2$/0.1 mM EDTA/10% glycerol) in a glass homogenizer with a Teflon pestle at 2000 rev./min. The supernatant of a brain homogenate centrifuged at 2000 × *g* for 10 min was centrifuged again at 100 000 × *g* for 60 min and the resulting supernatant was chromatographed on Blue-Sepharose CL-6B. The fractions containing guanylate cyclase activity were pooled and proteins precipitated by adding solid $(NH_4)_2SO_4$ up to 50% saturation (at +4°C). The precipitate was collected by centrifugation, resuspended in 5 ml chilled buffer A and dialysed for 6 h against buffer A at 4°C. The dialysed protein solution was then submitted to preparative isoelectric focusing. After localizing the focused protein bands with a paper print of the gel surface [20], 30 fractions of the gel were collected and the proteins were eluted from each fraction with 3 ml buffer A. Two major peaks of guanylate cyclase activity, here called form A and B of the enzyme, were obtained. Active fractions from each peak were collected and were separately applied to an Ultrogel AcA-34 column (80 × 2.9 cm). Elution was performed with buffer A containing 50 mM Tris-HCl, pH 7.5 at a rate of 18 ml/h, and 4 ml fractions were collected. The fractions containing guanylate cyclase activity were pooled, precipitated with solid $(NH_4)_2SO_4$ (50% saturation) and centrifuged. The precipitate was resuspended in 3.6 ml buffer A containing 50% glycerol and stored at −20°C.

Preparation of the activator fraction. Rat brains were homogenized in 3 vol. 10 mM Tris-HCl, pH 7.5. The homogenate was centrifuged at 100 000 × *g* for 60 min and the resulting supernatant was placed in a boiling water bath for 5 min, centrifuged at 8000 × *g* for 30 min, the precipitated protein discarded, and the supernatant, devoid of guanylate cyclase activity, was used as activator fraction [21].

Electrophoresis. Analytical gel electrophoresis under denaturing conditions was performed in horizontal slabs of acrylamide (115 × 115 × 3 mm). Slabs of 10% separating and 3% stacking gels were prepared in 370 mM Tris-HCl buffer, pH 8.6/0.1% SDS (w/v). *N,N'*-Methylene-bisacrylamide was 3% (w/w) of total acrylamide; gels were polymerized with ammonium peroxodisulfate and *N,N,N',N'*-tetramethylenediamine. The buffer in the electrolytic compartments was the same as in the gel. Samples were heated in 1% SDS/1% mercaptoethanol/25 mM Tris-HCl buffer (pH 8.6)/100 mM sucrose/0.01% bromphenol blue (w/v) at 80°C for 10 min and were then applied at the cathodic end of the gel. Electrophoresis was performed overnight at 4°C with 50 mA/gel on a flat bed apparatus. Gels were stained with 0.05% Coomassie brilliant blue R-250 in 40% methanol/5% acetic acid, and subsequently destained in 40% methanol/10% acetic acid.

Charge-shift electrophoresis was performed as described by Helenius and Simons [22] and Bjerrum et al. [23]. The method is based on the observation

that amphiphilic detergent-binding proteins exhibit bi-directional migration changes when electrophoresed in the presence of Triton and an anionic (deoxycholate) or a cationic (cetyltrimethylammonium bromide) detergent, compared with their migration in Triton alone. Electrophoresis was carried out in a 1.5 mm thick 1% (w/v) agarose C gel in 75 mM Tris-barbital pH 8.6 buffer, until human haemoglobin C had migrated 1 cm. Native haemoglobin migrates identically in all three electrophoretic systems and thus constitutes a convenient marker.

Immunological techniques. Antibodies against guanylate cyclase from rat brain were raised in rabbits by subcutaneous injection of 0.4 mg pure enzyme in complete Freund's adjuvant. Three booster injections were given at 3-week intervals. On the 7th day after the last injection, the animals were bled, the blood was allowed to clot by standing overnight at 4°C. Serum was obtained by centrifugation at $1000 \times g$ for 15 min and the γ-globulin fraction was purified by $(NH_4)_2SO_4$ fractionation as described by Campbell et al. [24].

Double immunodiffusion in 1% agarose A in barbiturate buffer, pH 8.6 was carried out in Ouchterlony plates for 48 h at 4°C [25]. Immunoelectrophoresis was performed according to Grabar and Williams [26].

Immunoprecipitation was carried out as follows: fixed amounts of guanylate cyclase were incubated with increasing dilutions of γ-globulin fraction at 37°C for 30 min and at 4°C for a further 60 h. Mixtures were then centrifuged ($3000 \times g$, 30 min) and guanylate cyclase activities determined in the supernatants. A control experiment was performed simultaneously with the purified γ-globulin fraction from non-immunized rabbit.

Results

Guanylate cyclase purification

Guanylate cyclase from the soluble fractions of rat brain has been purified to a specific activity of 72 nmol cyclic GMP formed/min per mg protein. Yields and enrichment of guanylate cyclase activity throughout purification are shown in Table I. Use of Blue-Sepharose chromatography as a first step (Fig. 1), followed by isoelectric focusing (Fig. 2) represents a new, convenient and relatively rapid method for purifying large amounts of guanylate cyclase. Final purification and removal of carrier ampholytes were achieved by gel filtration. During purification two enzymatic forms with p*I* values of 5.95 and 6.3 were obtained by preparative isoelectric focusing (Fig. 2). Both forms showed similar electrophoretic patterns and an identical precipitin band against antibody (see below). Moreover, upon refocusing of each purified form, the initial pattern (two peaks) is still obtained. As it was suggested [27], the two forms may correspond to an interacting system for which several possibilities have been described: ampholyte-induced association or dissociation, ampholyte-induced isomerization or pH-dependent conformational transitions.

Purified guanylate cyclase activity is known to be labile [28—29]. Inactivation of rat brain guanylate cyclase occurred when enzyme (Ultrogel step) was further chromatographed either on activated Thiol-Sepharose and eluted with cysteine or on Octyl-Sepharose and eluted with Triton X-100 (without any change in the electrophoretic pattern). Similar inactivation was also observed

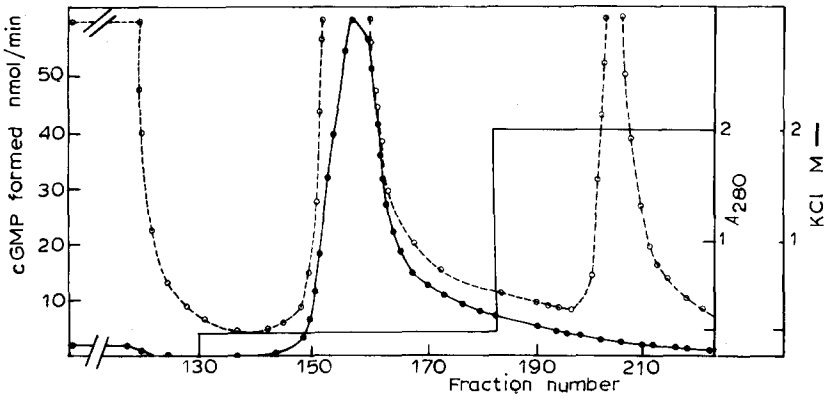

Fig. 1. Brain soluble guanylate cyclase purification on Blue-Sepharose CL-6B column. Brain homogenate supernatant in buffer A was applied to a 20 X 2.6 cm column of Blue-Sepharose CL-6B equilibrated with the same buffer. The column was sequentially eluted with 0.2 and then 2 M KCl in buffer A (elution rate 30 ml/h, 5 ml fractions). Pooled fractions: 152—166. •———•, Guanylate cyclase activity; o- - - - -o, A_{280}.

when purified enzyme (Ultrogel step) was concentrated by $(NH_4)_2SO_4$ precipitation. However, this lower activity (5.6 nmol cyclic GMP formed/min per mg prot) can be increased to 26.7 nmol by adding 45 μg of an endogenous heat-stable activator fraction. This effect was enhanced in presence of $1 \cdot 10^{-3}$ M sodium nitroprusside (from 9.4 to 49.5 nmol).

On SDS-polyacrylamide gel electrophoresis, guanylate cyclase stored with 50% glycerol exhibited a pattern similar to that reported for enzyme isolated from other tissue in the presence of glycerol [30]: two bands with molecular weights of 61 000 and 66 000 (Fig. 3). In contrast, only one band with a lower apparent molecular weight (59 000) was obtained on electrophoresis after removal of glycerol and 2-mercaptoethanol by dialysis. This single band electrophoretic pattern did not revert to the two band pattern on addition of glycerol and 2-mercaptoethanol. It is worthwhile to stress these data with the potent stabilizing effect of glycerol and mercaptoethanol on enzymatic activity during the purification and storage of the enzyme.

TABLE I

PURIFICATION OF SOLUBLE RAT BRAIN GUANYLATE CYCLASE

Fraction	Total protein (mg)	Total activity (mmol/min)	Specific activity (nmol/min per mg protein)
Homogenate	13 840	862	0.06 (1) *
Supernatant (100 000 X *g*)	4250	556	0.13 (2)
Blue-Sepharose	255	251	1.0 (17)
$(NH_4)_2SO_4$	164	220	1.3 (22)
Isoelectrofocusing **	14	108	7.7 (128)
Ultrogel	0.9	65	72 (1200)

* Specific activity relative to homogenate = 1.

** Corresponding to peak B.

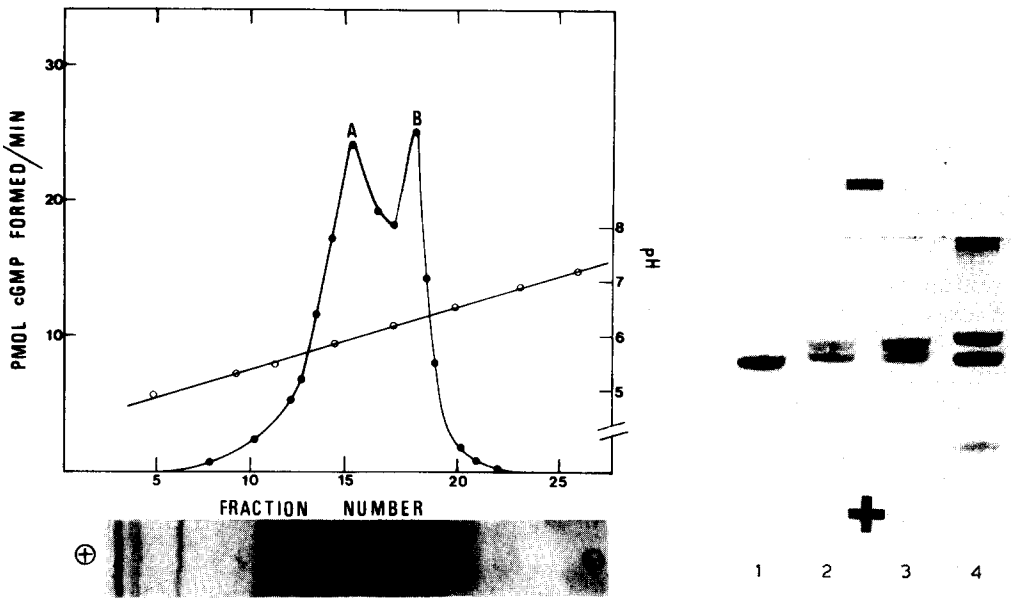

Fig. 2. Preparative isoelectric focusing of guanylate cyclase. Isoelectric focusing was carried out overnight, at a constant power of 8 W. A 4% Ultrodex gel containing 2% ampholine (pH gradient from 5 to 8)/ 10 mM 2-mercaptoethanol/1 mM $MnCl_2$ · 0.1 mM EDTA/15% glycerol was poured onto the LKB gel tray and evaporated to a water loss corresponding to 32% of the initial wet weight. The cooling block was regulated at −3° C. The dialysed enzyme preparation (130—170 mg protein) was applied at the cathodic end (corresponding to fractions 20—22). Peak A corresponds to p*I* 5.95, peak B to p*I* 6.3. •———•, guanylate cyclase activity; ○———○, pH gradient. Bottom: print stained with Coomassie brilliant blue R-250.

Fig. 3. Analytical SDS-polyacrylamide gel electrophoresis of purified guanylate cyclase. Electrophoresis was performed at alkaline pH as described under 'Experimental procedures'. Samples of 4 μg were applied. 1, common pattern of guanylate cyclase (either form A or B) after removal of glycerol and 2-mercaptoethanol by dialysis. 2, guanylate cyclase form B stored under standard conditions (50% glycerol). 3, guanylate cyclase form A stored under standard conditions. 4, standard proteins: from bottom to top, lactate dehydrogenase (M_r = 36 000), catalase (M_r = 60 000) and albumin (M_r = 67 000).

Properties of purified enzyme

Effect of ions. The presence of Mn^{2+} is necessary for optimal guanylate cyclase activity. Maximal activity was observed with 3 mM $MnCl_2$ at 0.185 mM GTP substrate concentration. The affinity of the enzyme for GTP (K_m value 34 μM) was found to be independent of Mn^{2+} concentration. Mg^{2+} is a less efficient cofactor than Mn^{2+}; replacing Mn^{2+} by 5 mM Mg^{2+} resulted in decreased activity (22% of the activity with Mn^{2+}); in the presence of $1 \cdot 10^{-4}$ M nitroprusside, however, purified guanylate cyclase with Mg^{2+} as cofactor had 39% of its activity with Mn^{2+} (data not shown). Ca^{2+} which is known to produce cellular accumulation of cyclic GMP in several systems [1] was not found to have any activating effect on the purified enzyme while Sr^{2+} was slightly activatory (Table II).

Heavy metal cations (Zn^{2+}, Hg^{2+}, Cd^{2+} and Cu^{2+}) were the most potent inhibitors of the activity of purified guanylate cyclase (Table II). This inhibition was partially reversed by dithiothreitol, suggesting a direct effect on free

TABLE II

EFFECT OF SEVERAL DIVALENT CATIONS ON RELATIVE GUANYLATE CYCLASE ACTIVITY

Unless otherwise indicated, concentration of cations is 1 mM. Guanylate cyclase assay system contained 7 mM $MnCl_2$ and 0.185 mM GTP. Activities are relative to that with no additions = 100.

Additions	Guanylate cyclase relative activity
None	100
Zn^{2+}, Cu^{2+}, Cd^{2+}, Hg^{2+}	2—4
Co^{2+}	34
Fe^{2+}	72
Ca^{2+} 0.01 mM	104
0.1 mM	102
1.0 mM	94
Sr^{2+} 0.1 mM	134
1.0 mM	123

thiol groups, present at a high level, as indicated by retention of guanylate cyclase by Thiol-Sepharose.

Effect of triphosphonucleotides. As it was shown in crude preparations [1], ATP, as well as CTP and UTP, inhibited the purified rat brain enzyme. This inhibition is the result of competition for GTP at the substrate site, as indicated by double-reciprocal plots (data not shown).

Effect of nitroprusside. Nitric oxide (NO)-forming products are known to stimulate guanylate cyclase in most crude tissue preparations. Sodium nitroprusside activated purified guanylate cyclase, but to a lesser extent than it did on crude enzyme (Fig. 4). As reported for rat liver [29], the purified enzyme was activated to a greater extent by nitroprusside with Mg^{2+} as cofactor than with Mn^{2+}. The effect of nitroprusside was increased by reducing agents such as dithiothreitol. Dithiothreitol might restore guanylate cyclase to a more reduced form (therefore it is more capable of being activated by nitroprusside), but it may also prevent oxidation of NO formed during the incubation. Our results obtained with the purified enzyme clearly show that, as has been suggested [31], the activation of the enzyme by NO-forming products is due to the direct action of NO on the enzyme molecule.

Effect of lipids-amphiphilic properties. Activation of crude enzyme by low concentrations of unsaturated fatty acids was previously reported in lysates of human platelets [11—13] and in synaptic plasma membranes [15]. Moreover, inhibition of crude guanylate cyclase by high concentrations of unsaturated fatty acids has been described [15]. We investigated the effects of phospholipids and fatty acids on the purified preparation of guanylate cyclase. Phosphatidylcholine and lysophosphatidylethanolamine (egg yolk) slightly inhibited the enzyme activity, while lysophosphatidylcholine activated it (Fig. 5). Maximal activation was observed at 0.5 mg/ml with lysophosphatidylcholine (egg yolk) and at 2 mg/ml with the synthetic lauroyllysophosphatidylcholine. Saturated fatty acids with chain lengths of 14—20 carbon atoms (myristic, palmitic, stearic and arachidic acid) either had no effect or slightly inhibited the purified guanylate cyclase. Each unsaturated fatty acid showed a different concentration-activity curve (Fig. 6). Oleic acid (18 : 1)

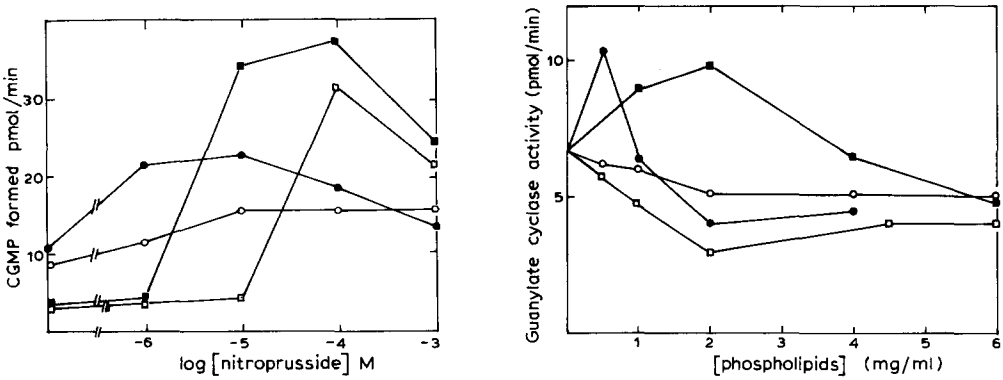

Fig. 4. Effect of sodium nitroprusside on guanylate cyclase activity. Soluble rat brain guanylate cyclase or purified enzyme was incubated with sodium nitroprusside at the concentration indicated, in the standard reaction mixture. Soluble guanylate cyclase without (□——□) and with (■——■) 2 mM dithiothreitol. Purified enzyme without (○——○) and with (●——●) dithiothreitol.

Fig. 5. Effect of phospholipids on the purified rat brain guanylate cyclase. Concentrations indicated are those present in the 10 min preincubation step, as described under 'Experimental procedures'. ●——●, L-α-lysophosphatidylcholine (egg yolk); ■——■, L-α-lauroyl-lysophosphatidylcholine; ○——○, L-α-lysophosphatidylethanolamine (egg yolk); □——□, L-α-phosphatidylcholine (egg yolk).

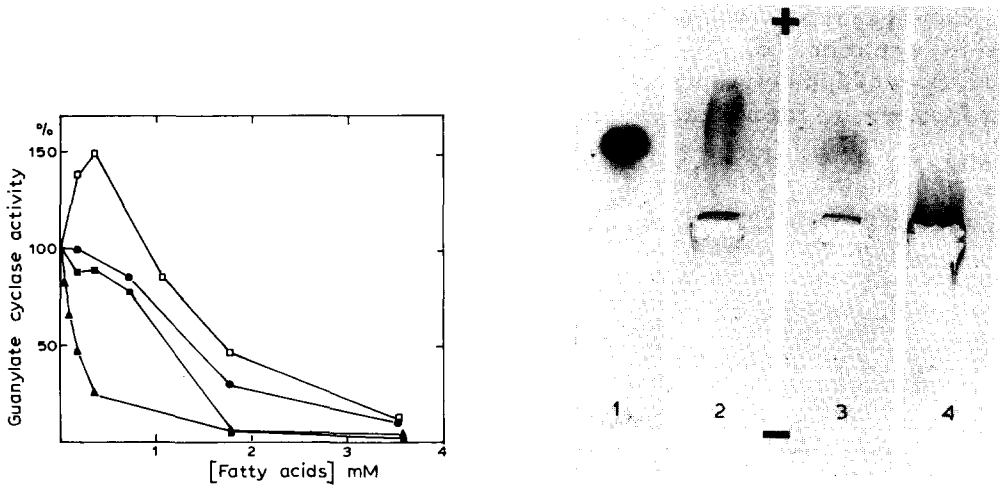

Fig. 6. Effect of unsaturated fatty acids on the purified guanylate cyclase. Guanylate cyclase activity is expressed as a percentage of the control (100%). Enzyme content (25 μg) and fatty acid concentrations indicated are those present in the 10 min preincubation. □——□, oleic acid (18 : 1); ●——●, linoleic acid (18 : 2); ■——■, linolenic acid (18 : 3); ▲——▲, arachidonic acid (20 : 4).

Fig. 7. Charge-shift electrophoresis of purified guanylate cyclase. Electrophoresis was performed at 15°C in 1% agarose C gel at 10 V/cm in 75 mM Tris-barbital buffer, pH 8.6, until haemoglobin had migrated 1 cm. 1, Human haemoglobin C. 2, 3 and 4, 4 μg guanylate cyclase. Electrophoresis with: 2, 0.5% (v/v) Triton X-100 plus 0.2% (w/v) deoxycholate present in agarose and buffer; 3, 0.5% Triton X-100 added to agarose, no detergent in the buffer; 4, 0.5% Triton plus 0.0125% (w/v) cetyltrimethylammonium bromide present in agarose and buffer.

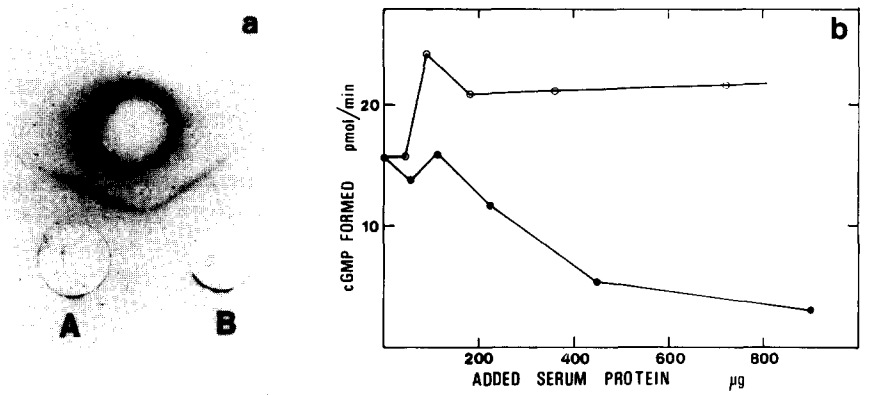

Fig. 8. (a) Double immunodiffusion of purified guanylate cyclase. The upper well contained 70 μg partial purified antibody and the lower wells 5 μg of each form (A and B as indicated) of purified guanylate cyclase. (b) Effect of antibody on purified guanylate cyclase. Enzyme (16 μg) was incubated with indicated quantities of immunized (●———●) or control rabbit (○———○) γ-globulin fraction. Mixtures were centrifuged before determining guanylate cyclase activity in the supernatant as described in 'Experimental procedures'.

stimulated enzyme activity, but the activation was reversed at higher concentration. The more unsaturated fatty acids, linoleic acid (18 : 2), linolenic acid (18 : 3) and arachidonic acid (20 : 4) dramatically inhibited guanylate cyclase in a manner related to their degree of unsaturation. No activation was observed even if arachidonic acid was used at lower concentration. How soluble guanylate cyclase is affected by lipids was further studied by charge-shift electrophoresis. We found that purified soluble guanylate cyclase possesses amphiphilic properties (Fig. 7), since its electrophoretic migration was influenced by the various detergents: greater migration velocity was observed

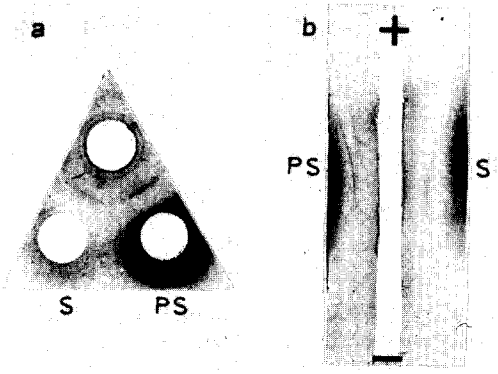

Fig. 9. Immunological characterization of crude guanylate cyclase. Rat brain 100 000 × *g* supernatant was prepared as described in 'Experimental procedures' (fraction S). The corresponding pellet was washed three times with 10 vol. of buffer A at 4°C, resuspended in 8 vol. of the same buffer and Triton X-100 was added at a final concentration of 0.5%. After 1 h incubation at 4°C, tbe mixture was centrifuged (100 000 × *g*, 1 h) and the supernatant recovered (fraction PS). 10 μl fractions S and PS as indicated were analysed (a) by double immunodiffusion and (b) by immunoelectrophoresis in presence of 0.5% (v/v) Triton X-100 using partial purified antibody against soluble guanylate cyclase.

in deoxycholate and lower migration velocity in cetyltrimethylammonium bromide than those seen in Triton alone.

Immunological characterization

Using the Ouchterlony double-diffusion test, a single precipitin band was observed between purified guanylate cyclase and antiserum against purified enzyme (Fig. 8a). Purified guanylate cyclase was precipited by the antibody preparation (Fig. 8b). When crude enzyme was used, we obtained, upon double immunodiffusion, the same precipitin band with the soluble enzyme and with the particulate 0.5% Triton X-100-extracted enzyme (Fig. 9a), showing immunological identity. Using immunoelectrophoresis, one band can also be shown with crude soluble guanylate cyclase (Fig. 9b) and two bands, showing partial identity, with particulate Triton X-100-extracted enzyme. The same results were obtained when the particulate enzyme was extracted with 1% Triton X-100 or with Zwittergent 3-14 (0.5 or 1%), or when the preparation was frozen, subsequently thawed, then twice submitted to hyposmotic shock prior to detergent extraction.

Discussion

Guanylate cyclase has been purified from *Caulobacter crescentus* [32] and *Escherichia coli* [33]. Purification of particulate guanylate cyclase from sea urchin sperm [34] and of the soluble enzyme from bovine lung [35], human platelets [36], rat liver [28—29], rat lung [30] and rat brain [37—38] have also been described. We report here a relatively simple procedure for the purification of rat brain soluble guanylate cyclase to a specific activity of 72 nmol cyclic GMP formed/min per mg protein. This value is of the same order as that reported for rat brain [38] or liver [28—29], and lower than that reported for rat lung [30]. Differences between specific activities of enzymes purified from different sources may be partially explained by the existence of an endogenous heat-stable activator fraction, which we separated from enzyme in rat brain, and which was also previously reported for rat liver [28]. Different degrees of inactivation may also result from different purification procedures used and from enzyme properties which may be dependent on the tissue of origin.

We report here the first preparation of antibody against mammalian soluble guanylate cyclase. The monospecificity of the antibody was demonstrated by double immunodiffusion (Fig. 8a), by immunoelectrophoresis (Fig. 9) and by crossed immunoisoelectric focusing (data not shown). In addition, the antibody preparation precipitated purified guanylate cyclase. All evidence indicating that our antibody is directed against guanylate cyclase and thereby supporting the purity of our enzyme preparation.

Antibody prepared against particulate guanylate cyclase from sea urchin sperm [39] was not found to affect the soluble form and gave two precipitin bands with the particulate enzyme, on Ouchterlony plates. In the present study, the same technique provides one identical precipitin line with both the soluble and the particulate detergent-solubilized rat brain enzyme when soluble rat brain guanylate cyclase antibody was used.

Soluble guanylate cyclase appears to possess a hydrophobic site as shown by its retention by Octyl-Sepharose. Such an apolar domain could also be indicated by the amphiphilic properties of purified soluble enzyme. Lipids may regulate the enzyme activity by binding to this apolar domain. Such a mechanism may also explain the activation of crude enzyme by detergents [13]. The presence of an apolar domain on soluble guanylate cyclase may also suggest [22] that soluble enzyme derives from membrane-bound enzyme. This hypothesis is supported by our immunological data since antibody against soluble guanylate cyclase cross-reacted with detergent-solubilized enzyme: by double immunodiffusion, particulate detergent-solubilized and soluble enzyme gave the same band, indicating some antigenic identity; by immunoelectrophoresis, particulate detergent-solubilized enzyme appeared to consist of two immunologically related components, one migrating in a similar manner to the soluble enzyme. These results indicate that soluble and particulate guanylate cyclase are structurally related and suggest that enzyme, called soluble, represents in fact one form of particulate enzyme detached or released from the membranes.

Acknowledgements

We are most grateful to Mrs. M.O. Revel for technical assistance and to Dr. K. Langley for critically reading the manuscript.

References

1 Goldberg, N.D. and Haddox, M.K. (1977) Ann. Rev. Biochem. 46, 823—896
2 White, A.A. and Aurbach, G.D. (1969) Biochim. Biophys. Acta 191, 686—697
3 Kimura, H. and Murad, F. (1975) J. Biol. Chem. 250, 4810—4817
4 Kimura, H., Mittal, C.K. and Murad, F. (1975) J. Biol. Chem. 250, 8016—8022
5 Katsuki, S., Arnold, W.P., Mittal, C.K. and Murad, F. (1977) J. Cyclic Nucl. Res. 3, 23—35
6 DeRubertis, F. and Craven, P.A. (1977) J. Biol. Chem. 252, 5804—5814
7 Fujimoto, M. and Okabayashi, T. (1975) Biochem. Biophys. Res. Commun. 67, 1332—1336
8 Shier, W.T., Baldwin, J.H., Nilsen-Hamilton, M., Hamilton, R.T. and Thanassi, N.M. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 1586—1590
9 Zwiller, J., Ciesielski-Treska, J. and Mandel, P. (1976) FEBS Lett. 69, 286—290
10 Aunis, D., Pescheloche, M. and Zwiller, J. (1978) Neuroscience 3, 83—93
11 Glass, D.B., Frey, W., Carr, D.W. and Goldberg, N.D. (1977) J. Biol. Chem. 252, 1279—1285
12 Hidaka, H. and Asano, T. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 3657—3661
13 Abigail, F.A. and Haslam, R.J. (1978) Biochem. J. 174, 23—35
14 Wallach, D. and Pastan, A. (1976) J. Biol. Chem. 251, 5802—5809
15 Asakawa, T., Takenoshita, M., Uchida, S. and Tanaka, S. (1978) J. Neurochem. 30, 161—166
16 Ferrendelli, J.A. (1978) Adv. Cyclic Nucl. Res. 9, 453—464
17 Zwiller, J., Basset, P. and Mandel, P. (1979) Symposium 'Cyclic Nucleotides and Therapeutic Perspectives' (Cehovic, G. and Robison, G.A., eds.), pp. 225—233, Pergamon Press, Oxford
18 Goridis, C., Zwiller, J. and Reutter, W. (1977) Biochem. J. 164, 33—39
19 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
20 Winter, A. (1977) in Electrofocusing and Isotachophoresis (Radola, B.J. and Graesslin, D., eds.), pp. 433—442, W. de Gruyter, Berlin
21 Takehawa, T. and Sacktor, B. (1979) Biochim. Biophys. Acta 566, 371—384
22 Helenius, A. and Simons, K. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 529—532
23 Bjerrum, O.J., Helle, K.B. and Bock, E. (1979) Biochem. J. 181, 231—237
24 Campbell, D.H., Garvey, J.S., Cremer, N.E. and Sussdorf, D.H. (1964) in Methods in Immunology, pp. 118—124, Benjamin, W.A., New York
25 Clausen, J. (1971) in Laboratory Techniques in Biochemistry and Molecular Biology (Work, T.S. and Work, E., eds.), Vol. 1, pp. 521—522, North-Holland Publishing Co., Amsterdam

26 Grabar, P. and Williams, C.A. (1953) Biochim. Biophys. Acta 10, 193—194
27 Cann, J.R., Stimpson, D.I. and Cox, D.J. (1978) Anal. Biochem. 86, 34—49
28 Tsai, S.C., Manganiello, V.C. and Vaughan, M. (1978) J. Biol. Chem. 253, 8452—8457
29 Braughler, J.M., Mittal, C.K. and Murad, F. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 219—222
30 Garbers, D.L. (1979) J. Biol. Chem. 254, 240—243
31 Arnold, W.P., Mittal, C.K., Katsuki, S. and Murad, F. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 3203–3207
32 Sun, I.C., Shapiro, L. and Rosen, O.M. (1974) Biochem. Biophys. Res. Commun. 61, 193—203
33 Macchia, V., Varrone, S., Weissbach, H., Miller, D.L. and Pastan, I. (1975) J. Biol. Chem. 250, 6214–6217
34 Garbers, D.L. (1976) J. Biol. Chem. 251, 4071—4077
35 White, A.A., Northup, S.J. and Zanser, T.V. (1972) in Methods in Cyclic Nucleotide Research (Cha sin, M., ed.), pp. 125—167, Dekker, New York
36 Asano, T. and Hidaka, H. (1977) Biochem. Biophys. Res. Commun. 78, 910—918
37 Zwiller, J. and Mandel, P. (1978) C.R. Acad. Sci. Paris 286, 423—426
38 Nakane, M. and Deguchi, T. (1978) Biochim. Biophys. Acta 525, 275—285
39 Garbers, D.L. (1978) J. Biol. Chem. 253, 1898—1901