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PURIFICATION AND PROPERTIES OF GUANYLATE CYCLASE FROM THE SYNAPTOSOMAL SOLUBLE FRACTION OF RAT BRAIN

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Summary

Guanylate cyclase (GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2) was purified 2250-fold from the synaptosomal soluble fraction of rat brain. The specific activity of the purified enzyme reached 41 nmol cyclic GMP formed per min per mg protein at 37°C. In the purified preparation, GTPase activity was not detected and cyclic GMP phosphodiesterase activity was less than 4% of guanylate cyclase activity. The molecular weight was approx. 480000.

Lubrol PX, hydroxylamine, or NaN_3 activated the guanylate cyclase in crude preparations, but had no effect on the purified enzyme. In contrast, NaN_3 plus catalase, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or sodium nitroprusside activated the purified enzyme.

The purified enzyme required Mn^{2+} for its activity; the maximum activity was observed at 3—5 mM. Cyclic GMP activated guanylate cyclase activity 1.4-fold at 2 mM, whereas inorganic pyrophosphate inhibited it by about 50% at 0.2 mM.

Guanylyl-(β,γ-methylene)-diphosphonate and guanylyl-imidodiphosphate, analogues of GTP, served as substrates of guanylate cyclase in the purified enzyme preparation. NaN_3 plus catalase or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine also remarkably activated guanylate cyclase activity when the analogues of GTP were used as substrates.

Introduction

Guanylate cyclase (GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2) catalyzes the formation of cyclic GMP from GTP and is found in various mammalian tissues, most of which is detected in the soluble cellular fraction [1—5].

Abbreviations: GMP-PCP, guanylyl-(β,γ-methylene)-diphosphonate; GMP-PNP, guanylyl-imidodiphosphate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

White and Aurbach [1] reported that among various tissues of rat, lung has the highest guanylate cyclase activity. Recently, we showed that guanylate cyclase in the synaptosomal soluble fraction of rat brain has much higher specific activity than that in lung. The total guanylate cyclase activity found in the synaptosomal soluble fraction was 6—7 times higher than that in the starting material [6], which was due to liberation of endogenous activating factor for guanylate cyclase in the soluble fraction [7]. We thought, therefore, that the synaptosomal soluble fraction would be the best starting material for the purification of guanylate cyclase of mammals and that the guanylate cyclase purified from the synaptosomes would offer the most suitable preparation for the studies on the mechanisms of cyclic GMP elevation in response to cholinergic neurotransmission. The present paper reports the purification and properties of guanylate cyclase from the synaptosomal soluble fraction of rat brain.

Materials and Methods

Chemicals. [8-^{3}H]GTP (11 Ci/mmol), cyclic [8-^{3}H]GMP (19 Ci/mmol), [8-^{3}H]guanylyl-(β,γ-methylene)-diphosphonate (GMP-PCP) (12 Ci/mmol) and [8-^{3}H]guanylyl-iminodiphosphate (GMP-PNP) (11 Ci/mmol) were purchased from The Radiochemical Centre (Amersham, U.K.). Creatin kinase from rabbit muscle, alkaline phosphatase from calf intestine, creatin phosphate, GTP, GMP-PCP, GMP-PNP and cyclic AMP were obtained from Boehringer (Mannheim, G.F.R.). Catalase from beef liver was purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.), cyclic GMP from Kohjin Co. (Tokyo, Japan), neutral aluminium oxide super I from Woelm (Eschwege, G.F.R.), and NaN_3 from Merck and Co. (Darmstadt, G.F.R.). Sepharose 4B, Sephadex G-25, Sephadex G-200, DEAE-Sepharose CL-6B, DEAE-Sephadex A-25 and QAE-Sephadex A-25 were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden), and DEAE-cellulose from Brown Co. (Berlin, N.H., U.S.A.).

Preparation of synaptosomal soluble fraction. Wistar rats weighing 150—200 g were supplied from Sizuoka Laboratory Agricultural Cooperative Association. The rats were killed by decapitation and brains were quickly removed and chilled on ice. All subsequent procedures were carried out at 0—4°C. The brains were homogenized with 9 vols. 0.32 M sucrose in a glass homogenizer with a Teflon pestle. Crude mitochondrial fraction was prepared according to the method of Whittaker and Barker [8]. Subfractionation of the crude mitochondrial fraction was carried out by a slight modification of the method of De Robertis et al. [9] as described previously [6]. The crude mitochondrial fraction was homogenized with ice-cold distilled water (6 ml/g wet tissue weight). The homogenate (fraction W) was centrifuged at 77 000 $\times$ g for 90 min to obtain a supernatant (synptosomal soluble fraction; fraction M_3) in a Hitachi model 80P preparative ultracentrifuge using a RP30-2 rotor.

Enzyme assays. Guanylate cyclase activity was estimated as described previously [6]. Unless specified, assay mixtures contained 50 mM Tris · HCl (pH 7.6), 5 mM $MnCl_2$, 5 mM creatin phosphate, 8 mM theophylline, 3.3 mM cyclic GMP, 4 units/ml creatin kinase and 0.25 mM [8-^{3}H]GTP (0.3 μCi/assay) in a volume of 150 μl. The incubations were carried out for 10 min at 37°C. The rate of product formation was linear over the time period studied and as a func-

tion of protein concentration. When indicated, [8-^{3}H]GMP-PCP or [8-^{3}H]GMP-PNP (1 μCi/assay) was used as a substrate instead of [8-^{3}H]GTP.

Cyclic GMP phosphodiesterase activity was assayed as described by Russell et al. [10] with a slight modification. Assay mixtures contained 40 mM Tris · HCl (pH 7.4), 10 μM [8-^{3}H]cyclic GMP (0.1 μCi/assay), 5 mM $MgCl_2$, 3.75 mM 2-mercaptoethanol and 1 μg (about 0.4 units) alkaline phosphatase in a final volume of 0.4 ml. After 10 min at 30°C, the reaction was terminated by the addition of 100 μl of 0.25 M formic acid, and diluted with an equal volume of distilled water. The solution was applied to a Dowex 1-X8 formate column (0.4 × 2.5 cm), and then washed with 1 ml distilled water and 4 ml 0.3 M formic acid successively. The washings were pooled and counted for radioactivity.

GTPase activity was determined by measuring the conversion of [^{3}H]GTP to [^{3}H]GDP. The reaction mixture contained 50 mM Tris · HCl (pH 7.6), 5 mM $MnCl_2$, 0.1 mM dithiothreitol and 0.25 mM [8-^{3}H]GTP (0.5 μCi/assay) in a total volume of 150 μl. After 10 min at 37°C, the reaction was terminated by heating in a boiling water bath for 2 min. The reaction tube was chilled on ice, and 100 μl 10 mM GDP and 2 ml 50 mM Tris · HCl (pH 7.6) were added followed by centrifugation at 1000 × *g* for 10 min. The radioactive GDP in the supernatant was isolated by a Dowex 1-X8 formate column (0.4 × 2.5 cm) according to the method of Krishnan and Krishna [11]. After the Dowex 1-X8 column was washed with 20 ml 4 M formic acid, the radioactive GDP was eluted with 6 ml 8 M formic acid containing 20 mM ammonium formate and counted for radioactivity.

Preparation of GTP affinity chromatography. The coupling of GTP to Sepharose 4B was performed as described by Lamed et al. [12] and Garbers [13]. The efficiency of coupling ranged from 3 to 5 μmol oxidized GTP/ml gel.

Protein determination. Protein was determined by the methods of Nakao ey al. [14], because the procedure is not affected by the presence of various substances including Tris, sucrose, glycerol and dithiothreitol. The protein was stained with amido black under the defined condition, filtered and washed on a membrane filter (Millipore, HAWP, pore size: 0.45 μm), and the dye on the filter was extracted with NaOH/ethanol. The intensity of the color was measured spectrophotometrically, which was linear from 2 to 20 μg protein.

SDS polyacrylamide gel electrophoresis. SDS polyacrylamide gel electrophoresis was performed as described by Laemmli [15]. The gels were prepared with 7.5% monomer concentrations in Tris/glycine buffer (pH 8.9), and were stained with 0.05% Coomasie brilliant blue.

Results

Purification procedure of guanylate cyclase

The synaptosomal soluble fraction (M_3), prepared from approx. 150 g of rat brain as described under Materials and Methods, was adjusted to pH 5.2 by dropwise addition of 2 M acetic acid with stirring for 20 min at 4°C. The precipitate was obtained by centrifugation (10000 × *g*, 20 min) and dissolved in 12 ml of a solution containing 0.4 M Tris · HCl (pH 8.1), 2 mM dithiothreitol, 0.1 mM EDTA and 20% glycerol. The solution was centrifuged at 3000 × *g* for 10 min. The precipitate was resuspended in 5 ml of the same buffer followed

by centrifugation at 3000 × *g* for 10 min. The supernatant fluids were combined, and denoted "pH 5.2 fraction".

The "pH 5.2 fraction" (20 ml) was applied to a Sepharose 4B column (4.8 × 80 cm). The guanylate cyclase activity was eluted in concert with one of the protein peaks (Fig. 1). The fractions containing guanylate cyclase activity were pooled (Sepharose 4B fraction).

Guanylate cyclase was retained by GTP affinity column in the presence of 2 mM $MnCl_2$ at pH 7.6 or 8.1. In the absence of Mn^{2+}, however, essentially all of the enzyme activity passed through the column at pH 8.1. Taking advantage of such properties, guanylate cyclase was purified as follows: the "Sepharose 4B fraction", to which NaN_3 was added to a final concentration of 10 mM to prevent the degradation of GTP, was applied on a GTP affinity column (1 × 8 cm) equilibrated with a solution containing 25 mM Tris · HCl (pH 8.1), 2 mM dithiothreitol, 0.1 mM EDTA, 10 mM NaN_3 and 20% glycerol. After application of the solution, the column was washed with the same buffer. Most of guanylate cyclase passed through the column, which was denoted "Mn(−) GTP affinity column fraction".

The "Mn(−) GTP affinity column fraction" was adjusted to pH 7.6 by a dropwise addition of 0.5 M HCl, and 100 mM $MnCl_2$ was slowly added to a final concentration of 2 mM. The resulting fluid was applied to the GTP affinity column (1 × 8 cm). Guanylate cyclase was eluted with the buffer containing 0.5 M NaCl and 5 mM EDTA (Fig. 2). To remove salts, the fractions containing guanylate cyclase activity were passed through a Sephadex G-25 column (1.5 × 20 cm). The resulting fluid was denoted "Mn(+) GTP affinity column fraction".

The "Mn(+) GTP affinity column fraction" was applied to a DEAE-Sepharose CL-6B column (1 × 8 cm). Guanylate cyclase was eluted with a linear gradient of NaCl (Fig. 3). The peak of guanylate cyclase activity was found at approx. 0.2 M NaCl. When other ion-exchange chromatographies such as

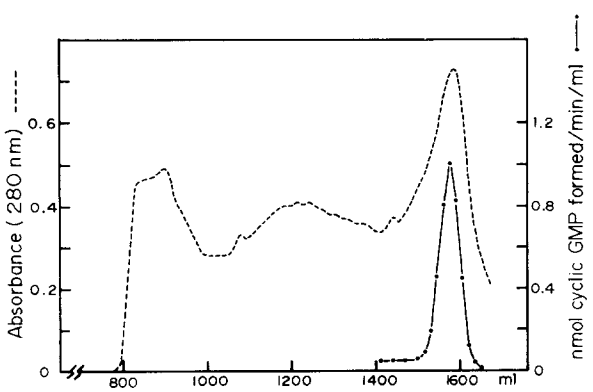

Fig. 1. Sepharose 4B column chromatography of guanylate cyclase. The "pH 5.2 fraction" prepared as described in the text was applied to a Sepharose 4B column (4.8 × 80 cm) equilibrated with a solution containing 25 mM Tris · HCl (pH 8.1), 2 mM dithiothreitol, 0.1 mM EDTA and 20% glycerol, and eluted with the equilibration buffer. Flow rate was 60 ml/h; fractions of 15 ml were collected. ●———●, guanylate cyclase activity; - - - - -, absorbance at 280 nm.

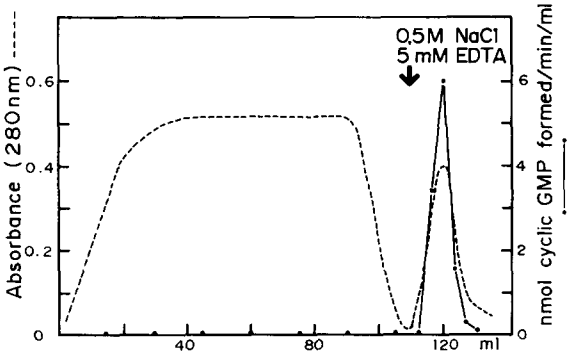

Fig. 2. GTP affinity column chromatography of guanylate cyclase. The "Mn(—) GTP affinity column fraction" prepared and treated as described in the text was applied to the GTP affinity column (1 × 8 cm) equilibrated with a solution containing 25 mM Tris · HCl (pH 7.6), 2 mM dithiothreitol, 2 mM $MnCl_2$, 10 mM NaN_3 and 20% glycerol, after which the column was washed with the equilibration buffer. Guanylate cyclase was eluted with 0.5 M NaCl and 5 mM EDTA in the buffer containing 25 mM Tris · HCl (pH 7.6), 2 mM dithiothreitol, 10 mM NaN_3 and 20% glycerol. Fractions of 3 ml were collected. ●———●, guanylate cyclase activity; - - - - - -, absorbance at 280 nm.

DEAE-cellulose, DEAE-Sephadex or QAE-Sephadex were used, the recovery of guanylate cyclase was very low, the reason of this remaining obscure. The peak fractions that contained guanylate cyclase activity were labaled "DEAE-Sepharose fraction".

The total activity and the specific activity of guanylate cyclase throughout the purification procedures are shown in Table I. The specific guanylate cyclase activity in the final preparation reached approx. 41 nmol of cyclic GMP formed per min per mg protein when assayed with 0.25 mM GTP at 37°C. A 2250-fold purification was achieved from the homogenate. The recovery was about 1.6% of the activity present in the homogenate. In fraction M_3, the total guanylate cyclase activity was 16-fold higher than that in fraction W. This was due to liberation of an endogenous activating factor for guanylate cyclase into the soluble fraction upon hypo-osmotic treatment [6,7]. Most of the endogenous

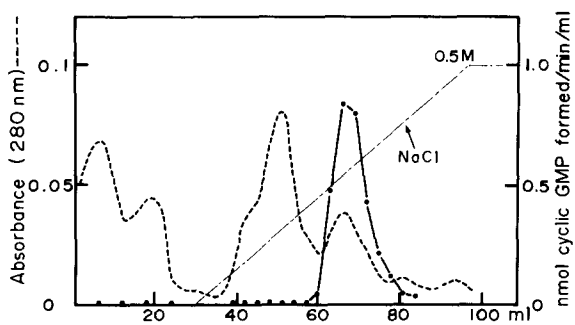

Fig. 3. DEAE-Sepharose CL-6B column chromatography of guanylate cyclase. The "Mn(+) GTP affinity column fraction" prepared as described in the text was applied to a DEAE-Sepharose CL-6B column (1 × 8 cm), equilibrated with 25 mM Tris · HCl (pH 7.6), 2 mM dithiothreitol, 0.1 mM EDTA and 20% glycerol, after which the column was washed with the equilibration buffer. Guanylate cyclase was eluted with 0—0.5 M linear gradient of NaCl. Fractions of 3 ml were collected. ●———●, gunaylate cyclase activity; - - - - - -, absorbance at 280 nm; - ———-, concentration of NaCl.

TABLE I

PURIFICATION OF RAT BRAIN GUANYLATE CYCLASE

Assays were performed as described in Materials and Methods.

Fraction	Total protein (mg)	Guanylate cyclase		Purification (-fold)
		total activity (nmol/min)	specific activity (pmol/min per mg protein)	
Homogenate	21 420	391	18.2	1.0
Fraction W	6 106	202	33.1	1.8
Fraction M_3	1 396	3176	2 275	125
pH 5.2	516	199	385	21.2
Sepharose 4B	98.0	97	976	53.6
Mn(—) GTP affinity	81.6	95	1 181	64.9
Mn(+) GTP affinity	1.6	10.1	5 988	329
DEAE-Sepharose	0.14	6.0	40 950	2250

activating factor, however, was removed from guanylate cyclase by pH 5.2 treatment.

Purity of the purified enzyme preparations

Table II shows GTPase and cyclic GMP phosphodiesterase activities throughout the purification procedures. Most of the GTPase and cyclic GMP phosphodiesterase activities were removed by Mn(+) GTP affinity column and DEAE-Sepharose chromatography. In the final preparation, GTPase activity was not detected. Cyclic GMP phosphodiesterase activity was less than 4% of guanylate cyclase activity.

When the purified enzyme preparation was applied to a Sephadex G-200 column, guanylate cyclase migrated as a single and symmetrical peak approximately the same elution volume of apoferritin with an apparent molecular weight of 480000 (data not shown). On a SDS-gel electrophoresis, the purified

TABLE II

OTHER ENZYME ACTIVITIES IN GUANYLATE CYCLASE PREPARATIONS

Assays were performed as described in Materials and Methods.

Fraction	Cyclic GMP phosphodiesterase		GTPase	
	total activity (nmol/min)	specific activity (nmol/min per mg protein)	total activity (nmol/min)	specific activity (nmol/min per mg protein)
Homogenate	134 946	6.30	436 968	20.4
Sepharose 4B	3 969	40.5	2 626	26.8
Mn(+) GTP affinity	4.3	2.7	6.3	3.9
DEAE-Sepharose	0.22	1.6	n.d. *	n.d. *

* n.d., not detected.

enzyme preparation exhibited four major protein bands with apparent molecular weights of 145000, 135000, 58000 and 46000 (data not shown).

Effect of various agents on guanylate cyclase activity

Several agents have been shown to activate guanylate cyclase in crude enzyme preparations [3,6,16—21]. In order to elucidate the activation mechanism, the effect of the agents was examined on guanylate cyclase activity throughout the purification procedures (Table III). Lubrol PX (0.5%) activated guanylate cyclase in the homogenate, while it inhibited the enzyme activity in fraction M_3. The activation in the homogenate is probably due to the solubilization of particulates. The inhibition in fraction M_3 is due to blockade of the activation of guanylate cyclase by an endogenous activating factor [6,7]. In the highly purified enzyme preparations, Lubrol PX was without effect. Hydroxylamine or NaN_3 activated guanylate cyclase in the homogenate, which is probably due to the presence of macromolecules necessary for activation [22,23]. Hydroxylamine inhibited guanylate cyclase activity in fraction M_3. The inhibition was due to blockade of the activation by an endogenous activating factor [7]. In the highly purified enzyme preparation, hydroxylamine or NaN_3 had no effect.

NaN_3 plus catalase, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) or sodium nitroprusside activated guanylate cyclase in all fractions throughout the purification procedures. These agents activated guanylate cyclase in fraction M_3 to a lesser degree than that in other fractions, because the enzyme in fraction M_3 was already activated by an endogenous activating factor [6,7].

Properties of guanylate cyclase in the purified preparation

Guanylate cyclase activity was measured in the highly purified preparation with various concentrations of $MnCl_2$. The maximum activity was observed at 3—5 mM. When assayed with 3 mM $MgCl_2$, guanylate cyclase activity was less than 10% of that with $MnCl_2$ (data not shown).

TABLE III

EFFECT OF AGENTS ON GUANYLATE CYCLASE ACTIVITY

Assays were performed as described in Materials and Methods.

Fraction (protein/assay)	Guanylate cyclase activity (% of no addition)						
	No addition	Lubrol PX (0.5%)	NH_2OH (2 mM)	NaN_3 (1 mM)	NaN_3 + Catalase (1 mM + 10 μg)	MNNG (0.1 mM)	Nitro-prusside (1 mM)
Homogenate (150 μg)	100	320	1172	227	2910	1469	1311
Fraction M_3 (16 μg)	100	23	33	88	221	191	156
Mn(+) GTP affinity (1 μg)	100	101	100	103	1015	550	539
DEAE-Sepharose (0.5 μg)	100	104	110	101	1121	740	645

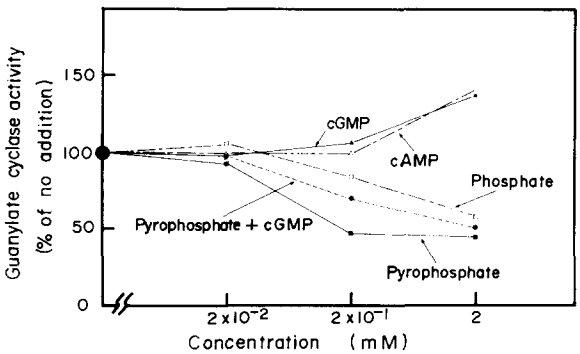

Fig. 4. Effect of products on guanylate cyclase activity. Assays were performed as described in Materials and Methods using 3 μg protein of the DEAE-Sepharose CL-6B chromatographed fraction. ▲———▲, cyclic GMP; △———△, cyclic AMP; ■———■, pyrophosphate; □———□, phosphate; ●———●, pyrophosphate plus cyclic GMP.

The effect of the products and related compounds on the guanylate cyclase activity was examined in the purified preparation. Cyclic GMP slightly activated the enzyme activity, while pyrophosphate inhibited it (Fig. 4). Cyclic AMP also activated the enzyme to the same extent as cyclic GMP. Inorganic phosphate inhibited the enzyme less markedly than pyrophosphate. The increase of guanylate cyclase activity by cyclic GMP or cyclic AMP is presumably not due to an inhibition of cyclic GMP phosphodiesterase activity, because the final enzyme preparation has only a trace of cyclic GMP phosphodiesterase activity (Table II) and a high concentration of phosphodiesterase inhibitor was included to the assay mixture.

GMP-PCP and GMP-PNP were examined for a substrate specificity. The apparent K_m value for GMP-PCP was 370 μM, while that for GTP was 70 μM. In contrast, GMP-PNP exhibited a biphasic pattern by a double reciprocal plot. The high K_m was 370 μM, which was the same as that for GMP-PCP, and the low K_m was 70 μM, same as that for GTP. The V values for both GMP-PCP and GMP-PNP were about 50% of that for GTP (data not shown). The effects of activating agents were examined using GTP, GMP-PCP and GMP-PNP as substrates. NaN_3 did not activate guanylate cyclase, while NaN_3 plus catalase or

TABLE IV

EFFECT OF AGENTS ON GUANYLATE CYCLASE ACTIVITY BY USING GTP, GMP-PCP OR GMP-PNP AS A ASUBSTRATE

Assays were performed with 1 mM substrates using 3 μg of the DEAE-Sepharose CL-6B chromatographed fraction.

Substrate	Guanylate cyclase activity (pmol/min per ml (%))			
	No addition	NaN_3 (1 mM)	NaN_3 (1 mM) + Catalase (10 μg)	MNNG (0.1 mM)
GTP	1735 (100)	1785 (103)	13 045 (752)	5733 (331)
GMP-PCP	927 (100)	844 (91)	13 715 (1480)	4972 (537)
GMP-PNP	840 (100)	908 (108)	5 525 (658)	2495 (297)

MNNG activated it when either GTP, GMP-PCP or GMP-PNP was used as a substrate (Table IV). The activation was most remarkably with GMP-PCP reaching the same level of enzyme activity as with GTP.

Discussion

Purification of guanylate cyclase has been performed from bacteria, sea urchin and mammalian tissues in several laboratories. Sun et al. partially purified guanylate cyclase from cell extracts of *Caulobacter crescentus*, and estimated the molecular weight to be approx. 140000 [24]. Macchia et al. purified guanylate cyclase from extracts of *Escherichia coli* to an apparently homogeneous enzyme. The molecular weight of the enzyme was estimated to be approx. 30000 [25]. Recently, Garbers purified the Lubrol-dispersed guanylate cyclase from sea urchin sperm by use of GTP affinity chromatography to approx. 12 μmol of cyclic GMP formed per min per mg protein, and estimated the molecular weight as approx. 182000 [13]. However, only a partial purification has been achieved from mammalian tissues [26,27].

We purified guanylate cyclase from the synaptosomal soluble fraction of rat brain to a specific activity of approx. 41 nmol of cyclic GMP formed per min per mg protein at 37°C. This represents the highest specific activity of guanylate cyclase ever reported from mammalian tissues. GTPase activity was completely removed, and cyclic GMP phosphodiesterase activity was less than 4% of the guanylate cyclase activity.

Several reports described the enzymic properties and activation of guanylate cyclase by various agents using crude enzyme preparations [3,6,16–21]. Activation of guanylate cyclase by NaN_3 or hydroxylamine is variable from tissue to tissue, which is probably due to the presence or absence of the macromolecules required for the activation [18,22,23]. In contrast, guanylate cyclase was activated by nitroprusside, MNNG or nitric oxide in most of enzyme preparations suggesting that macromolecular factors are not needed for the activation [20,21,28,29]. However, very crude enzyme preparations were used in these studies. To study the activation mechanisms by these agents, a highly purified enzyme preparation was required.

As shown in Table III, Lubrol PX, hydroxylamine or NaN_3 activated guanylate cyclase in crude enzyme preparations, whereas they did not activate it in the highly purified enzyme preparations. In contrast, NaN_3 plus catalase, MNNG or sodium nitroprusside activated guanylate cyclase even in the highly purified fraction. It appears that these agents do not require any other factors for the activation [23,28], and activate guanylate cyclase directly through an interaction with the enzyme. Activation of guanylate cyclase by NaN_3 or hydroxylamine is thought to be due to the formation of nitric oxide in incubations [29].

Cyclic GMP and cyclic AMP activated guanylate cyclase activity, while pyrophosphate inhibited it. During the preparation of this report, Asano and Hidaka reported that a partially purified guanylate cyclase from human platelet was stimulated by cyclic GMP [27]. On the other hand, Sun et al. reported that partially purified guanylate cyclase from *Caulobacter crescentus* was unaffected by cyclic GMP and cyclic AMP, while it was inhibited by pyrophosphate [24].

These discrepancies would be due to the difference of the enzyme source and the degree of purification.

Rodbell et al. reported that ATP and adenylyl-imidodiphosphate served as substrates for adenylate cyclase in rat liver plasma membrane [30]. On the other hand, Mittal et al. reported that GMP-PNP was a very poor substrate for rat liver guanylate cyclase [31], but a detailed kinetic study was not presented. In our experiment with the highly purified guanylate cyclase from rat brain, both GMP-PCP and GMP-PNP were shown to serve as good substrates of guanylate cyclase. The apparent K_m value for GMP-PCP was 370 μM, while that for GTP was 70 μM. GMP-PNP exhibited a biphasic pattern by a double reciprocal plot. The high K_m was the same as that for GMP-PCP, and the low K_m was the same as that for GTP.

A number of studies have suggested that cyclic GMP may serve as an intracellular mediator of muscarinic neurotransmission in nervous tissues [32—35]. The observation that guanylate cyclase in brain is mostly localized in the synaptosomes [6] would support the concept. However, the mechanism by which guanylate cyclase is activated in response to muscarinic agents has not been elucidated yet at the cellular level as well as at the molecular level. Although various agents have been shown to stimulate guanylate cyclase activity, very crude enzyme preparations were employed in these studies. We have obtained a highly purified guanylate cyclase from the synaptosomal soluble fraction and characterized its properties. Using this preparation, we are studying at the molecular level how the activating agents interact with the enzyme. Although we have shown that most of guanylate cyclase is localized in the synaptosomal soluble fraction in brain using a subfractionation technique [6], a more detailed localization of the enzyme in cells of brain and other tissues has to be established. The application of an immunohistochemical procedure for guanylate cyclase will be one of the effective approach to this problem. Although our enzyme preparation is not homogeneous enough to afford the formation of antibody, we are looking for a possible way to do this.

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