Sea Urchin Sperm Guanylate Cyclase

PURIFICATION AND LOSS OF COOPERATIVITY*

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The Lubrol-dispersed guanylate cyclase from sea urchin sperm was purified and isolated essentially free of detergent by GTP affinity chromatography, DEAE-Sephadex chromatography, and gel filtration. After removal of the detergent, the enzyme remained in solution in the presence of 20% glycerol.

The specific activity of the purified enzyme was about 12 μmol of guanosine 3':5'-monophosphate (cyclic GMP) formed min⁻¹ mg⁻¹ at 30°C, an activity about 4600 times that of a soluble guanylate cyclase purified recently from Escherichia coli (Macchia V., Varrone, S., Weissbach, H., Miller, D. L., and Pastan, I. (1975) J. Biol. Chem. 250, 6214-6217). The cyclic GMP phosphodiesterase activity was negligible and adenosine 3':5'-monophosphate (cyclic AMP) phosphodiesterase was not detectable in the purified preparation. Cyclic AMP formation from ATP occurred at a rate of 0.002% of that of guanylate cyclase. In the absence of phosphodiesterase or guanosine triphosphatase inhibitors, 100% of the added GTP was converted to cyclic GMP.

The purified enzyme required Mn²⁺ for maximum activity, the relative rates in the presence of Mg²⁺ or Ca²⁺ being less than 0.6% of the rates with Mn²⁺. The purified enzyme displayed classical Michaelis-Menten kinetics with respect to MnGTP (apparent Kₘ = 170 μM) in contrast to the positively cooperative kinetic behavior displayed by the unpurified, detergent-dispersed, or particulate guanylate cyclase.

The molecular weight of the purified enzyme was approximately 182,000 as estimated on Bio-Gel A-0.5m columns equilibrated in the presence or absence of 0.1 M NaCl. The unpurified, detergent-dispersed enzyme also migrated with an apparent molecular weight of 182,000 on columns equilibrated with 0.5% Lubrol WX and 0.1 M NaCl, but it migrated as a large aggregate (molecular weight > 5 × 10⁶) on columns equilibrated in the absence of either the detergent or NaCl. After gel filtration, the unpurified, dispersed enzyme still yielded positive cooperative kinetic patterns as a function of MnGTP. Na dodecyl-SO₄ gel electrophoresis of the enzyme after the DEAE-Sephadex or the gel filtration steps resulted in two major protein bands with estimated molecular weights of 118,000 and 75,000. Whether or not these protein bands represent the subunit molecular weights of guanylate cyclase is unknown at present.

Guanylate cyclase (GTP pyrophosphatase-lyase, EC 4.6.1.2) is found in both the soluble and particulate fractions of most tissue homogenates, the relative distribution being dependent upon the homogenization technique and the tissue being studied (1-3). The enzyme activity in particulate fractions appears to be mainly associated with plasma membranes (3-5) and has been shown to differ from the enzyme found in soluble fractions with respect to its kinetics, metal sensitivity, apparent molecular weight, and inhibition or activation by nucleotides (6-9). The particulate and soluble forms of the enzyme can apparently also change in activity independently of each other, since after partial hepatectomy in rats, the liver particu-late enzyme activity increases, while the soluble enzyme activity decreases or remains the same (10, 11). Despite these apparent differences between the soluble and particulate forms of guanylate cyclase, it is not known whether they represent distinct proteins or the same protein perhaps capable of translocation and interconversion. It also remains to be established that some of the apparent differences between the soluble and particulate forms of the enzyme are not generated by the necessity of adding guanosine triphosphatase inhibitors or GTP-regeneration systems, and phosphodiesterase inhibitors to the guanylate cyclase assay mixtures. Purification and complete characterization of both the soluble and particulate forms of guanylate cyclase probably will be required to establish whether or not the two forms of activity are, in fact, distinctly different proteins.
We present here a description of the purification of the particulate enzyme from sea urchin sperm. Sea urchin sperm were chosen as the source of the particulate enzyme because (a) the enzyme activity is located entirely in particulate fractions (4, 6, 12), (b) the enzyme activity in homogenates is at least 1 to 2 orders of magnitude higher in activity than that in homogenates of various mammalian tissues (12), and (c) the characteristics of the enzyme from sea urchin sperm appear to be the same as those of the particulate enzymes from various mammalian tissues (6-8, 13-15).

**EXPERIMENTAL PROCEDURES**

**Materials**

Live sea urchins (Lytechinus pictus) were obtained from Pacific Bio-Marine Supply Co., Venice, Calif. Polyethyleneimine-cellulose was obtained from Serva Feinbiochemica and Bio-Gel A-0.5m (100 to 200 mesh) from Bio-Rad Laboratories. Unlabeled nucleotides were purchased from the DuPont Co. of Delaware-Mannheim and [3H]GTP, 12 Ci/mmol, from Schwarz/Mann. Metabolic (chloride salts) were obtained from Fisher, theophylline from Merck & Co., Lubrol WX and triethanolamine from Sigma, and dithiothreitol from P-L Biochemicals. Adipic acid dihydrazide was obtained from Eastern Chemicals and sodium metaperiodate from Baker Chemical Co. DEAE-Sepharose A-25, and cyagenic bromide-activated Sepharose were purchased from Pharmacia. [14C]Lubrol PX was a gift from Dr. G. Levy, University of Miami.

**Methods**

**Sperm Collection**—Sperm were collected from sea urchins as previously described (16), resuspended in a solution containing 454 mM NaCl, 9.7 mM KCl, 24.9 mM MgCl2, 9.6 mM CaCl2, 27.1 mM MgSO4, and 4.4 mM NaHCO3 at pH 7.8 to 8.0, and centrifuged at 10,000 x g for 15 min. The wet weight of the packed cells was determined; the pellet was then frozen at −20°C until use.

**GTP Affinity Chromatography**—The coupling of GTP to Sepharose was essentially as described by Lamed et al. (17). Sepharose hydrazide was prepared by coupling cyagenic bromide activated Sepharose with adronic acid dihydrazide; the Sepharose hydrazide was then coupled with GTP which had been previously oxidized with periodate. The resulting Sepharose was stored in 50 mNa of oxidized GTP/g of gel. The GTP-Sepharose was washed, successively, with 16 ml each of 0.1 M LiCl, 0.2 M LiCl, and 0.5 M LiCl. After these washes, 1 ml of 1 M LiCl was applied to the column and the effluent was discarded; this was followed by the addition of 6 ml of 1 M LiCl, and this fraction was collected and counted for radioactivity. In some experiments, unlabeled GTP was added to the sample with the 50 mM acetic acid to monitor recovery, but this was not done routinely, since recoveries of [3H]GTP were consistently 80 to 90%, and could thus be corrected for with the time zero control samples.

**Other Methods**—Protein was determined by the method of Lowry et al. (20). Na dodecyl-SO4 gel electrophoresis was as described by Weber and Osborn (21). Bio-Gel A-0.5m columns were calibrated with ribonuclease, chymotrypsinogen, ovalbumin, bovine serum albumin, and dextran blue in the presence of an equilibration buffer containing 25 mM triethanolamine at pH 7.4, 2 mM dithiothreitol, 1 mM MnCl2, and 0.1 M NaCl. The ribonuclease and dextran blue also were used as molecular weight standards on columns equilibrated with the above buffer containing 0.5% Lubrol WX; these substances eluted in the same peak fractions in the presence or absence of the detergent. Since some Lubrol WX adsorbs to the Bio-Gel A 0.5m column (a small white band of adsorbed detergent can be observed at the top of the gel), the column was washed with the triethanolamine-dithiothreitol buffer without Lubrol after each experiment to remove this adsorbed material, and then re-equilibrated by washing the column with 2 column volumes of the triethanolamine-dithiothreitol buffer containing 0.5% Lubrol WX.

**RESULTS**

**Step I: Enzyme Solubilization**—After treatment of sperm particles with 1% Triton X-100, Lubrol PX, or Lubrol WX, sperm guanylate cyclase remained in the supernatant fluid after centrifugation at 100,000 x g for 1 hour. Enzyme activity in the supernatant fluid was 10 to 20% greater with the Lubrol detergent than with Triton X-100. Because of the slightly lower activity with Triton X-100 and the strong absorbance of this detergent at 280 nm, the Lubrol detergents were used during the subsequent purification procedures.

The final washed sperm pellet (15.5 g wet weight), prepared as described under “Experimental Procedures,” was diluted with 300 ml of a solution containing 25 mM triethanolamine buffer at pH 7.4 and 2 mM dithiothreitol. The sperm were then briefly homogenized at 0-2°C for 30 s with an Ultraturrax homogenizer (Jahnke and Kunkel, Staufen, Germany) at full speed. A 2-ml aliquot of this solution was saved and labeled “Homogenate 1.” The remaining suspension was then centrifuged at 30,000 x g for 45 min. After centrifugation, the supernatant fluid was discarded, the pellet was resuspended in 300 m/l of the triethanolamine-dithiothreitol buffer and the suspension was again homogenized as before. A 2-ml aliquot of this suspension was retained and labeled “Homogenate 2.” The rest of the suspension was centrifuged at 39,000 x g for 45 min, and the resulting supernatant fluid was discarded. The pellet was resuspended in 300 ml of a solution containing 25 mM triethanolamine at pH 7.4, 2 mM dithiothreitol, and 1% Lubrol WX and homogenized as described above, and a 2-ml aliquot was saved (“Homogenate 3”). The remaining suspension was centrifuged at 39,000 x g for 45 min. The resulting supernatant fluid was saved, and 3 ml of 100 mM MnCl2 were slowly added. The Mn**+-treated solution was centrifuged at 39,000 x g for 2 hours, and the resulting supernatant fluid (“Lubrol-supernatant”) was saved. The MnCl2 was added at this step for the purposes of GTP-affinity chromatography.

The removal of detergent at this step by either gel filtration or dialysis resulted in the reappearance of particulate material, with which all of the guanylate cyclase activity was associated.

**Step II: GTP Affinity Chromatography**—The detergent-dispersed guanylate cyclase would not bind effectively to the
GTP-column unless Mn²⁺ (1 mM) was included in the equilibration buffer (Fig. 1). Some enzyme activity was retained by the column in the presence of 1 mM Ca²⁺, but essentially none of the enzyme activity was retained by the column in the absence of metal or in the presence of 1 mM Mg²⁺. It was also shown that 100 mM NaCl, 5 mM EDTA, 5 mM MnGTP (nucleotide and MnCl₂ were equimolar) or 5 mM MnADP, but not 5 mM MnGMP, 5 mM cyclic GMP, or 50 mM acetylcholine could elute the enzyme from the GTP column in the presence of 1 mM free Mn²⁺. These results suggest that the GTP column is actually functioning as an affinity column since in previous studies it was shown that free GTP and MgGTP bind only very weakly or not at all to guanylate cyclase (6, 22).

The supernatant fluid from Step I was applied to the GTP-column equilibrated with a solution containing 25 mM triethanolamine at pH 7.4, 2 mM dithiothreitol, 1 mM MnCl₂, 0.2% Lubrol WX, and 20 mM sodium azide. Guanylate cyclase was eluted after the addition of 50 mM EDTA to the column (Fig. 2A). Most of the adenylate cyclase, and cyclic AMP and cyclic GMP phosphodiesterase activities were not retained by the column (Fig. 2B). The fractions containing guanylate cyclase activity were pooled and dialyzed for about 12 hours against a solution containing 25 mM triethanolamine at pH 7.4, 2 mM dithiothreitol, 1 mM MnCl₂, and 20% glycerol. Glycerol stabilized the enzyme during purification, but when added to incubation mixtures it did not directly affect enzyme activity.

Step III: DEAE-Sephadex.—The dialyzed, pooled fractions from the GTP affinity column were applied to a DEAE-Sephadex A-25 column equilibrated with the triethanolamine-glycerol buffer. After application of the sample, the column was washed with the buffer to remove most of the Lubrol WX, and the enzyme was then eluted with a linear NaCl gradient (Fig. 3). The peak of guanylate cyclase activity occurred at approximately 0.4 M NaCl, and the fractions containing the activity were pooled and immediately concentrated with an Amicon ultrafiltration unit (PM-30 membrane). Adenylate cyclase, and cyclic AMP and cyclic GMP phosphodiesterase activity were retained by the column, and cyclic GMP phosphodiesterase activity was lost in the fractions containing the activity. The GTP and NaCl were both made up in the equilibration buffer. The GTP (5 mM) and NaCl (0.5 M) were added to elute the guanylate cyclase activity; this was followed with 0.5 M NaCl to ensure that all of the activity was removed from the column. The GTP and NaCl were both made up in the equilibration buffer. Aliquots (50 μl) of each fraction (4.5 ml) were assayed for guanylate cyclase as described under "Experimental Procedures." A, absorbance at 280 nm and guanylate cyclase activity; B, adenylate cyclase, and cyclic AMP and cyclic GMP phosphodiesterase activity.

![Fig. 1. Chromatography of the Lubrol WX-dispersed guanylate cyclase on GTP affinity columns.](http://www.jbc.org/)

The sample applied to the column was also assayed, and the activity in each fraction was normalized to a percentage of the total enzyme activity applied.
activities also were estimated in the fractions collected from the ion exchange column (Fig. 3). Cyclic GMP phosphodiesterase activity was dispersed rather broadly across the column, while adenylate cyclase and cyclic AMP phosphodiesterase activities were not detectable; these latter activities were detectable, however, after pooling and concentrating the guanylate cyclase-containing fractions. When the DEAE-Sephadex step was attempted before the GTP column, guanylate cyclase activity chromatographed as a very broad peak after initiation of the linear NaCl gradient.

In other experiments, the enzyme was solubilized with 1% [14C]Lubrol PX in order to determine the per cent contamination by Lubrol in the final enzyme preparation. The enzyme was treated as described in Steps I and II in the presence of [14C]Lubrol PX and chromatographed on DEAE-Sephadex as described in the legend to Fig. 3. After Step III less than 0.00001% of the detergent (weight/volume) remained associated with the guanylate cyclase-containing fractions. The detergent was not detectable after Step IV.

**Step IV: Bio-Gel A-0.5m Filtration**—The concentrated sample obtained after DEAE-Sephadex chromatography (4 ml, 1.04 mg of protein) was applied to a Bio-Gel A-0.5m column (2.6 × 35 cm) equilibrated with a solution containing 25 mM triethanolamine at pH 7.4, 2 mM dithiothreitol, 1 mM MnCl₂, and 20% glycerol. After addition of the enzyme, the column was washed with about 250 ml of the equilibration buffer. At fraction 30, a linear NaCl gradient (250 ml of equilibration buffer, mixing chamber; 250 ml of equilibration buffer containing 0.5 M NaCl, reservoir chamber) was started. Aliquots were removed from each 16-ml fraction and assayed for enzyme activity as described under 'Experimental Procedures.'

Step IV have ranged from 4 to 12 µmol of cyclic GMP formed·min⁻¹·µg protein⁻¹ at 30°, and the recoveries have ranged from 0.3 to 1.3% of the activity originally present in the Lubrol-dispersed preparation (Homogenate 3). The contamination by cyclic GMP phosphodiesterase activity was negligible, and cyclic AMP phosphodiesterase activity was not detectable. The very low rate of formation of cyclic AMP from ATP may have been due to slight adenylate cyclase activity or to a side activity of the guanylate cyclase.

In order to test the purified guanylate cyclase preparation for interfering enzyme contamination, MnGTP was allowed to incubate with 1 µg of protein in the absence of inhibitors of phosphodiesterase or guanosine triphosphatase (Table II). Essentially 100% of the added GTP (250 nmol) was converted to cyclic GMP, indicating that interfering enzyme activity was negligible. These data should not be construed to suggest that a reverse reaction does not occur, since a slight white precipitate (possibly MnPPi) was observed to form in the incubation tubes as the reaction progressed.

**Homogeneity**—The actual purity of the final enzyme preparation will remain in question until the purification procedure has been carried out on a larger, preparative scale. At this moment the major obstacle to a large scale purification procedure is the availability of the sea urchins. The specific activity of 12 µmol of cyclic GMP formed·min⁻¹·µg protein⁻¹ at 30° compares favorably, however, to the specific activity of 30 µmol of cyclic AMP formed·min⁻¹·µg protein⁻¹ at 33° for the crystalline-soluble adenylate cyclase from *Brevibacterium liquifaciens* (23).

After Na dodecyl-SO₄, gel electrophoresis (15 or 30 µg of protein applied/gel), two protein-staining bands were visible (not shown) with apparent molecular weights of 118,000 and 75,000; these proteins could represent the guanylate cyclase subunits, but one of the bands also could represent a contaminant.

**Metal Requirements and pH Optimum**—A strong dependency of the purified guanylate cyclase on Mn²⁺ for activity is shown in Table III. Rates in the presence of Mg²⁺ or Ca²⁺ were only 0.6% of that seen with Mn²⁺. The cation dependence of the purified preparation is thus essentially the same as that...
Guanylate Cyclase Purification

Purification of sea urchin sperm guanylate cyclase

The units for the values given in the table are as follows: protein, milligrams; activity, nanomoles of product formed min\(^{-1}\); specific activity, nanomoles of product formed min\(^{-1}\) mg of protein\(^{-1}\). Enzyme assays were conducted as described under "Experimental Procedures."

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein</th>
<th>Guanylate cyclase</th>
<th>Adenylate cyclase</th>
<th>Cyclic AMP phosphodiesterase</th>
<th>Cyclic GMP phosphodiesterase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total activity</td>
<td>Specific activity</td>
<td>Total activity</td>
<td>Specific activity</td>
<td>Total activity</td>
</tr>
<tr>
<td>Homogenate 1</td>
<td>3,240</td>
<td>32,340</td>
<td>9.9</td>
<td>11,400</td>
<td>3.5</td>
</tr>
<tr>
<td>Homogenate 2</td>
<td>2,880</td>
<td>30,870</td>
<td>10.7</td>
<td>9,000</td>
<td>3.1</td>
</tr>
<tr>
<td>Homogenate 3</td>
<td>3,120</td>
<td>143,400</td>
<td>46.0</td>
<td>7,200</td>
<td>2.3</td>
</tr>
<tr>
<td>Step I</td>
<td>381</td>
<td>138,100</td>
<td>415</td>
<td>6.5</td>
<td>0.09</td>
</tr>
<tr>
<td>Step II</td>
<td>75</td>
<td>32,340</td>
<td>415</td>
<td>6.5</td>
<td>0.09</td>
</tr>
<tr>
<td>Step III</td>
<td>1.04</td>
<td>7,696</td>
<td>7,400</td>
<td>0.3</td>
<td>0.29</td>
</tr>
<tr>
<td>Step IV</td>
<td>0.18</td>
<td>2,100</td>
<td>11,700</td>
<td>0.04</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*S.D., not detectable.

TABLE II

Total conversion of GTP to cyclic GMP by purified guanylate cyclase

The assay mixture (30°) contained 1 pg of protein from Step IV, 250 nmol of GTP, 2.5 µmol of MnCl\(_2\), and 20 µmol of triethanolamine at pH 7.6 in a final volume of 0.4 ml. The values represent the mean ± S.E. of three determinations in two separate experiments.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Cyclic GMP formed</th>
<th>GTP utilized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol</td>
<td>nmol</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>51.5 ± 2.6</td>
<td>56 ± 3.5</td>
</tr>
<tr>
<td>10</td>
<td>102.2 ± 4.8</td>
<td>100 ± 0.5</td>
</tr>
<tr>
<td>20</td>
<td>158 ± 0.5</td>
<td>144 ± 0.5</td>
</tr>
<tr>
<td>40</td>
<td>206 ± 1.0</td>
<td>199 ± 2.0</td>
</tr>
<tr>
<td>90</td>
<td>237 ± 2.0</td>
<td>234 ± 0.5</td>
</tr>
<tr>
<td>120</td>
<td>269 ± 14.5</td>
<td>240 ± 0.5</td>
</tr>
</tbody>
</table>

Previously observed with the unpurified particulate or detergent-dispersed preparations from sea urchin sperm (12, 13). Maximum activity of the enzyme in the presence of Mn\(^{2+}\) was observed at about pH 7.6.

Molecular Weight—The enzyme preparations obtained after Lubrol-dispersion (Step I) and after DEAE-Sephadex chromatography (Step III) were used for the determination of apparent molecular weights. When aliquots of either preparation were applied to Bio-Gel A-0.5m columns equilibrated with a buffer containing 25 mM triethanolamine at pH 7.4, 2 mM dithiothreitol, 1 mM MnCl\(_2\), 0.5% Lubrol WX, and 0.1 M NaCl, the guanylyl cyclase activity was retained by the column and migrated with an apparent molecular weight of 182,000 (Fig. 4). In the absence of Lubrol WX, the enzyme purified through Step III still migrated with an apparent molecular weight of 182,000, but the Lubrol-dispersed (Step I) enzyme migrated at the column void volume as a large aggregate (Fig. 4). In the absence of Lubrol WX, the enzyme purified through Step III still migrated with an apparent molecular weight of 182,000, but the Lubrol-dispersed (Step I) enzyme migrated at the column void volume as a large aggregate (Fig. 4). In the absence of Lubrol WX, the enzyme purified through Step III still migrated with an apparent molecular weight of 182,000, but the Lubrol-dispersed (Step I) enzyme migrated at the column void volume as a large aggregate (Fig. 4). In the absence of Lubrol WX, the enzyme purified through Step III still migrated with an apparent molecular weight of 182,000, but the Lubrol-dispersed (Step I) enzyme migrated at the column void volume as a large aggregate (Fig. 4). In the absence of Lubrol WX, the enzyme purified through Step III still migrated with an apparent molecular weight of 182,000, but the Lubrol-dispersed (Step I) enzyme migrated at the column void volume as a large aggregate (Fig. 4). In the absence of Lubrol WX, the enzyme purified through Step III still migrated with an apparent molecular weight of 182,000, but the Lubrol-dispersed (Step I) enzyme migrated at the column void volume as a large aggregate (Fig. 4). In the absence of Lubrol WX, the enzyme purified through Step III still migrated with an apparent molecular weight of 182,000, but the Lubrol-dispersed (Step I) enzyme migrated at the column void volume as a large aggregate (Fig. 4).

Kinetic Behavior—Positively cooperative kinetic patterns as a function of MnGTP were observed when the particulate or Lubrol-dispersed (Step I) guanylate cyclases were studied (Fig. 5); this type of cooperative behavior is similar to that previously observed with particulate guanylate cyclases from sea urchin sperm and other sources (3, 6-8, 14, 15). After gel filtration of the detergent-dispersed enzyme in the presence of Lubrol WX and NaCl, positive cooperative kinetic patterns were still observed (Hill coefficient = 1.2).

Unlike the detergent-dispersed guanylate cyclase, normal Michaelis-Menten kinetics as a function of MnGTP were observed with the purified enzyme (Fig. 6). The apparent Michaelis constant for MnGTP (3.5 nM free Mn\(^{2+}\)) of 161 to 181 µM was similar to the S\(_{50}\) value of 141 µM for the positively cooperative enzyme.

DISCUSSION

Sea urchin sperm guanylate cyclase has been solubilized essentially free of detergent and purified to a specific activity of about 12,000 nmol of cyclic GMP formed min\(^{-1}\) mg of protein\(^{-1}\) at 30°. This represents the first description of a purification method for the particulate form of guanylate cyclase, and the first time that it has been possible to assay the enzyme in the absence of guanosine triphosphatase or phosphodiesterase inhibitors, or GTP-regeneration systems. The specific activity greatly exceeds those of the partially purified enzymes from bovine lung (6 nmol of cyclic GMP formed...
Fig. 4. Filtration of the Lubrol-dispersed (Step I) and the DEAE-Sephadex (Step III) guanylate cyclase preparations on Bio-Gel A-0.5m columns. The column (2.6 x 35 cm) was equilibrated with a solution containing 25 mM triethanolamine at pH 7.4, 2 mM dithiothreitol, 1 mM MnCl₂, 0.1 M NaCl, and 20% glycerol (O, Δ) or with this solution containing in addition 0.5% Lubrol WX (●, △). The enzyme sample (1 to 2 ml) was added to the column, 50 fractions of 2.2 ml each were collected, and aliquots of each fraction were assayed for guanylate cyclase activity as described under “Experimental Procedures.” The recovery of enzyme activity ranged from about 40 to 60% for both the Step I and Step III preparations. Vₒ = void volume. Inset, standard proteins and dextran blue were used to calibrate the column in the absence of Lubrol WX. The standards were: 1, ribonuclease; 2, chymotrypsinogen, 3, ovalbumin; 4, bovine serum albumin; and 5, dextran blue. Ribonuclease and dextran blue also were used in the presence of 0.5% Lubrol WX and migrated with peak elution volumes of 156 and 55 ml, respectively.

Fig. 5. Reciprocal plots as a function of MnGTP for the unpurified guanylate cyclase. Incubation mixtures contained 50 mM triethanolamine buffer at pH 7.9, 0.2 mM dithiothreitol, 3.5 mM Mn²⁺ in excess of GTP, the GTP concentrations given in the figure, 0.075 μg of protein (lower line) or 0.2 μg of protein (upper line) and 7 x 10⁶ cpm of ['H]GTP. Incubations were for 10 and 20 min at 30° and product formation was linear. The two plots represent the kinetic behavior of two different enzyme preparations. The same linear kinetics were observed when the incubation mixtures contained 8 mM theophylline and 8 mM sodium azide in addition to the ingredients listed above.

Fig. 6. Reciprocal plots as a function of MnGTP for the purified guanylate cyclase. Incubation mixtures contained 50 mM triethanolamine buffer at pH 7.9, 0.2 mM dithiothreitol, 3.5 mM Mn²⁺ in excess of GTP, the GTP concentrations given in the figure, 0.075 μg of protein (lower line) or 0.2 μg of protein (upper line) and 7 x 10⁶ cpm of ['H]GTP. Incubations were for 10 and 20 min at 30° and product formation was linear. The two plots represent the kinetic behavior of two different enzyme preparations. The same linear kinetics were observed when the incubation mixtures contained 8 mM theophylline and 8 mM sodium azide in addition to the ingredients listed above.

Since previous reports have outlined several apparent differences between the soluble and the particulate enzymes from a number of different tissues (3, 6-9), it is possible to examine the purified sea urchin sperm enzyme to determine whether it has retained the basic characteristics of a particulate guanylate cyclase or acquired those of a soluble one.

The particulate and soluble forms of guanylate cyclase appear to be different with respect to their general kinetic behavior. While only normal Michaelis-Menten type kinetics as a function of MnGTP have been reported for the soluble guanylate cyclases (3, 6-9, 14, 26-30), positively cooperative kinetics as a function of MnGTP have been observed with various particulate guanylate cyclases (3, 6-8, 14, 15). The Hill coefficients for MnGTP for the guanylate cyclases associated with the particulate fractions have generally ranged from 1.4 to 1.7 (6-8, 14, 15), but the coefficient for the enzyme associated with rat small intestine villous cell microsomes can be lower (n = 1.2) (14), and is the same as the coefficient for the Lytechinus pictus guanylate cyclase. In contrast to its cooperative behavior in the unpurified state, purified guanylate cyclase from sea urchin sperm exhibits classical Michaelis-Menten kinetic behavior. Thus, purification results not only in the conversion of an
initially particulate guanylate cyclase to an apparently soluble enzyme, but also results in the loss of positively cooperative kinetic behavior. Since this loss of cooperativity does not seem to involve a major change in the enzyme’s molecular weight, the loss of an essential low molecular weight cofactor(s) or partial denaturation of the enzyme may account for the change in kinetics.

The particulate and soluble forms of guanylate cyclase also appear to differ with respect to metal sensitivity (3, 6-8). In general, the rates of cyclic GMP formation are greater with Mn⁺⁺ relative to those seen with Mg⁺⁺ or Ca⁺⁺ when using the particulate form of the enzyme as compared to the soluble form of the enzyme (6-8). The particulate guanylate cyclase also is more sensitive to Mn⁺⁺ or Ca⁺⁺ inhibition (3, 6-8). No major changes in metal requirements seem to have occurred during purification of the sea urchin sperm enzyme. The purified sperm guanylate cyclase was considerably more active with Mn⁺⁺ than with Mg⁺⁺ or Ca⁺⁺ (Table III), as has been observed with the particulate or the detergent-dispersed guanylate cyclases from sea urchin sperm (12, 13). However, different metal concentrations and metals in various combinations were not studied for effects on the purified enzyme.

Based on gel filtration data, it has been suggested that the apparent molecular weight of the detergent-dispersed guanylate cyclase in a number of different tissues is larger than that of the soluble enzyme (3, 7-9). The apparent molecular weight of the purified enzyme is 182,000. This is similar to the molecular weight of 148,000 to 154,000 reported for the soluble enzyme (3, 7-9). The apparent molecular weight (3, 6-8). The particulate guanylate cyclase also is not studied for effects on the purified enzyme.

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