Purification of the regulatory component of adenylate cyclase
(adenosine 3',5'-cyclic monophosphate/hormone receptors/reconstitution/guanine nucleotide)

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ABSTRACT The regulatory component (G/F) of adenylate cyclase [ATP pyrophosphate-lyase (cycling), EC 4.6.1.1.] from rabbit liver plasma membranes has been purified essentially to homogeneity. The purification was accomplished by three chromatographic procedures in sodium cholate-containing solutions, followed by three steps in Lubrol-containing solutions. The specific activity of G/F was enriched 2000-fold from extracts of membranes to 3-4 μmol·min⁻¹·mg⁻¹ (reconstituted adenylate cyclase activity). Purified G/F reconstitutes guanine nucleotide-, fluoride-, and hormone-stimulated adenylate cyclase activity in the adenylate cyclase-deficient variant of S49 murine lymphoma cells. G/F also recouples hormonal stimulation of the enzyme in the uncoupled variant of S49. Preparations of pure G/F contain three polypeptides with approximate molecular weights of 52,000, 45,000, and 35,000. The active G/F protein behaves as a multisubunit complex of these polypeptides. Treatment of G/F with [32P]NAD⁺ and cholera toxin covalently labels the molecular weight 52,000 and 45,000 polypeptides with [32P]

The purification of adenylate cyclase [ATP pyrophosphate-lyase (cycling), EC 4.6.1.1.] has been an elusive goal for more than 20 years since the description of this important enzyme (1). Although the activity can readily be extracted from plasma membranes with nonionic detergents, attempts to fractionate these preparations have accomplished little. Either the enzyme has behaved as a polydisperse species or its activity has been lost.

Insight toward resolution of this problem was provided by experiments that demonstrated the multicomponent nature of adenylate cyclase. Pfueuffer (2) demonstrated that a guanine nucleotide binding protein could be partially resolved from the putative catalytic subunit of adenylate cyclase by affinity chromatography with GTP-Sepharose. Such chromatography resulted in a partial loss of guanine nucleotide- and fluoride-stimulated enzymatic activity. These activities could be restored by the combination of material that flowed through the column with fractions that were eluted by the addition of a guanine nucleotide.

Study of the requirements for reconstitution of adenylate cyclase activity in genetic variants of the murine S49 lymphoma cell line then indicated that adenylate cyclase consists of a labile catalytic moiety (C) and a relatively stable regulatory component (G/F), which confers upon the catalyst the ability to utilize its physiological substrate, MgATP, and the ability to be activated by fluoride and guanine nucleotides (3-5). The activity of G/F is absent in the adenylate cyclase-deficient cyc⁻ variant of S49, and reconstitution of adenylate cyclase activity in cyc⁻ membranes thus becomes a method for the assay of the regulatory component. Because G/F is considerably more stable than the catalytic moiety of adenylate cyclase, purification of the regulatory component was initiated.

The methods and materials involved in the purification of adenylate cyclase were described in detail elsewhere (6). The assays to be described here were performed essentially as described.
sucrose in buffer A with 2 mM MgCl₂. Portions (100 ml) of this suspension were poured into 250-ml bottles, overlaid with 80 ml of 43% sucrose solution in buffer A, and centrifuged at 14,000 rpm for 120 min in a Beckman JA14 rotor. The membranes floating over the layer of 43% sucrose were collected and homogenized, diluted to approximately 2.5 liters with buffer A, and sedimented at 14,000 rpm for 60 min in a JA14 rotor. The pellets from this and subsequent centrifugations were composed of three distinct layers. An upper pink layer was decanted with the supernatant after it was loosened with a spatula. The light-brown middle layer, which contains a higher proportion of plasma membranes, was then separated from the dark bottom pellet by agitation, and the former was suspended in buffer A. This procedure was repeated twice additionally. The final pellet (2 g of protein) was homogenized in 50 ml of buffer A and frozen quickly at −80°C.

**Purification Procedures.** Purification of G/F was performed in a common buffer solution (TED), consisting of 20 mM Tris-HCl at pH 8.0, 1 mM EDTA, and 1 mM dithiothreitol. In several steps, solutions also contained 1 mM ATP, 6 mM MgCl₂, and 10 mM NaF (AMF), a condition that stabilizes G/F activity and activates the protein* (11). All steps were carried out at 4°C unless otherwise stated.

Frozen hepatic membranes (about 10 g of protein) were thawed and washed with 2 liters of TED/500 mM NaCl prior to extraction. After collection by centrifugation, the membranes were suspended to a final volume of 1 liter of TED containing 50 mM NaCl and 1.0% sodium cholate and extracted for 60 min by stirring at 0°C. Membranes were then removed by sedimentation for 75 min at 35,000 rpm in a Beckman 35 rotor. The clear supernatant was removed carefully from beneath a turbid floating layer and was diluted with 2 vol of TED/AMF/0.9% cholate. The diluted supernatant (2.5 liters) was applied to a column of DEAE-Sepharose (Pharmacia) (5 × 60 cm), which had been equilibrated with 3 liters of TED/AMF/0.9% sodium cholate. After application of the extract, the column was eluted with a 2-liter linear gradient of NaCl (0–250 mM) in TED/AMF/0.9% sodium cholate. G/F eluted as a symmetrical peak in the middle of the gradient. Peak fractions were pooled and concentrated to approximately 90 ml by filtration with an Amicon PM-30 membrane in a stirred cell.

The concentrated pool was next fractionated on a column (5 × 60 cm) of Ultrogel AcA 34 (LKB), which was equilibrated and eluted with TED/AMF/0.9% sodium cholate/100 mM NaCl. A peak of G/F was obtained at a Kₐ of approximately 0.45. Fractions with peak activity (50–70 ml) were pooled and diluted with 1.5 vol of TED/AMF/100 mM NaCl.

The diluted Ultrogel AcA 34 pool was applied to a 50-ml column (1.5 × 27 cm) of heptylamine-Sepharose, which had been equilibrated with TED/AMF/0.4% sodium cholate/100 mM NaCl. The column was washed successively with 50 ml of TED/AMF/0.4% sodium cholate/100 mM NaCl and with 50 ml of TED/AMF/0.3% sodium cholate/500 mM NaCl. The gel was then eluted with a 250-ml linear gradient of TED/AMF/0.3% sodium cholate/250 mM NaCl and TED/AMF/1.3% sodium cholate/100 mM NaCl. The increasing concentration of cholate eluted a peak of G/F in the middle of the gradient. At this stage and beyond, fractions containing G/F were collected in silicone-treated tubes (Sili clad).

Peak fractions from the heptylamine-Sepharose column were pooled and applied directly to a 5-ml column (0.9 × 8 cm) of hydroxyapatite (Bio-Rad HTP), which had been equilibrated in TED/0.1% Lubrol/100 mM NaCl. During hydroxyapatite chromatography, the concentration of EDTA in TED was reduced to 0.1 mM. After application of the protein, the column was washed with 20 ml of TED/0.1% Lubrol/100 mM NaCl. In general, the sample was allowed to remain bound to the column overnight after the initial wash with the Lubrol-containing buffer. Minimally, several hours were allowed to elapse before elution to allow deactivation of G/F after removal of AMF (11). The column was then washed with 10 ml of TED/0.1% Lubrol/100 mM NaCl/30 mM potassium phosphate, followed by 20 ml of TED/0.1% Lubrol/100 mM NaCl/300 mM potassium phosphate. Most of the G/F activity eluted with the 300 mM phosphate wash; this fraction was desalted by passage through a column of Sephadex G-25.

Protein was next bound to 1 ml of GTP-Sepharose by incubation in the presence of TED/0.3% Lubrol/2.5 mM MnCl₂ for 30 min at 30°C. The gel was poured into a column and washed at room temperature with 7 ml of TED/0.5% Lubrol/2.5 mM MnCl₂ and 7 ml of TED/0.5% Lubrol/3.5 mM MnCl₂/1 mM GTP. G/F was eluted (by removal of free Mn²⁺) with 10 ml of TED/0.5% Lubrol.

The peak fractions from the GTP-Sepharose column were activated by addition of AMF and applied to a column of DEAE-Sepharcel (0.5 × 7 cm) that had been equilibrated with TED/AMF/0.5% Lubrol. After washing with 5 ml of TED/AMF/0.5% Lubrol, the gel was eluted with a linear gradient (40 ml) of NaCl from 50 to 300 mM in TED/AMF/0.5% Lubrol. Peak fractions of G/F activity were eluted at approximately 150 mM NaCl.

Materials. Heptylamine-Sepharose was prepared as described by Shaltiel (12). However, Sepharose CL-4B (Pharmacia) was used as the support, and only 1 mol of heptylamine was used per mol of cyanogen bromide. As a precaution, the derivatized gel was incubated with 1 M ethanolamine for 2 hr prior to storage in H₂O at 4°C.

GTP was immobilized on Sepharose CL-4B through its γ phosphate as described by Pfeuffer (2). A modification of the published procedure was the use of only 50% of the specified amount of γ-aminobutyric acid. The product contained 0.65 μmol of nucleotide per ml of packed gel.

(α₂³²P)ATP (13) and [³²P]NAD (³²P in the α position of the ADP moiety) (14) were synthesized as described; sodium cholate was purified by chromatography on DEAE-cellulose as described (6). Lubrol 12A9 was obtained from Imperial Chemical Industries and was deionized with a mixture of Dowex 1 and 50. Cells were cultured and harvested as described (11).

**RESULTS**

Purification of G/F was the logical outgrowth of the ability to assay its activity by reconstitution of a functional adenylate cyclase complex. Because the reconstitution of G/F depends on its prior extraction from membranes with detergents, such extracts represent the first point at which the specific activity of the protein can be measured. The source of G/F chosen for purification was rabbit liver, because large quantities of partially purified plasma membranes can be obtained with moderate ease and the specific activity of G/F is comparable to or better than that observed from several other sources. Detergent extracts of membranes prepared as described under **Methods and Materials** exhibit a 5-fold greater specific activity of G/F than do those obtained from the total particulate fraction of a liver homogenate.

The purification of G/F is documented in Table 1. The
method allows a 2000-fold purification of the activity in the extract, with a 3–4% yield. The six chromatographic steps employed can be divided into two stages. The first stage, consisting of the first three steps, has proven to be very reliable and consistently yields G/F with a specific activity greater than 1000 nmol-min⁻¹-mg⁻¹. The specific activity of the initial extract shown here is unusually high; a specific activity of 1–1.5 nmol-min⁻¹-mg⁻¹ is usual and purification of 1000-fold is normally achieved with these three steps. At this stage of the purification, G/F is about 30% pure and the recovery of activity is reasonably high. Overall recovery of activity can be closer to 30% at this point without significant sacrifice of specific activity if a greater percentage of the heptalamine-Sepharose eluate is pooled (see Discussion). The last three steps of the purification scheme are inefficient but, to date, have been necessary to remove the specific contaminants that remain in the preparation. Because G/F at this stage of purification is unstable to further manipulation in cholate and does not bind to GTP-Sepharose in this detergent, further purification proceeds in Lubrol 12A9. Hydroxypatite provides an excellent medium to effect this exchange of detergents, because neither cholate nor Lubrol binds significantly to the gel, whereas G/F binds quantitatively. If the protein is then allowed to deactivate on the gel by the removal of AFM, some purification (often 2-fold) can be achieved by the elution procedure described. G/F can then be quantitatively adsorbed to GTP-Sepharose. Prior to such binding, gel filtration of the peak from hydroxypatite was necessary, because salt, especially phosphate, prevents adsorption of G/F to the derivatized Sepharose. Activation of the G/F with guanosine 5’-[β,γ-imido]triphosphate, guanosine 5’-[-γ-thio]triphosphate (GTP[γS]), or AFM also prevents adsorption; however, these ligands were ineffective in eluting G/F from the matrix once bound. The G/F can be recovered efficiently by elution of divalent cations from the eluting buffer. The binding capacity appears to exceed 20 μg/ml of packed gel. A final step, chromatography on DEAE-Sepharose (in Lubrol), yields nearly homogeneous G/F. This is an effective procedure if performed in the presence of AMF and a relatively high concentration of detergent.

Analysis of the purified preparation of G/F in NaDodSO4/polyacrylamide gels is presented in Fig. 1A. Two major bands of protein are observed, with molecular weights of approximately 45,000 and 35,000. A third protein, with a molecular weight of 52,000, is also present in much smaller quantities and is thought to be a component of G/F (see Discussion). Scanning densitometry of the Coomassie blue-stained gel indicates that greater than 95% of the protein is contained in these three bands. The relative proportion of stain bound to these polypeptides was 1:5:4 for the 52,000-, 45,000-, and 35,000-dalton bands, respectively. Lane 1 of Fig. 1A shows G/F that had been purified through the first three steps of the procedure; the 35,000- and 45,000-dalton polypeptides are clearly distinguished at this point. The last three steps then remove the remaining impurities, including an excess of the 35,000-dalton polypeptide (see Discussion).

FIG. 1. (A) Polyacrylamide gel electrophoresis in NaDodSO4 of purified fractions of G/F. Samples were run in 11% acrylamide gels by the method of Laemmli (15). Samples were prepared for electrophoresis by precipitation with 15% trichloroacetic acid in the presence of 2% NaDodSO4 in order to remove cholate or Lubrol from the proteins. The pellets were rinsed with 1 ml of diethyl ether to extract residual trichloroacetic acid and were then dissolved in the sample buffer. Samples were applied to the gel as follows: lane 1, 7.5 μg of heptalamine-Sepharose peak; lane 2, 3 μg of DEAE-Sepharose peak; lane 3, 8 μg of DEAE-Sepharose peak. The arrows indicate the migration of calibrating proteins, with masses in kilodaltons of: β-galactosidase, 118; phosphorylase b, 93; glycogen synthase, 85; bovine serum albumin, 68; catalase, 58; aldolase, 50; α-chymotrypsinogen, 27.5; and cytochrome c, 12.5.

(B) Labeling of purified G/F with [35S]-NAD+ and cholera toxin. The cholera toxin labeling pattern of G/F was obtained by reconstitution of cyc- with purified G/F and incubation with [35S]-NAD+ and cholera toxin as described (16). After a 60-min incubation the reaction was terminated by addition of NaDodSO4 sample buffer, and the sample was applied to a 11% polyacrylamide gel. Lane 1 shows the Coomassie blue-staining pattern of the purified G/F, run as described in A. Lanes 2 and 3 are autoradiograms of the cholera toxin-labeled G/F developed for 16 and 48 hr, respectively. Under the conditions utilized, there is essentially no labeling of cyc- membranes in the absence of prior reconstitution with G/F (see figure 1 of ref. 16).
peptide was not labeled in this experiment and has never been labeled, even with prolonged incubation with high concentrations of NAD$^+$ and toxin. Although not visible in Fig. 1B, two very minor bands in the G/F preparation (38,000 and 42,000 daltons) can also be labeled and are discernible with prolonged exposure of autoradiograms or, more readily, in less pure preparations.

The regulatory component of adenylyl cyclase is assayed by its reconstitution with the catalytic subunit in cytc- membranes. The saturation of this catalytic component by purified G/F is shown in Fig. 2. Reconstituted activity increases in a hyperbolic fashion with added G/F. Adenylyl cyclase activity, stimulated by fluoride, was restored to a specific activity of 350 pmol-min$^{-1}$(mg mem-brane protein)$^{-1}$; this activity is comparable to that observed in membranes from wild-type cells (10). Higher specific activities (up to 1000 pmol-min$^{-1}$(mg cytc protein)$^{-1}$) have been observed with better preparations of cytc membranes. The reconstituted activity in cytc membranes is essentially linear with small amounts of G/F (up to $\approx$150 pmol-min$^{-1}$(mg mem-brane protein)$^{-1}$); this linear relationship constitutes a quantitative assay for G/F. Apparent half-maximal saturation of cytc membranes is obtained with 100 ng of G/F per mg of cytc membranes. This value is entirely consistent with the degree of purification achieved. Similar saturation profiles for reconstitution of cytc membranes are also observed for GTP($\gamma$S)-activated adenylyl cyclase activity. The specific activity of G/F, calculated for GTP($\gamma$S)-activated adenylyl cyclase, is approximately 2 pmol-min$^{-1}$(mg mem-brane protein)$^{-1}$.

The purified G/F reconstitutes adenylyl cyclase activatable by hormone. In Table 2, cytc- and uncoupled (UNC) membranes were reconstituted with purified G/F in cholate (6). Clearly, the restoration of adenylyl cyclase activity in cytc- is accompanied by the capability of stimulating this activity with isoproterenol. The stimulation in reconstituted UNC membranes is, however, less than that previously reported for membranes reconstituted with cholate extracts of wild-type membranes (6). It is not known if this reflects some substantive difference in the properties of purified G/F from liver.

There is also a quantitative difference between the reconstituted activities shown in Fig. 2 and those in Table 2. The procedure utilized in Table 2, which involves recovery of reconstituted membranes by centrifugation prior to assay, requires considerably greater concentrations of G/F to reconstitute the same level of fluoride-stimulated enzymatic activity.

FIG. 2. Saturation of cytc- membranes with purified G/F. Purified G/F was diluted into 20 mM Hepes, pH 8.1 mM EDTA/0.1% Lubrol. Then 15 $\mu$L of the dilutions containing the indicated amounts of G/F was added to 25 $\mu$L of cytc- membranes (1.6 mg/ml). The samples were assayed for NaF-stimulated adenylyl cyclase activity.

**Table 2. Reconstitution of hormone-stimulable adenylyl cyclase with purified G/F**

<table>
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<tr>
<th>Reconstituted membrane*</th>
<th>Adenylyl cyclase activity,†</th>
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<th>NaF</th>
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<td>GTP</td>
</tr>
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<td>cyc$^-$</td>
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</tr>
<tr>
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<td>33</td>
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</table>

* cytc- or UNC membranes (250 $\mu$L at 1.5 mg/ml or 2.0 mg/ml, respectively) were mixed with 150 $\mu$L of purified G/F in TES/100 mM NaCl/0.9% sodium cholate. After a 20-min incubation on ice, samples were diluted with 200 $\mu$L of 150 mM NaHepes, pH 8.15 mM MgCl$_2$/300 $\mu$L of bovine serum albumin per ml/9 mM phospho- enolpyruvate/30 $\mu$L of pyruvate kinase per ml/1.25 mM ATP/100 $\mu$L GTP and incubated for 10 min at 30°C. Reconstituted membranes were then collected at 0°C by centrifugation at 140,000 $\times$ g for 20 min and resuspended for assay in 300 $\mu$L of 50 mM NaHepes, pH 8.2 mM MgCl$_2$/1 mM EDTA.

† Reconstituted membranes (50 $\mu$L) were assayed for adenylyl cyclase as described (10) in the presence of the indicated effectors: GTP (100 $\mu$M), isoproterenol (4 $\mu$M), NaF (10 mM).

This is explained in part by the increased thermal lability of both G/F and the catalytic moiety in the presence of cholate. In addition, activation of soluble G/F by fluoride, which can occur in the single-step reconstitution procedure used in Fig. 2, facilitates incorporation of G/F into membranes (11).

**DISCUSSION**

Previous experiments have demonstrated that adenylyl cyclase requires at least two separable components for the expression of MgATP-dependent enzymatic activity (2-5). We have described herein a procedure for the purification of one of these proteins, the regulatory component (G/F), to near homogeneity. The protein has been purified approximately 2000-fold from detergent extracts. This can be extrapolated to represent a purification of 5000- to 10,000-fold from plasma membranes or nearly 100,000-fold from total cellular protein. Upon reconstitution with the catalytic subunit and activation by fluoride, 1 mg of G/F can stimulate the synthesis of 3-4 $\mu$mol of cyclic AMP per minute. If an approximate molecular weight of 130,000 is assumed for G/F (17), a molar turnover number of 7 sec$^{-1}$ is obtained for this component of adenylyl cyclase.

Analysis of the purified preparation of G/F by NaDodSO$_4$/polyacrylamide gel electrophoresis identified the presence of three predominant polypeptide bands with molecular weights of 52,000, 45,000, and 35,000. From the molecular weight of native G/F (130,000), calculated from hydrodynamic properties (17), we predict that G/F has a multisubunit structure of one or more of these polypeptides. Evidence indicates that the three polypeptides in the purified preparation of G/F are all relevant to the activity of the protein. They are not resolved by the several chromatographic procedures described here, during centrifugation in sucrose gradients, or by several other fractionation procedures that allow preservation of activity.

A powerful method for identification of the components of the regulatory protein is by their labeling with [$^{32}$P]NAD$^+$ and cholera toxin. Cholera toxin is believed to ADP-ribosylate G/F specifically; this appears to constitute the mechanism by which the toxin activates adenylyl cyclase (14, 18). Prior studies of
labeling with cholera toxin in the wild-type and variant clones of S49 have shown cholera toxin-specific incorporation of \(^{32}P\) into two bands with molecular weights of about 52,000 and 42,000 (19). Such labeling is absent in the cys\(^-\) variant, which lacks G/F activity. We have confirmed these findings, and have also demonstrated that both the 45,000-dalton polypeptide and the 52,000-dalton polypeptide have altered isoelectric points in the UNC S49 cell variant (16).\(^1\) The 45,000- and 52,000-dalton bands of purified G/F can both be labeled with \(^{32}P\)-NAD\(^+\) and cholera toxin, and they migrate identically with the corresponding bands in S49 cell membranes. The data, taken together, strongly suggest the relevance of both of these polypeptides to G/F. Previously, Cassel and Pfeuffer (14) observed identically migrating bands at 42,000 daltons when pigeon erythrocytes were labeled with either cholera toxin or a photoaffinity analog of GTP (2). The single toxin-labeled band from turkey erythrocytes migrates identically with the 45,000-dalton band of pure G/F (unpublished data).

An apparent excess of the 35,000-dalton polypeptide is removed during the purification of G/F. During elution of the hydroxyapatite column with 50 mM potassium phosphate, 35,000-dalton protein is removed as an essentially pure species, such that the stoichiometric ratio of the 35,000 band to the two other bands is near 1:1 in the peak of G/F activity. Rechromatography of the G/F peak under the same conditions does not remove more of the 35,000-dalton protein. The isolated 35,000-dalton protein has no identifiable activity. It and G/F were examined by two-dimensional electrophoresis (electrofocusing and NaDodSO\(_4\) electrophoresis). Identical patterns of stained protein in the 35,000-dalton region were obtained with both preparations, confirming the suspected identity of the two 35,000-dalton polypeptides. When purified G/F is inactivated (by incubation at 4\(^\circ\)C in detergent solution) and is then rechromatographed over hydroxyapatite, all of the 35,000-dalton protein elutes during the low phosphate wash and it is resolved from the 45,000- and 52,000-dalton species. One interpretation of this result is that the observed inactivation is coincident with dissociation of the subunits of G/F. Furthermore, it is suggested that the apparent excess of the 35,000-dalton protein is a result of inactivation of G/F during purification. In support of this contention, inactivation also leads to partial resolution of the 35,000- and 45,000-dalton peptides during chromatography on DEAE-Sephacel or GTP-Sepharose. Additional evidence for the identity of the 35,000-dalton protein as a subunit of G/F has been obtained by its purification from turkey erythrocytes. G/F that has been purified to near homogeneity from this source contains both the 35,000-dalton and the 45,000-dalton proteins in a ratio of approximately unity (unpublished data). Definitive proof of the contribution of all three species to the activity of G/F will require their resolution and reconstitution; unfortunately, combination of resolved 35,000- and 45,000-dalton polypeptides has not yet resulted in restoration of G/F activity.

We have obtained some evidence for the existence of complexes of G/F with different subunit composition. There is a partial resolution of the 52,000- and 45,000-dalton polypeptides during chromatography on heptylamine-Sepharose. The 52,000-dalton band is more prominent in the front of this peak, while the 45,000-dalton species dominates the back. The 35,000-dalton protein distributes across the entire peak. The

\(^1\) The predominant toxin-labeled bands from S49 cells, rabbit liver, and turkey erythrocytes migrate identically during NaDodSO\(_4\)/polyacrylamide gel electrophoresis. We have estimated the molecular weight of this protein to be 45,000. This is the protein band estimated by others (e.g., ref. 19) to have a molecular weight of 42,000. 

45,000- and 52,000-dalton species are not completely resolved from each other, and no further separation is observed upon rechromatography of portions of this peak. In the preparation documented above, the peak from the heptylamine-Sepharose was taken such that most of the 52,000-dalton protein was eliminated; this contributed to a lower cumulative recovery than can be achieved when a wider peak is taken.

The 50-μg yield of pure G/F from this six-step procedure permits detailed study of the biochemical properties of this protein. G/F has been postulated to hydrolyze GTP (20) and to alter the affinity of hormone receptors for agonist ligand (5). These and other hypotheses concerning the biochemical activities of G/F may now be tested directly. In this regard it may be noted that the GTPase activity of the preparation described herein is less than 1 nmol·min\(^{-1}\)·mg\(^{-1}\) (less than 0.001 of the specific activity of reconstituted adenylate cyclase). If G/F can in fact catalyze the hydrolysis of GTP, it seems probable that other proteins (e.g., hormone receptors or the catalytic subunit) will be necessary for the observation of a reasonable specific activity. Finally, it is hoped that portions of this procedure can be modified to allow preparation of greater quantities of material for physical and chemical analysis. Membrane preparation is currently rate-limiting, and, as noted, the final three steps of the procedure are inefficient.

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