Catalytic unit of adenylyl cyclase: Purification and identification by affinity crosslinking

(GTP-binding protein/forskolin/affinity chromatography/HPLC)

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Communicated by Manfred Eigen, December 26, 1984

ABSTRACT The guanosine 5'-[β,γ-imido]triphosphate (p[NH]ppG)-activated adenylyl cyclase from rabbit myocardial membranes was purified 60,000-fold to a specific activity of 15 μmol/mg min⁻¹ by Lubrol PX extraction, affinity chromatography, and gel permeation HPLC. The major purification (>2000-fold) was achieved by affinity chromatography on forskolin-Sepharose, a method previously developed in this laboratory. The final product appeared as two major peptides of Mr, 150,000 and 42,000, and one minor peptide of Mr, 45,000 when analyzed by NaDdSO₄/polyacrylamide gel electrophoresis. It is suggested that the Mr, 42,000 and 150,000 components represent the α-subunit of the stimulatory guanine nucleotide binding regulatory protein (Gₐ) and the catalytic unit, respectively, because upon crosslinking of a reconstituted adenylyl cyclase containing the [³²P]ADP-ribosylated α-subunit of Gₐ (Mr, 42,000), a single radiolabeled peptide of Mr, 190,000 appeared on NaDdSO₄/polyacrylamide gels. Further purification of the Mr, 150,000/42,000 hands with enzymatic activity when the purified enzyme was analyzed by various chromatographic procedures.

The hormone-regulated adenylyl cyclase is a multicomponent system composed of signal receivers (receptors for hormones and neurotransmitters), transducer elements (GTP-binding proteins), and the signal amplifier, the catalytic unit (1-4). Comprehension of the mode of interaction between these components requires their characterization, purification, and functional reconstitution. Recently, efforts toward purification of the β-adrenergic receptor and the stimulatory Gₐ as well as the inhibitory Gₜ-binding proteins (Gₐ and Gₜ, respectively) have been successful (5-9). This has allowed reconstitution of purified β-receptor and Gₐ into lipid vesicles that restored both hormone-dependent binding of nucleotides and hormonally stimulated GTPase activity (10, 11). However, to approach the membrane-bound system as closely as possible, the reconstituted system should also include the catalyst. Its presence becomes indispensable when the natural ligand GTP (and not its nonhydrolyzable analogs) is used as the regulatory ligand, because of the transient nature of the GTP-ligated Gₐ. The inclusion of the catalytic protein was not possible in the past, because efforts to purify this entity were unsuccessful due to its scarcity and its extreme lability. We are impressed by the high specificity of the hypotensive diterpene forskolin for adenylyl cyclase (12), and we have synthesized a matrix-bound derivative of forskolin, which proved to be a powerful tool for purifying that enzyme from various sources.

This affinity support also bound to different forms of the enzyme (13, 14). When adenylyl cyclase was preactivated with GTP analogs or F⁻ prior to affinity chromatography, a stable activity was recovered that was not influenced by nucleotides, F⁻, or Gₐ (13, 14). On the other hand, when nonactivated adenylyl cyclase was chromatographed on the immobilized diterpene, a preparation was obtained that had become refractory toward stimulators acting through Gₐ, but not to forskolin or activated Gₐ. The most plausible explanation for this was that the inactive form represented the resolved catalytic function (C), while the active form represented the G-C complex. In the present report, we have improved the affinity chromatographic procedure, which now enables purification of the Lubrol PX-solubilized enzyme by 2000-fold in one step. Furthermore, gel permeation HPLC is introduced as a subsequent purification step, achieving a final 60,000-fold purification starting from rabbit myocardial membranes. In addition, we describe here a procedure that allows identification of the catalytic unit of adenylyl cyclase by crosslinking it to ³²P-labeled Gₐ.

EXPERIMENTAL PROCEDURES

Materials. Guanosine 5'-[β,γ-imido]triphosphate (p[NH]ppG), guanosine 5'-[γ-thio]triphosphate (GTP-[γS]), NAD, creatine phosphate, and creatine kinase were purchased from Boehringer Mannheim. Cholic acid and Tween 60 were obtained from Serva, Heidelberg. Hydroxyapatite and silica gel 60 (70–230 mesh) were from Merck. Dicu-ccinimidyl suberate, dibithiole(succinimidyl propionate), succinimidyl 4-azidobenzoate, and dimethyl suberimidate were from Pierce. The disuccinimidyl esters of succinic acid and sebacic acid were prepared according to ref. 15. Cholera toxin, thymidine, ATP, ADP-ribose, GTP, benzamidine, benzothionium chloride, and Lubrol PX were obtained from Sigma. Sepharose CL-4B, Sephadex G-25, and wheat germ agglutinin Sepharose 6 MB are products of Pharmacia. TSK 4000 SW gel permeation HPLC columns were bought from Varian. Tween 60 and Lubrol PX were deionized by passage through a mixed bed ion exchanger (AG 501 X8, Bio-Rad). Rabbit hearts from mature animals were obtained from Pel-Freez. [α-³²P]ATP (760 Ci/mmol and 20-30 Ci/mmol; 1 Ci = 37 GBq) was obtained from New England Nuclear. [³²P]NAD was prepared from [α-³²P]ATP according to Cassel and Pfeuffer (16). [³H]cAMP (15-30 Ci/mmol), 1-fluoro-2,4-dinitro[3,5-H]benzene (10-30 Ci/mmol), and Na⁺[³²P] (carrier free) were purchased from the Radiochemical Centre.

Synthesis of Succinylated Derivatives of Deacteylforskolin. Deacteylforskolin (0.35 g) was dissolved in a solution of 1.2 g of succinic anhydride in 6 ml of anhydrous pyridine, and the mixture was maintained at 40°C for 80 hr. Product formation was marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.
was controlled by TLC [Silica gel sheets from Merck, developed in toluene/ethylacetate (1:1), containing 1% formic acid; detection was by charring with concentrated H2SO4]. The first major spot was 7-deacetylforskolin; RF, 0.4) followed by compound B (most likely 1,7-disuccinyl-7-deacetylforskolin; RF, 0.22). After this period, 80%–85% of the deacetylforskolin (RF, 0.6) disappeared, while the formation of compound A reached a maximum (35%–40%). Further extension of the reaction time resulted in the accumulation of compound B. The reaction was stopped by the addition of 2 ml of water and was left at 0°C for 2 hr. After evaporation of the reaction mixture under reduced pressure at 40°C, the resulting solid residue was extracted 4 times with 20 ml each of ethyl acetate under vigorous stirring. The combined extracts were dried over anhydrous Na2SO4, evaporated, and chromatographed at room temperature on an 80-ml Silicagel column using CHCl3/propanol-2 9/3 as eluant. The 7-succinyl-7-deacetyl derivative eluted after residual deacetylforskolin and appeared as a colorless semisolid after evaporation of the solvent (yield, 35% starting from deacetylforskolin). When chromatography was continued, compound B (most likely 1,7-disuccinyl-7-deacetylforskolin) was eluted.

The 90 MHz PMR spectrum of compound A was in agreement with a 7-succinyl-7-deacetyl derivative of forskolin.

Preparation of Forskolin-Sepharose. 7-Succinyl-7-deacetyl forskolin (16 μmol) was dissolved in 200 μl of anhydrous acetonitrile containing 24 μmol each of dicyclohexyl-carbodiimide and N-hydroxysuccinimide, and the mixture was left for 4 hr at 22°C. Formed dicyclohexylurea was removed by centrifugation and extracted 3 times with 20 μl each of acetonitrile. The combined supernatants were added to 10 ml of aminomethyl-Sepharose CL-4B (20–40 μmol of amino groups per ml, prepared according to ref. 17) in 15 ml of dry dimethylformamide and were shaken for 24 hr at 22°C. The Sepharose derivative was collected by filtration and washed successively with 30 ml of dimethylformamide, 20 ml of ethanol, and 30 ml of 70% aqueous ethanol. The latter solvent was also used to store the beads at −20°C. Quantitation of matrix-bound ligand was as described (13). The hydrophilic spacer, to which both the succinyl and the ethylenediamine moieties are contributing, appeared to provide optimal distance between the Sepharose matrix and the forskolin derivative, because further extension by 13- and 17-atom spacers did not improve the properties of this affinity support.

Adenylate Cyclase Preparations. Crude membranes from rabbit cardiac muscle were prepared and adenylate cyclase was solubilized at 10 mg of protein per ml with the Lubrol PX containing buffer A (10 mM 3-(N-morpholino)propanesulfonic acid (Mops), pH 7.4/1 mM MgCl2/1 mM EDTA/1 mM benzamidine/10 mM benzethonium chloride/5 mM Lubrol PX/0.02% Na2N3) as described (13). Preparation of p[NH]ppG-activated solubilized adenylate cyclase was as described except that 30 μM instead of 100 μM p[NH]ppG was used for activation (13).

Adenylate Cyclase Assay. Adenylate cyclase activity was estimated in a medium consisting of 20 mM Mops, pH 7.4/10 mM creatine phosphate/50 μg of creatine kinase per ml/5 mM MgCl2/1 mM [α-32P]ATP (2–8 cpm/pmol). Incubation was for 10 min at 37°C. Protein was estimated according to Lowry et al. (18) or by the method of Schultz et al. (19).

Purification of p[NH]ppG-Activated Adenylate Cyclase by Forskolin-Sepharose Affinity Chromatography and Gel Permeation HPLC. The following steps were carried out at 4°C. A Lubrol PX-solubilized adenylate cyclase preparation (p[NH]ppG-activated; 750 ml containing 600–800 mg of protein) in buffer A was adjusted to 500 mM NaCl and over 12 hr was loaded onto an 18-ml column of forskolin-Sephaserose that had been equilibrated with buffer C (same as buffer A except 500 mM NaCl is added and 5 mM Lubrol PX is replaced by 1 mM Tween 80). The loaded column was first washed with 375 ml of buffer C at 70 ml/hr and then the resin was removed so the following wash steps could be carried out batchwise, because this appeared to result in more efficient removal of nonspecifically bound protein than did column washes. The resin was transferred to a 60-ml plastic syringe (equipped with a nylon net) with 40 ml of buffer C. The slurry was adjusted to 2 M NaCl and gently shaken. After 20 min, the liquid was removed and the resin was first washed with 30 ml of buffer B (same as buffer C but without NaCl) and 2 M NaCl and then with 70 ml of buffer C. A slurry of the resin and 40 ml of buffer C was made, brought to 300 mM MgCl2, and then agitated for 20 min. The fluid phase was removed and the beads were washed with 40 ml of buffer C and 300 mM MgCl2, followed by 30 ml of buffer C. Adenylate cyclase was released by passing 150 ml of buffer C and 100 μM forskolin (added as concentrated ethanolic solution) through the forskolin-Sepharose at 50 ml/hr. To concentrate the effluent, it was allowed to drop directly onto 0.5 ml of hydroxyapatite (equilibrated with buffer C) in a 2-ml plastic syringe. The hydroxyapatite containing bound adenylate cyclase was washed with 2 ml of buffer C and then shaken with 1.5 ml of 400 mM Na phosphate, pH 7.4/2 mM Lubrol PX for 15 min in order to remove the cyclase. The fluid phase was removed, saved, and combined with a subsequent rinse with 0.5 ml of the same buffer. Prior to gel permeation HPLC, the hydroxyapatite effluents were concentrated to ~500 μl in an Amicon CF-50 filter cone and passed through an Acro LC 13 0.45-μm filter (Gelman). The filtrate (400–500 μl) was applied to a system of two connected Toyo Soda TSK 4000 SW columns (30 cm and 50 cm; i.d., 7.5 mm) equilibrated with buffer B and 100 mM NaCl, and it was chromatographed at 0.15 ml/min with an LKB HPLC pump. Fractions of 500–600 μl were collected and checked for adenylate cyclase activity in the presence and absence of 100 μM forskolin.

Preparation of [32P]ADP-Ribosylated G Protein ([32P]Gα). Pigeon erythrocyte membranes (10 mg/ml) were treated with cholera toxin (100 μg/ml) and [32P]NAD as described (16) except that 0.2 μM [32P]NAD (5–7 × 105 cpm/pmol) was used. Toxin-treated membranes were solubilized with 2.5% cholate at a protein concentration of 10 mg/ml as described for the purification of Gα from duck erythrocyte membranes (14). Since cholate interfered with the subsequent reconstitution step, it was removed by centrifugation of 500–μl aliquots of the cholate extract through 5 ml of dehydrated Sephadex G-25 in plastic syringes equilibrated with buffer C. For preparation of GTP[yS]-activated [32P]-labeled Gα, toxin-treated membranes were incubated with 20 μM GTP[yS]/100 μM DL-iso-proterenol/20 mM phosphate buffer, pH 7.4/3 mM MgCl2/1 mM EDTA/150 mM NaCl, for 15 min at 37°C before solubilization with cholate as described above.

Reconstitution of [32P]-labeled Gα with Forskolin-Sepharose-Bound Catalytic Moiety and Crosslinking. Three small chromatographic columns (plastic syringes) were supplied with 0.3 ml each of packed forskolin-Sepharose in buffer C. Each column was loaded with 10 ml of solubilized myocardial adenylate cyclase (nonactivated) in buffer A containing 300 mM NaCl during 2 hr at 4°C. The columns were washed 4 times each with 2 ml of cold buffer C and were then loaded with 400 μl of either 300 mM GTP[yS]-activated [32P]-labeled Gα, GTP[yS]-activated [32P]-labeled Gα, or GTP[yS]-activated [32P]-labeled Gα, and 800 ng of nonlabeled Gβ, (from duck erythrocytes, p[NH]ppG form (14)), respectively. The mixtures were shaken for 10 min at 22°C and for 50 min at 4°C. After removal of unbound material, the resins were washed 4 times each with 2 ml of cold buffer C. For release of bound protein 400 μl of buffer C and 100 μM forskolin were added to each column, and the slurry was agitated for 60 min at 4°C.
Released protein was removed, saved, and combined with a subsequent rinse with another 400 µl of buffer C and 100 µM forskolin. The combined eluates were concentrated to ~200 µl by centrifugation in an Amicon CF-50 filter cone.

Crosstlinking. We treated 200-µl samples of the 35P-labeled Gs preparations and of reconstituted 32P-labeled Gs-C in buffer C with 0.2-2 mM of the desired disuccinimidylester, which had been diluted from freshly prepared stock solutions in dry dimethylformamide. The reaction was allowed to proceed for 120 min at 4°C and was quenched by the addition of 10 mM taurine.

NaDodSO4/Polyacrylamide Gel Electrophoresis. Samples were adjusted to 1% NaDodSO4/10% (vol/vol) glycerol and where indicated, 1% dithiotretiol, heated for 5 min at 95°C, and electrophoresed on 5%-20% or 5%-15% gradient pore slab gels using the gel system of Laemmli (20). When the gels were stained for protein, the silver-staining method of Oakley et al. (21) was used. Mr standards were pig neurofilament triplet proteins (22) (Mr, 200,000; 160,000; 68,000), phosphorylase (Mr, 94,000), ovalbumin (Mr, 43,000) and carbonic anhydrase (Mr, 30,000). These were iodinated according to ref. 23.

RESULTS

The purification of [pNH]ppG-activated adenylate cyclase from rabbit myocardial membranes by Lubrol extraction, forskolin-Sepharose chromatography, and HPLC is summarized in Table 1. Affinity chromatography with the type of forskolin-Sepharose described in this study had to be carried out in the presence of at least 500 mM NaCl to prevent ionic interactions with excessive aminoethyl groups. A strict wash protocol that included sequential treatment with high concentrations of NaCl (2 M) and MgCl2 (0.3 M) was used to isolate the enzyme, which was 20%-30% pure in one step. The continuous presence of a detergent in the wash fluids and in the forskolin-containing buffer for release was required. The subsequent hydroxyapatite step did not achieve purification; it was chosen to rapidly concentrate adenylate cyclase in the forskolin-Sepharose eluate for the gel permeation HPLC step. A further advantage of this method is that it does not concentrate the detergent, which would interfere with the HPLC separation. The final purification step on a TSK 4000 SW gel filtration matrix achieved a further 3- to 4-fold purification of the enzyme. A representative elution diagram of the HPLC step is shown in Fig. 1.

Fig. 2A shows an autoradiograph of a NaDodSO4/polyacrylamide gel of a radiotriniodinated fraction of the HPLC step (Fig. 1) containing adenylate cyclase activity. Two prominent bands corresponding to Mr, 150,000 and 42,000 and a weaker band of Mr, 45,000 are apparent. The latter was occasionally found to be incompletely separated from the Mr, 42,000 band. Fig. 2B shows a NaDodSO4/polyacrylamide gel of the same sample as in Fig. 2A, but it is visualized by silver staining. This gel had to be heavily overloaded to stain the Mr, 42,000 band.

The Mr, 150,000 peptide exhibited a quite unusual electrophoretic mobility. In gel systems according to Laemmi (20), with acrylamide monomer concentration >6%, this protein partially or fully aggregated and failed to enter the separation gel. In different gel systems, such as those introduced by Neville and Glossmann (24) or Davies and Stark (25), the electrophoretic mobility of this protein corresponded to that of a macromolecule of Mr, 130,000 rather than 150,000. Because of the absence of high Mr contaminants, this peptide is readily identified in less pure adenylate cyclase preparations. However, most important, this protein is completely missing when the loaded forskolin-Sepharose is eluted with diethoxyforskolin instead of forskolin. This analog does not share the hypotensive properties of forskolin (26), fails to activate adenylate cyclase, and, more importantly, does not antagonize forskolin stimulation of adenylate cyclase.

The most likely candidate for the Mr, 42,000 protein is the α-subunit of Gs, because the enzyme was isolated in its [pNH]ppG-activated form and therefore should represent the Gs-C complex. This is supported by recent experiments that showed a portion of protein-bound 35S-labeled GTP[yS] copurified with adenylate cyclase activity through the forskolin-Sepharose affinity step and showed a constant ratio of 35S radioactivity and enzymatic activity when analyzed by subsequent sucrose density gradient centrifugation (14). Fig. 2C shows the autoradiograph of a NaDodSO4/polyacrylamide gel of cholera toxin[32P]NAD-treated myocardial membranes, demonstrating that the respective Gs-α has a Mr of 42,000. Indeed, as reported previously, reconstitution

![Fig. 1. HPLC elution profile of forskolin-Sepharose-purified, [pNH]ppG-activated adenylate cyclase. The concentrated forskolin-Sepharose eluate was chromatographed on two connected TSK G 4000 SW columns (total vol, 38 ml). Adenylate cyclase activity (●) was measured in the presence and absence of 100 µM forskolin, but it was found to be same in at least five similarly conducted experiments. Protein (●) was determined by the [14C]fluorodinitrobenzene method (19).](image-url)
experiments with pure 3H-labeled G, dimer (M, 42,000/35,000) indicated that only the a-component of G, combined with the catalyst and was therefore sufficient for activation (14).

To identify the catalytic subunit, we exploited its specific interaction with the stimulatory regulatory component G, by affinity crosslinking of a 32P-labeled G, C complex. A convenient means to covalently label G, consists of the 32P]ADP-riboseylation of its a-subunit by cholera toxin (16). However, purified G, (and probably also G, C) can only be labeled by cholera toxin in the presence of an ADP-ribosylation factor, which has been purified to apparent homogeneity very recently (27). As an alternative, we decided to reconstitute the G, C complex from the myocardial catalytic component and any G, that is readily 32P]ADP-ribosylated in its membrane-bound form. G, from pigeon erythrocyte membranes was chosen because cholera toxin-catalyzed 32P]ADP-ribosylation was clearly highest when compared to a variety of other tissues (including rabbit myocardial). This may be due to the absence of nonspecific ADP-ribosyltransferase in pigeon erythrocyte membranes. Note that the 32P Radioactivity incorporated into myocardial G, was only 2% that found in pigeon erythrocyte G, at 1 mM [32P]NAD (Fig. 2C). As we have previously shown, such reconstitution could be conveniently performed with a catalytic function that was immobilized onto forskolin-Sepharose (14). An advantage of this technique is that the G, must not be necessarily pure, because the ethe red lipids (membrane) can be easily removed while the G, C complex is bound to the forskolin-Sepharose matrix. Indeed, for the experiment here we used a crude cholate extract from 33P]NAD/cholera toxin-treated pigeon erythrocyte membranes as a source of labeled G, Cholate extracts from pigeon erythrocyte membranes were essentially devoid of C activity, although membranes had been treated with GTP[yS] prior to solubilization.

The protocol for formation and crosslinking of the 32P-labeled G, C complex is summarized in the flow diagram shown in Fig. 3.

The results of this approach can be obtained from the autoradiograph in Fig. 4. Lane 1 shows the 32P-labeled G, (GTP[yS]-activated), exhibiting one major band representing the a-subunit of G, (16). As seen from lane 2, no crosslinked products were appearing when the G, preparation was treated with the crosslinker disuccinimidylsulphate, although at higher concentrations (>2 mM) of this reagent, high M, aggregates were present that did not enter the gel. Lane 3 shows the radiochemically pure M, 42,000 peptide of the 32P-labeled G, C complex eluted from the forskolin column.

![Diagram](image_url)
When such a preparation was treated with disuccinimidyl suberate, an additional radiolabeled band appeared that corresponded to a crosslinked product of $M_r$ 190,000 (lane 6). These findings strongly suggested that the crosslinked product of $M_r$ 190,000 was formed between $G_s\alpha$ and only one additional peptide of $M_r$ 150,000. A $M_r$ 190,000 species was also observed when the purified homologous $G_s\beta\gamma$ complex from myocardial membranes was treated with various crosslinkers.

To demonstrate the specificity of the $G_s\rightarrow C$ interaction, two control experiments were included in which the GTP-[yS]-activated $^{32}$P-labeled $G_s$ was replaced by nonactivated $^{32}$P-labeled $G_s\alpha$, or unlabeled $G_s\alpha(p[NH]ppG form, from duck erythrocyte membranes) was used to compete with $^{32}$P-labeled $G_s$ for the catalytic protein. Virtually no radiolabeled $M_r$ 42,000 protein bound to the catalyst when nonactivated $^{32}$P-labeled $G_s$ was applied (lane 5), in agreement with earlier findings of this laboratory (28), which also indicated that this form of $G_s$ does not confer activity to the catalyst. Radiolabeling of the $G_s\beta\gamma$ complex was considerably reduced when nonlabeled $G_s$ was simultaneously added in the reconstitution step (lanes 4 and 7 compared to lanes 3 and 6). It should be noted that a peptide of $M_r$ 150,000 has been labeled with $^{[125]}$I-2izobenzoyl-calmodulin by Andreassen et al. (29) and could possibly represent the calmodulin-stimulated adenylate cyclase.

**DISCUSSION**

On NaDodSO$_4$/polyacrylamide gels, the purified adenylate cyclase exhibited a simple protein pattern consisting of three bands of $M_r$ 42,000, 45,000, and 150,000. The $M_r$ 42,000 and $M_r$ 150,000 peptides appeared to represent the $\alpha$-subunit of $G_s$ and the catalytic subunit, respectively, because crosslinking of a reconstituted enzyme containing the $[^{32}]$ADP-ribosylated $\alpha$-component ($M_r\sim 42,000$) of $G_s$ produced a single $M_r\sim 190,000$ species, believed to be the $G_s\alpha\gamma$ complex. The protein pattern of the purified adenylate cyclase did not change when further fractionation by chromatography on wheat germ agglutinin Sepharose, by a second chromatography on forskolin-Sepharose, by ion exchange chromatography on DEAE-Sepharose, or by sucrose density gradient centrifugation was attempted. By each criterion so far applied, the relative intensity of the protein bands remained constant and followed adenylate cyclase activity. The identity of the minor component of $M_r\sim 45,000$ remains to be clarified. It is possible that myocardial membranes contain a small population of a second $G_s\alpha$-subunit (as most tissues) that has escaped detection by choleratoxin. This would be compatible with the crosslinking experiments, which consistently failed to reveal a product of $M_r\sim 190,000$. The $\beta$-subunit of $G_s$ appeared to be absent in the purified adenylate cyclase preparation (Fig. 2 A and B). This was in agreement with the results obtained from the crosslinking experiments (Fig. 4), which likewise ruled out an involvement of that entity in the reconstituted complex.

Our results indicate that the $M_r$ of adenylate cyclase is $150,000$ (C) or $190,000$ in the activated form (G$_s$C), although the molecular size in its native environment could be different. However, using target size theory for estimation of the size of membrane-bound rat liver adenylate cyclase, $M_r$ values of $150,000$ (for the nonactivated enzyme) and $230,000$ (for the $p[NH]ppG$-activated enzyme) were deduced (30), which are in good agreement with the data we have obtained. Somewhat higher $M_r$ values resulted from hydrodynamic measurements from a detergent-solubilized enzyme: $190,000$ for the catalytic function of the cyt$^-$ mutant of S49 lymphoma cells (31) and $250,000$ for the $p[NH]ppG$-activated enzyme from S49 wild-type cells (32). The rather large $M_r$ of the adenylate cyclase catalytic subunit together with the finding that it specifically binds to wheat germ lectin suggest that $C$ may be a glycoprotein that traverses the plasma membrane. This aspect raises the question of the physiological significance of the catalyst’s accessibility from the external face of the membrane bilayer.

We thank Mrs. B. Weber and Mr. M. Brenner for expert technical assistance and Mrs. C. Möller for help with the preparation of the manuscript. We thank Drs. Norman Geisler and K. Weber (Göttingen) for a gift of purified neurofilament proteins. The generous supply with forskolin by Drs. Schöne and Schorr (Hoechst Company, Frankfurt) is gratefully acknowledged. We are indebted to Dr. Suzanne Lohmann for critical reading of the manuscript. This work was supported by grants from the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie.