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Biochemistry

Identification of the catalytic subunit of brain adenylate cyclase: A calmodulin binding protein of 135 kDa

(synaptosomes/gel overlay/proteolysis/N_s regulatory subunits)

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ABSTRACT **The partial purification of the eukaryote adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] catalytic subunit has been achieved by a procedure based on the calmodulin (CaM) sensitivity of the enzyme. Small amounts of rat brain synaptosomal membranes depleted of CaM were solubilized with Lubrol and subjected to a three-step chromatographic procedure involving gel filtration, a CaM-Sepharose affinity step, and fast protein liquid chromatography. About 20% of the adenylate cyclase activity contained in the membranes was recovered in the final enriched fraction with a specific activity of 200 nmol·mg^{-1}·min^{-1}. The α subunits of the adenylate cyclase stimulatory proteins N_s were absent from this final fraction. The addition of CaM, of forskolin, or of preactivated N_s-containing fractions to this preparation greatly increased the enzyme activity. A CaM-binding polypeptide of 135,000 Da copurified with the adenylate cyclase activity in each of the three steps. Polyacrylamide gel electrophoresis of the final fraction showed that this polypeptide represented 35% of the total protein. We propose that this polypeptide is likely to be the adenylate cyclase catalytic subunit. This enzyme would represent close to 0.5% of the synaptosomal membrane proteins. Its low turnover number would be due to the absence of the α subunits of the N_s regulatory proteins and would correspond to the enzymic basal level.**

Cyclic AMP-mediated signals, hormones, or neurotransmitters are collected by receptors that, by interacting with two sets of regulatory proteins, N_s and N_i, stimulate or inhibit adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1]. N_s and N_i are inserted between the receptors and the catalytic subunit of adenylate cyclase (1, 2). The regulatory proteins have been purified to homogeneity. Each set consists of three polypeptides—α, β, and γ. Both α N_s (42–52 kDa) and α N_i (40 kDa) are GTP-binding proteins possessing a GTPase activity, and both can be ADP-ribosylated by, respectively, cholera or pertussis toxins. The information gained recently on adenylate cyclase regulatory components sharply contrasts with the paucity of data concerning the enzyme catalytic subunit.

In the brain, where adenylate cyclase is thought to play a crucial role and has a very high specific activity (3), the enzyme activity largely depends on Ca^{2+} calmodulin (CaM) levels (4, 5). CaM may interact with one or several components of the membrane-bound adenylate cyclase complex. Several recent independent studies tend to show that CaM stimulation occurs in the absence of GTP (6, 7) or of functional N_s subunits (8). CaM would thus interact with the adenylate cyclase catalytic subunit itself. These observations formed the basis of the approach of Andreasen *et al.* to the identification of the catalytic subunit (9). Following the same line, we have set up a simple three-step procedure allowing progress in the purification of the adenylate cyclase catalytic subunit on the basis of its CaM sensitivity. We obtained a fraction containing a high yield of a CaM-activated enzyme with a specific activity of 200 nmol·mg^{-1}·min^{-1} and completely devoid of α subunits of the stimulatory N_s proteins. We propose that the major component of this fraction, a 135-kDa CaM-binding polypeptide, is likely to be the adenylate cyclase catalytic subunit.

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MATERIALS AND METHODS

CaM-Sepharose-CL-4B resin, a mono Q column and a fast protein liquid chromatography (FPLC) apparatus were from Pharmacia. Disuccinimidylsuberate and Iodogen were from Pierce, Bio-Gel A 5 m was from Bio-Rad, [α-^{32}P]ATP (30 Ci/mmol; 1 Ci = 37 GBq) was from New England Nuclear, [^{3}H]cAMP (23 Ci/mmol) was from Commissariat à l'Energie Atomique (France), and ^{125}I-labeled Na (2500 Ci/mmol) was from Amersham.

Preparation of Rat Brain Synaptosomal Membranes and Membrane Solubilization. This was performed according to d'Alayer *et al.* (10). Briefly, rat brains were homogenized (1 g/ml) in cold buffer A [50 mM Tris·HCl, pH 7.5/5 mM $MgCl_2$/10% (wt/vol) sucrose/2 mM EGTA/2 mM dithiothreitol/0.1 mM phenylmethylsulfonyl chloride ($PhMeSO_2Cl$)/0.2 mg of leupeptin per ml/0.2 mg of aprotinin per ml]. Aprotinin (Trasylol) was a gift from the Bayer Company (Federal Republic of Germany). The synaptosomes were prepared by flotation as described (10). CaM-depleted membranes (18 mg per rat brain) were obtained by three consecutive shocks of the synaptosomes, by lysis in a buffer containing 1 mM triethanolamine (pH 8.1), 2 mM dithiothreitol, 0.1 mM $PhMeSO_2Cl$, 1 mM EGTA for the first two shocks, and 1 mM EDTA for the third shock. Control membranes were obtained by preparing synaptosomes and synaptosomal membranes in the absence of Ca^{2+} chelators. Membranes were solubilized as described (10) in a medium containing 50 mM triethanolamine (pH 8.1), 0.9% Lubrol, 2 mM dithiothreitol, 1 mM EDTA, and 1 mM $MgCl_2$. After centrifugation, the supernatant was used.

Adenylate Cyclase Purification. Adenylate cyclase was purified in three steps, carried out at 6°C.

(*i*) Gel filtration: 3 ml of Lubrol supernatant (6 mg of protein) was chromatographed on a Bio-Gel A 5 m column (1.9 × 85 cm) equilibrated in buffer B (50 mM triethanolamine, pH 8.1/75 mM sucrose/2 mM dithiothreitol/2 mM EDTA/1 mM $MgCl_2$/0.05% Lubrol-PX/0.02% NaN_3/0.1 mg of leupeptin per liter/0.1 mg of aprotinin per liter/0.1 mM $PhMeSO_2Cl$) and eluted with the same buffer as described (10).

Abbreviations: CaM, calmodulin; FPLC, fast protein liquid chromatography.

(*ii*) CaM-Sepharose affinity chromatography: The Bio-Gel eluate fractions, from 22 to 40 ml (Fig. 1) (0.7 mg of protein), were pooled and complemented with 2 mM $MgCl_2$ and 1 mM $CaCl_2$. This "Bio-Gel pool" was incubated 1 hr at 4°C with 3 ml of CaM-Sepharose-CL 4B resin equilibrated with buffer I (buffer B lacking EDTA and $MgCl_2$, and containing 1 mM $CaCl_2$). The mixture was then poured onto a column (1 × 5 cm), washed, and eluted as described in the legend of Fig. 3.

(*iii*) Anion exchange chromatography: The selected CaM-Sepharose fractions (peak A in Fig. 3) were pooled (48 μg of protein) and chromatographed on a Mono Q column equilibrated with buffer C (20 mM triethanolamine, pH 7.8/0.1 mM dithiothreitol/1 mM EDTA/0.05% Lubrol). The column was eluted with a 50-ml linear KCl gradient, from 0.1 to 1 M, at a flow rate of 1 ml·min^{-1}.

Adenylate Cyclase Assay. The assay contained, in a final vol of 300 μl, 5–150 μl of the enzyme sample, 0.2 mM [α-^{32}P]ATP (2–6 × 10^5 cpm), 50 mM triethanolamine·HCl buffer (pH 8.0), 0.5 mM [^{3}H]cAMP (3 × 10^4 cpm), 2 mM dithiothreitol, 5 mM theophylline, 0.017% Lubrol, 10 mM $MgCl_2$, 0.2 mM EGTA, 0.25 mM $CaCl_2$, and, when indicated, 2 mM $MnCl_2$. The assay was performed with and without 1 μM CaM. In some cases, 0.1 mM forskolin was added in the absence of CaM. The method of Salomon *et al.* (11) was used to quantify cAMP.

Iodination of CaM and CaM Gel Overlay. CaM was prepared according to ref. 12. CaM (5 μg) was iodinated by the Iodogen procedure, at room temperature for 5 min, with 1.5 mCi of ^{125}I-labeled Na. Iodinated CaM was separated from free iodide by filtration on a PD10 column (Pharmacia) in the presence of 250 μg of bovine serum albumin. CaM gel overlays were prepared as described in the legend to Fig. 5.

Other Procedures. (*i*) Protein was measured according to ref. 13. Also, fractions corresponding to CaM-Sepharose peak A, of known protein content, were iodinated, and the protein content of the fractions from the FPLC column was deduced from their ^{125}I content. Densitometry of silver-stained gels scanned with a Vernon microdensitometer was used in parallel.

(*ii*) To label the α subunits of N_s and N_i, ADP-ribosylation of membranes by cholera and pertussis toxins was carried out as described (10, 14, 15).

(*iii*) Complementation assays were done by mixing and incubating enzyme-containing fractions with N_s-containing fractions (Fig. 1, 68–73 ml) as described (16).

(*iv*) The reconstitution of CaM-depleted membranes with exogenous CaM was studied. CaM-depleted membranes (4 mg/ml) were incubated for 15 min at 30°C with 1 μM CaM (containing 5 × 10^4 cpm of ^{125}I-labeled CaM) in 50 mM triethanolamine·HCl, pH 8.0/0.2 mM EGTA/0.25 mM $CaCl_2$/10 mM $MgCl_2$/2 mM dithiothreitol, and inhibitors of proteases as in buffer A; membranes were then washed. CaM-reconstituted membranes were solubilized and subjected to gel filtration (see Fig. 2 legend).

RESULTS

Preparation of a Brain Lubrol-Solubilized Fraction Containing the Adenylate Cyclase Activity. Since brain adenylate cyclase activity largely depends on CaM, we chose as a starting material a well-defined brain compartment: synaptosomes, in which both Ca^{2+} and adenylate cyclase are thought to play major roles (3). This material was carefully protected from proteolysis (10). Hypotonic shocks of the synaptosomes eliminated most of the soluble CaM content, albeit leaving some membrane-bound CaM (17). The adenylate cyclase-specific activity of CaM-depleted membranes was 90 pmol·mg^{-1}·min^{-1}, lower than that of control membranes by a factor of 2.5. It regained a value of 220–260 pmol·mg^{-1}·min^{-1} when membranes were incubated with 1 μM CaM.

Next, the membranes were solubilized with Lubrol, a procedure well known to increase the apparent specific activity of the enzyme (18) (Table 1). The Lubrol soluble adenylate cyclase was only marginally stimulated by 1 μM CaM (1.3-fold). Indeed, solubilization of the membranes by Lubrol resulted in the release of the residual membrane-bound CaM, thus obscuring the effect of exogenous CaM on the enzyme activity (17).

Gel Filtration Step. A high level of CaM activation of adenylate cyclase was again observed in the gel filtration eluate (Fig. 1). Two peaks of CaM-dependent enzyme activity were detected upon elution. The first one, in the void volume, was of low specific activity (1.3 nmol·mg^{-1}·min^{-1}) and thus was not further studied. The second peak, of higher specific activity (7.5 nmol·mg^{-1}·min^{-1}), corresponded to particles of Stokes radii of 10–12 nm (Fig. 1, 20–45 ml) and was thus completely separated from the peak of free CaM, which has a Stokes radius of 2.1 nm (19). In the peak fraction, the adenylate cyclase activity was increased 4.5-fold by including 1 μM CaM in the assay, in the presence of Mg^{2+} (3.5-fold in the presence of Mg^{2+} and Mn^{2+}). The addition of CaM to the assay resulted in an apparent gain in the recovery of adenylate cyclase activity in the eluate fractions, with respect to that of the supernatant applied to the column (≈150%). For further fractionation, we selected the eluate fractions from 20 to 40 ml, or Bio-Gel pool, containing close to 50% of the total CaM-sensitive enzyme activity (Table 1).

The gel filtration step achieved the separation of adenylate cyclase activity from the α N_s and α N_i subunits (Fig. 1, 70–85 ml); 0.8% of the α N_s subunits applied to the Bio-Gel column were recovered in the Bio-Gel pool, accounting for a 1.4-fold increase in the enzyme activity of this pool observed upon incubation with 0.1 mM guanylylimidodiphosphate. By comparison, the adenylate cyclase found in the Lubrol unfractionated supernatant was stimulated 2.8-fold by the GTP analogue.

When adenylate cyclase was assayed in the presence of forskolin, an additional peak of lower particle size became apparent (Stokes radius, 8.5–10 nm; not shown). More α N_s subunits were present in the fractions containing this CaM-

Table 1. Partial purification of adenylate cyclase catalytic subunit

Material selected at each step	Protein, mg	Activity,* nmol·min^{-1}	Specific activity,* nmol·min^{-1}·mg^{-1}	Yield, %	Purification, -fold
Synaptosomal membrane	16.21	2.76	0.17	100	1
Lubrol-solubilized membrane	15.06	5.27	0.35	191	2
Lubrol supernatant	6.65	4.65	0.7	168	4
Selected Bio-Gel pool	1.08	3.77	3.5	137	20
CaM-Sepharose peak A	0.09	2.26	25.1	82	148
Selected FPLC fraction	0.008	0.61	76.1	22	448

The purification procedure applies to 16 mg of membrane proteins.
*Enzyme activities were measured in the presence of 10 mM $MgCl_2$, 2 mM $MnCl_2$, and 1 μM CaM.

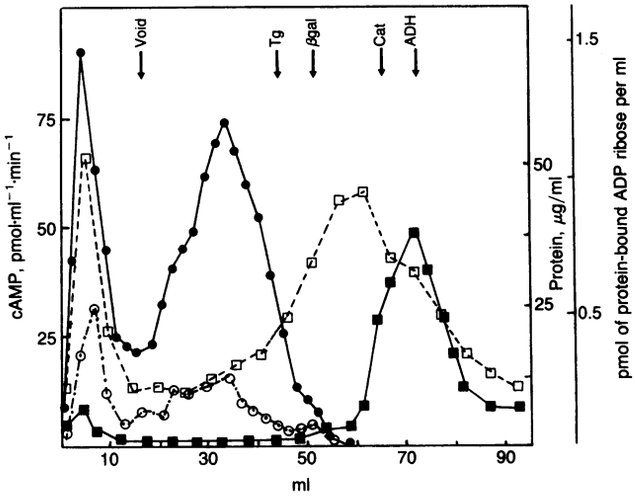

FIG. 1. Fractionation of the Lubrol supernatant by Bio-Gel A 5 m chromatography. Lubrol-soluble ADP-ribosylated extract (3 ml) was layered on the column and eluted with buffer B at 4°C. Adenylate cyclase was assayed with (●) or without (○) 1 μM CaM in the presence of 10 mM $MgCl_2$. Dried gel bands corresponding to proteins ADP-ribosylated by both cholera and pertussis toxins were excised, dissolved in NCS tissue solubilizer (Amersham), and their radioactivity was determined (■); protein (□). Arrows point to enzymes of known Stokes radius, as indicated in ref. 10. Tg, thyroglobulin; βgal, β-galactosidase; Cat, catalase; ADH, alcohol dehydrogenase.

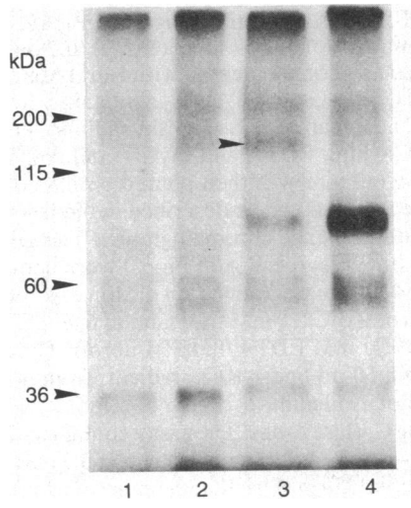

FIG. 2. Analysis of the CaM-binding proteins in the eluate of the Bio-Gel column by cross-linking with ^{125}I-labeled CaM. CaM-depleted membranes were reconstituted with ^{125}I-labeled CaM, washed, and solubilized in the presence of 0.20 mM EGTA and 0.25 mM Ca^{2+}. The supernatant was layered on the column and eluted with buffer B lacking EDTA and containing 5 mM $MgCl_2$. Fractions were treated with 0.25 mM disuccinimidylsuberate at 0°C for 5 min; the reaction was stopped by adding 10 mM Tris·HCl (pH 7.5). Electrophoresis of the trichloroacetic acid-precipitated fractions was performed on 7–12% polyacrylamide gels, and the gels were autoradiographed. Lanes: 1, fractions corresponding to 20–26 ml; 2, 27–30 ml; 3, 31–40 ml; 4, 41–47 ml (see Fig. 1). The fractions corresponding to lane 3 contain the highest amount of adenylate cyclase activity. Size markers were myosin (200 kDa), β-galactosidase (115 kDa), catalase (60 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa).

insensitive adenylate cyclase activity than in those containing the CaM-sensitive activity.

The binding of CaM to membrane proteins was studied by using ^{125}I-labeled CaM reconstituted membranes. The corresponding eluate fractions were briefly treated with the cross-linking reagent disuccinimidylsuberate, and the protein content was analyzed by PAGE and autoradiography (Fig. 2). In the adenylate cyclase-containing fractions, CaM was cross-linked to several polypeptides, one of which was specific to these fractions and corresponded to a radioactive complex of 150,000 Da.

CaM-Sepharose Affinity Step. The Bio-Gel pool was incubated with CaM-Sepharose in the presence of 1 mM Ca^{2+}; 52–60% of the applied adenylate cyclase activity was retained on the affinity column. Upon washing, the adsorbed activity was recovered in two peaks by elution with 1 mM EDTA (Fig. 3). Peak A was eluted with a low ionic strength buffer. As measured in the presence of Mn^{2+} and 1 μM CaM, it contained 38% of the enzyme activity applied to the column and had a specific activity of 25 (±4) nmol·mg^{-1}·min^{-1} (Table 1). At least a 4.5-fold stimulation by CaM was obtained in the presence of Mg^{2+}, 3.5-fold in the presence of Mg^{2+} and Mn^{2+}. Forskolin enhanced the basal catalytic activity by a factor of 10. The fractions corresponding to peak A contained only 3% of the α N_s subunits present in the Bio-Gel pool. Not surprisingly, the enzyme activity in peak A was not stimulated by guanylylimidodiphosphate, but it was activated 5-fold upon complementation with crude fractions containing N_s (not shown). Peak B was eluted with a high ionic strength buffer (Fig. 3). It contained 16.5% of the applied enzyme activity and otherwise resembled peak A.

All fractions were studied by PAGE analysis with respect to their protein and CaM-binding protein composition. A polypeptide of 135 kDa was detected by silver staining of the gels only in fractions containing adenylate cyclase activity. It accounted for 10–17% of the total proteins in peaks A and B (Fig. 4*A*). In gels treated with the CaM overlay technique, several CaM-binding proteins were revealed, one of which, a polypeptide of 135 kDa, was present only in fractions containing adenylate cyclase activity (Fig. 5*A*).

Anion Exchange Chromatography. Peak A of the CaM-Sepharose column was further analyzed by chromatography on a FPLC Mono Q column. A sharp peak of enzyme activity was eluted at a concentration of 0.25 M KCl. Its specific

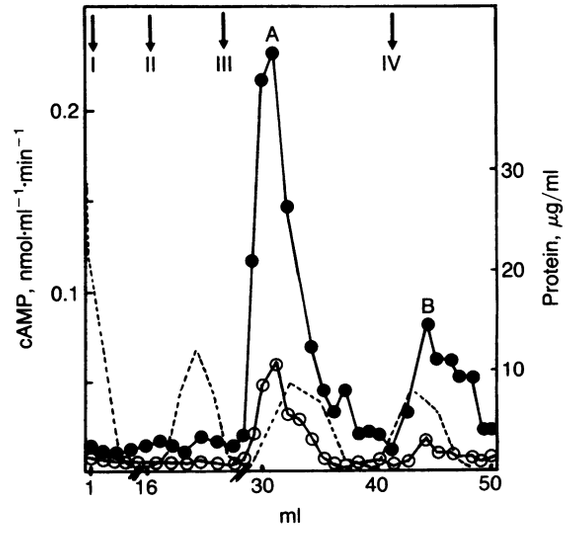

FIG. 3. Elution of adenylate cyclase from the CaM-Sepharose column. The selected Bio-Gel pool (675 μg of protein) was incubated with CaM-Sepharose as described. The column was washed with 13 ml of buffer I, 13 ml of buffer II (buffer I/0.2 M NaCl), 10 ml of buffer III (buffer I without $CaCl_2$ and with 1 mM EDTA), and 10 ml of buffer IV (buffer III/0.5 M NaCl). Fractions (1 ml) were collected. Adenylate cyclase was assayed with (●) or without (○) 1 μM CaM and in the presence of 2 mM $MnCl_2$ and 10 mM $MgCl_2$; protein (----).

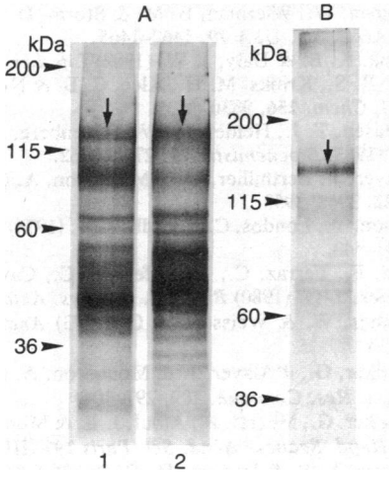

FIG. 4. Analysis of the proteins eluted from the CaM-Sepharose column and from the FPLC anion exchange column, by silver staining of the gels according to ref. 20. (*A*) CaM-Sepharose eluate fractions. Pooled fractions (1 ml) corresponding to peak A (lane 1) and to peak B (lane 2) were precipitated with trichloroacetic acid and analyzed. (*B*) Fraction from the anion exchange column, corresponding to KCl molarities of 0.25–0.28 M KCl, and treated as in *A*. Size markers are as in Fig. 2. Arrows point to a 135-kDa polypeptide.

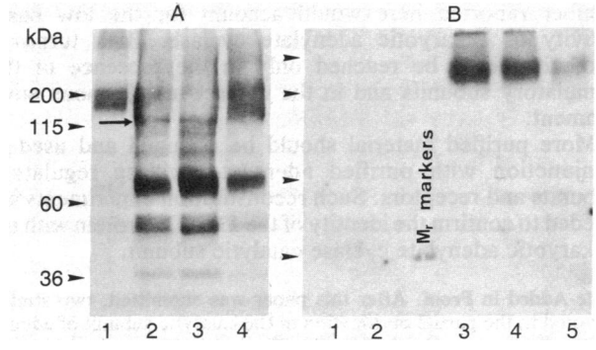

FIG. 5. Analysis of the CaM-binding proteins by CaM gel overlays. After electrophoresis, gradient gels (7–12%) were fixed with 25% isopropanol/10% acetic acid, washed with water, treated with 6 M guanidium chloride in buffer D [50 mM Tris·HCl, pH 7.5/0.15 M NaCl/20% (vol/vol) glycerol/1 mM Mg acetate] for 30 min, washed in buffer D, and then in buffer D without glycerol. Gels were incubated in buffer D containing 1 mg of bovine serum albumin per ml, 1 mM $CaCl_2$, and 4–5 × 10^7 cpm of ^{125}I-labeled CaM per gel for 24 hr. Gels were washed with several changes of buffer D. For control gels, 1 mM $CaCl_2$ was replaced by 2 mM EDTA. Gels were stained with Coomassie blue, dried, and autoradiographed. Arrows point to size markers. (*A*) CaM-Sepharose eluate fractions. Lane 1, fractions eluted with buffer II (Fig. 3, 17–26 ml); lanes 2 and 3, fractions corresponding to peak A (Fig. 3, 27–36 ml; lane 2, 27–31 ml; lane 3, 32–36 ml); lane 4, fractions corresponding to 37–41 ml. Arrow points to a 135-kDa CaM-binding protein. (*B*) Fractions from anion exchange column. Lane 1, fractions corresponding to KCl molarities of 0.13–0.17 M; lane 2, 0.18–0.29 M; lane 3, 0.24–0.26 M; lane 4, 0.27–0.30 M; lane 5, 0.31–0.38 M.

activity was 200 nmol·mg^{-1}·min^{-1}, as measured in the presence of forskolin, 76 nmol·mg^{-1}·min^{-1} in the presence of Mn^{2+} and 1 μM CaM (Table 1). Five-fold stimulation by 1 μM CaM was obtained in the presence of Mn^{2+} and even more was obtained in the presence of Mg^{2+}. The protein composition of the corresponding fractions was less complex than that of the CaM-Sepharose peaks A or B (Fig. 4*B*). A major polypeptide of 135 kDa, present only in adenylate cyclase-containing fractions, accounted for 35% of the protein content of the enzymic peak fraction, as deduced from the scanning of the gels. This polypeptide avidly bound CaM in gels in a Ca^{2+}-dependent manner (Fig. 5*B*). No other CaM-binding proteins could be detected in those fractions by this technique, but we cannot exclude the possibility that other components of this fraction could bind CaM when in the native state.

DISCUSSION

In this study, brain adenylate cyclase activity is shown to be recovered in a high yield within a fraction obtained within 24 hr by a simple three-step chromatographic procedure. This fraction contains one major polypeptide of 135 kDa, which is the only CaM-binding protein detectable by the overlay method. The α subunits of N_s and N_i are absent from this fraction.

Taking advantage of the considerable variation occurring in membrane protein associations upon solubilization with different detergents, we have repeated the same study with material solubilized with the zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate at high ionic strength. We found that the fractions that contained the highest adenylate cyclase activity were also enriched in a CaM-binding 135-kDa polypeptide. This is the basis for our proposal that this protein is the CaM-sensitive catalytic subunit of adenylate cyclase. The fact that a cross-linking agent binds CaM in the membrane to a polypeptide of ≈135 kDa may also be recalled, because adenylate cyclase activity in membranes depends so much on the presence of CaM.

A protein of 150 kDa has already been proposed as the catalytic subunit by Andreasen *et al.* (9), who followed the presence of azido-calmodulin-labeled polypeptides in their fractions containing CaM-sensitive adenylate cyclase activity. However, these authors did not analyze the protein composition of their fractions. Another approach has been followed by Pfeuffer *et al.* (21), based on the known interaction of forskolin with the adenylate cyclase subunit. By using a forskolin affinity column, these authors obtained an adenylate cyclase-enriched fraction with a specific activity of 1.1 μmol·mg^{-1}·min^{-1} when assayed with forskolin and added N_s subunits. No further analysis of the fractions was shown. Comparisons of adenylate cyclase-specific activities determined in different laboratories are hazardous, because the enzyme assay conditions, as well as the procedures used to measure protein, are different. For example, when we determined protein in our final fractions by the procedure used by Andreasen *et al.* (9) or by Pfeuffer *et al.* (21), we obtained protein values that were 1/10th the values obtained with our usual assay (13). Furthermore, this discrepancy was noted only in fractions containing very diluted protein. This would lead to considerable overestimation of the enzymic specific activity of our final fraction and of the purification of the enzyme. Concerning the enzyme assay conditions, the presence and amount of N_s subunits in the fractions to be tested determine at least in part the level of activity of the enzyme. The purification procedure that we describe eliminates the α N_s subunits, contrary to the procedure based on forskolin affinity columns (21).

The final step of our procedure yielded a fraction with an adenylate cyclase-specific activity of 200 nmol·mg^{-1}·min^{-1}. If we presume that the 135-kDa polypeptide is the adenylate cyclase catalytic subunit, it would represent ≈0.5% of the synaptosomal membrane proteins. Following this line, the isolated catalytic subunit would have a turnover number of ≈80 min^{-1}. This is in the range of values found for the *Escherichia coli* adenylate cyclase enzyme obtained from the isolated gene (22). Proteins exerting the same enzymic role are indeed expected to have rather similar active sites, even in very different organisms. The relatively low turnover

number reported here would account for the low basal activity of eukaryotic adenylate cyclase. High turnover numbers would be reached only in the presence of the stimulatory subunits and in the correct membranous environment.

More purified material should be obtained and used in conjunction with purified adenylate cyclase regulatory subunits and receptors. Such reconstitution experiments are needed to confirm the identity of the 135-kDa protein with the eukaryotic adenylate cyclase catalytic subunit.

Note Added in Proof. After this paper was submitted, two studies devoted to the partial purification of the catalytic subunit of adenylate cyclase were published by Pfeuffer *et al.* (23) and by Yeager *et al.* (24). In ref. 24, the purification procedure is also based on the calmodulin sensitivity of the enzyme. All three studies are compatible with a catalytic subunit of 135–150 kDa.

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