Purification and Characterization of an Inhibitor Protein of Brain Adenylate Cyclase and Cyclic Nucleotide Phosphodiesterase

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A heat-labile inhibitor protein of adenylate cyclase (EC 4.6.1.1) and phosphodiesterase (EC 3.1.4.17) has been purified to apparent homogeneity from bovine brain cerebrum by a simple two-column procedure. The inhibitor exerts its effect on adenylate cyclase or phosphodiesterase by forming a complex with the Ca$^{2+}$-dependent activator protein, thereby competing with the apoenzyme for the activator. The protein was estimated to have a molecular weight of 80,000 and a Stokes radius of 39 Å by gel filtration. The inhibitor was resolved in a sodium dodecyl sulfate-polyacrylamide gel into two equal molar subunits, with molecular weights of 60,000 and 18,500. In the presence of the activator and Ca$^{2+}$, the thermal stability of the inhibitor was increased, indicative of a new conformation. The effectiveness of the inhibitor varied considerably, depending on its sequence of addition to the reaction mixture relative to phosphodiesterase and the activator protein, presumably because the activator appeared to have a greater affinity for the inhibitor than for phosphodiesterase.

The cellular level of cAMP is regulated by the activity of adenylate cyclase, which catalyzes the synthesis from ATP and by that of cyclic nucleotide phosphodiesterase, which catalyzes its hydrolysis to 5'-AMP. In mammalian brain, both enzyme systems require a common Ca$^{2+}$-dependent activator protein for maximum activity (2, 3). The activator protein, a heat-stable, ubiquitous Ca$^{2+}$-binding protein in animal cells, has been extensively characterized (1, 4-18); it interacts with the relatively inactive apoenzyme of adenylate cyclase, phosphodiesterase, or erythrocyte (Ca$^{2+}$-Mg$^{2+}$)-ATPase to form an activated holoenzyme, resulting in an increase of the $V_{max}$ and little or no change in the $K_M$ (19-21).

Recently, the activator has been shown to stimulate skeletal muscle actomyosin ATPase (22, 23), human erythrocyte (Ca$^{2+}$-Mg$^{2+}$)-ATPase (24, 25), Ca$^{2+}$ efflux in erythrocytes (26), myo-inositol lipid chain protein kinase (27), phosphorylation of synaptic membranes (28), and disassembly of microtubules (29). Thus, it appears that the protein activator serves as a multifunctional modulatory protein in animal cells.

In addition to the activator protein, the activity of brain adenylate cyclase and phosphodiesterase may also be regulated by inhibitor proteins (15, 30-34). Of particular interest is a heat-labile inhibitor protein found in bovine brain (31). This protein diminished the activity of the holoenzyme and not that of the apoenzyme, presumably by forming a Ca$^{2+}$- dependent inhibitor-activator complex, in direct competition with the apoenzyme for the activator (15, 32-34). Recently, the inhibitor has been purified to apparent homogeneity (33), but the yield appears rather low.

In this communication, we describe a simple procedure which allows the purification of the inhibitor protein to apparent homogeneity from bovine brain in two column steps with some 10 fold increase in the yield. In addition, we have characterized its molecular properties and subunit structures.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—[3H]cAMP (20 Ci/mmol) and ovalbumin were purchased from Schwarz/Mann. cAMP, RNAase, and bovine serum albumin were obtained from Sigma. Affi-Gel blue (agarose beads covalently linked to Cibacron blue F3GA, 100 to 200 mesh, 70 to 100 μm), Affi-Gel 10 (an activated agarose with a 10 Å spacer arm), acrylamide, N,N'-methylenebisacrylamide, and Coomassie brilliant blue were supplied by Bio-Rad. Goat immunoglobulin G was a gift from Dr. William Walker. Sephacryl S-200 superfine was obtained from Pharmacia. All other reagents were of highest analytical grades.

**Preparation and Assay of Phosphodiesterase—Ca$^{2+}$-dependent phosphodiesterase was partially purified from bovine brain to the stage of a DEAE-cellulose column (35). The enzyme at this stage was activator-deficient and required an exogenous activator for full activity. Phosphodiesterase was assayed by a two-stage procedure with a batch use of an anionic exchange resin AG 1-X2; the data were corrected for binding of adenosine to the resin (36). The reaction mixture contained 40 mM Tris-HCl (pH 8.0), 5 mM MgCl$_2$, 50 μM Ca$^{2+}$, 2 μM [3H]cAMP, and an appropriate amount of enzyme in a final volume of 0.1 ml. Incubation was at 30°C for 10 to 20 min. One unit of phosphodiesterase activity is that amount of protein catalyzing the hydrolysis of 1 pmol of cAMP/min under the standard conditions.

**Preparation and Assay of Activator Protein**—The activator protein, prepared from bovine brain as described previously (37), was assayed by its ability to stimulate activator-deficient phosphodiesterase in a reaction mixture containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl$_2$, 50 μM Ca$^{2+}$, 5 milliunits of phosphodiesterase, an appropriate amount of activator and 2 μM [3H]cAMP; the final reaction mixture was 0.1 ml. One unit of activator is that amount of protein giving half-maximal stimulation of phosphodiesterase.

**Assay of Inhibitor Protein**—The inhibitor was determined by its ability to suppress activator-supported phosphodiesterase activity in a reaction mixture containing the standard ingredients, 2 units of activator protein, an appropriate amount of inhibitor protein, and 5 pmol of [3H]cAMP.

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*Because of its multifunctions, this ubiquitous protein has been referred to in the literature under various designations: activator, activator protein, phosphodiesterase activating factor, phosphodiesterase activator protein, Ca$^{2+}$-dependent regulatory (CDR), troponin C-like modulator protein, and Ca$^{2+}$-dependent modulator. The term calmodulin has been proposed by Cheung et al. as a proper name; it is meant to denote a Ca$^{2+}$-modulated protein or a protein modulating the concentration of Ca$^{2+}$, or both (1).]

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milliliters of phosphodiesterase. After the addition of phosphodiesterase, the reaction was initiated immediately by the addition of cAMP and was allowed to proceed for 5 min. Phosphodiesterase was assayed as described above. One unit of inhibitor is the amount of protein causing 50% inhibition of the activator-supported phosphodiesterase activity. Under the assay conditions, one unit of inhibitor corresponds approximately to one unit of activator.

All assays were done in duplicates.

**Preparation of Affi-Gel Blue Column**—A column (3.2 × 46 cm) was packed with 400 ml of wet Affi-Gel blue, washed with 4 liters of 0.5 M NaCO₃ (pH 11), 4 liters of 2 M NaCl, and equilibrated with 4 liters of 50 mM Tris-HCl (pH 7.8), 3 mM MgSO₄, 1 mM dithiothreitol, and 1 mM EGTA (Buffer A). At the end of a run, the column was regenerated with the same procedure. After several runs, the Affi-Gel blue was drained into a large beaker and washed with 0.5 M NaCO₃ and then 2 M NaCl. This was done to remove insoluble fatty materials that accumulated in the column. The washed gel was then repacked into the column, was equilibrated with Buffer A, and was ready for use again.

**Chemical Coupling of Activator Protein to Affi-Gel 10**—Affi-Gel 10 is a N-hydroxysuccinimide ester of a succinylated aminoalkylagarose support. The activated arm, 10 Å long, reacts with the amino group of proteins under mild conditions. Briefly, 20 ml of 0.1 M phosvitin (pH 7.0) was added to 1 g of dehydrated Affi-Gel 10, which swelled to 10 to 15 ml when hydrated. To this was added 25 mg of bovine brain activator. The mixture was incubated overnight at 4°C with gentle shaking. Twenty-five per cent of activator (6.3 mg) was covalently bound to the gel. The unbound protein was removed by washing first with Buffer A containing 1 M NaCl, and then with Buffer A. When not in use, the gel was kept in Buffer A containing 0.02% NaN₃. One preparation was used repeatedly over a period of more than 6 months with no apparent decrease in functional capacity.

**Polyacrylamide Gel Electrophoresis**—Electrophoresis under non-denaturing conditions was done in a 7% acrylamide, 3% N,N'-methylenebisacrylamide gel using a continuous 0.188 M Tris, 0.188 M glycine buffer system (pH 6.0) at 4°C. Discontinuous sodium dodecyl sulfate gel electrophoresis was performed at 22°C in a slab gel which contained a linear 5 to 15% acrylamide gradient. N,N'-Methylenebisacrylamide was kept constant at 2.7% and the buffer system was that described by Laemmli (38). Proteins were denatured by boiling in 1% sodium dodecyl sulfate containing 2 mM dithiothreitol immediately before electrophoresis. The marker dye was bromophenol blue; proteins were stained with Coomassie brilliant blue.

**Protein Determination**—Protein was determined according to Lowry et al. (39) with slight modification. The protein was precipitated with 10 volumes of 10% perchloric acid and 1% phosphotungstic acid. Bovine serum albumin was used as a standard.

## Results

### Purification of Inhibitor Protein from Bovine Brain

In an attempt to further purify bovine brain Ca²⁺-dependent phosphodiesterase with an activator-agarose affinity column, we noted that a partially purified phosphodiesterase preparation (35) contained what appeared to be several other proteins which bound to the affinity column in a Ca²⁺-dependent, reversible manner. One of the proteins proved to be an inhibitor of brain adenylate cyclase and phosphodiesterase (15, 34).

Using an Affi-Gel blue column, which binds phosphodiesterase tenaciously, we have isolated a highly purified inhibitor protein from such a preparation. However, the final preparation was contaminated by a trace of Ca²⁺-dependent phosphodiesterase; the elution profile of this peak was reproducible. The trace of phosphodiesterase which preceded the major inhibitor peak was Ca²⁺-independent.

In this Affi-Gel blue column, the Ca²⁺-dependent phosphodiesterase present in the initial extract was retained by the column and could be eluted after the inhibitor with Buffer A containing 1.5 M NaCl and 1 mM cAMP. The amount of phosphodiesterase activity recovered from the column was about 30% of the activity originally loaded on the column.

**Activator-Affinity Column Chromatography**—This step takes advantage of the fact that the inhibitor protein is bound to the affinity column in the presence of Ca²⁺ and is released when the concentration of Ca²⁺ is lowered (34). The active fractions from the second inhibitor peak of the Affi-Gel blue column were pooled, adjusted to 1.5 mM CaCl₂, and subjected to activator-affinity chromatography. Fig. 2 shows that the bulk of the protein passed through the column unhindered. The column was then eluted with Buffer A. All the inhibitor came out in two and sometimes three tubes. Note that there was no detectable endogenous phosphodiesterase in any of the fractions collected. The amount of protein pooled from the peak fraction ranged from 1.5 to 2 mg with a specific activity of about 2,000 units/mg of protein.

### Aliquot of Inhibitor Protein from the Brain Extract.

The inhibitor usually emerged under two active fractions. The activity associated with the first fraction was eluted with the bulk of the hemoglobin in the extract (see inset); the amount of assayable inhibitor was variable. The activator, present in overwhelming quantities in the brain extract, passed through the column as a broad peak, and the descending side of the activator peak partially overlapped with the first inhibitor peak. Because the assay of the inhibitor is based on its suppression of activator-supported phosphodiesterase activity, contamination by activator would give an apparently variable inhibitor peak. The second and major inhibitor peak, which emerged coincident with the minor hemoglobin peak, was free of the activator and phosphodiesterase; the elution profile of this peak was reproducible. The trace of phosphodiesterase which preceded the major inhibitor peak was Ca²⁺-independent.

**An Inhibitor Protein of CAMP Metabolic Enzymes**

Fig. 1. Affi-Gel blue column chromatography. Six hundred milliliters of a bovine brain extract was loaded on the column (3.2 × 46 cm) at a rate of 1 ml/min. The column was washed with 200 ml of Buffer A, and then eluted with 600 ml of Buffer A containing 0.2 M NaCl. Fractions of 20 ml were collected. An aliquot was assayed for inhibitor (——O), activator (— — —), and phosphodiesterase (C—C) activities. The endogenous phosphodiesterase (PDE) emerging along with the activator was Ca²⁺-independent. The elution pattern of hemoglobin was monitored at 550 nm (inset); the second hemoglobin peak coincided with the major inhibitor peak, which was pooled as indicated. Other details are present in the text.

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An Inhibitor Protein of cAMP Metabolic Enzymes

CaC₁₂, 1 mM DTT, and then eluted with Buffer A. Fractions of 10 ml were collected, an aliquot was assayed for inhibitor protein was stained with Coomassie brilliant blue; the polyacrylamide gel electrophoresis of the inhibitor in a 7% gel. The washed with 60 ml of 50 mM Tris-HCl (pH 7.8), 3 mM MgSO₄, 0.1 mM CaCl₂, 1 mM dithiothreitol, and then eluted with Buffer A. Fractions of 10 ml were collected; an aliquot was assayed for inhibitor by measurement with water. The column was eluted at a rate of 10 ml/h; 2-ml fractions were collected. An aliquot was assayed for inhibitor (○-○) and phosphodiesterase (PDE) (□-□) activities. Inset, acrylamide gel electrophoresis of the inhibitor in a 7% gel. The protein was stained with Coomassie brilliant blue; the lower mark identifies the location of the tracking dye, bromophenol blue. Protein in the gel, 20 μg.

Homogeneity of the Inhibitor—The inhibitor from the affinity column appeared as a single component in a 7% polyacrylamide gel under nondenaturing conditions (Fig. 2, inset). In a Sephacryl S-200 filtration column, the inhibitor was eluted in a single symmetrical peak (Fig. 3). A trace amount of protein, which appeared in the void volume of the column, exhibited no inhibitor activity; it may be an aggregate form of the phosphodiesterase system. For the tube containing the activator and Ca²⁺ or activator and EGTA, the sample was assayed for residual inhibitor activity with 5 millimoles of phosphodiesterase; for the tube containing Ca²⁺ or EGTA, the aliquot was assayed for residual inhibitor activity in the presence of 2 units of activator and 5 millimoles of phosphodiesterase. The concentration of Ca²⁺ in the assayed mixture was 250 μM. The per cent inhibitor activity remaining was calculated as follows:

\[
\frac{1 - \left( \frac{(E + A + I) - (E)}{(E + A) - (E)} \right)}{100}
\]

where \((E)\) stands for phosphodiesterase (basal activity), \((A)\) for activator, \((I)\) for inhibitor, \((E + A)\) for stimulated phosphodiesterase activity, and \((E + A + I)\) for phosphodiesterase activity after I was precubated for times indicated. Basal \((E)\) and stimulated \((E + A)\) phosphodiesterase activities were 200 and 530 nmol/mg/min, respectively.

General Properties—The inhibitor protein displays a typical ultraviolet spectrum with maximum absorption at 278 nm. The extinction coefficient of a 1% solution at 280 nm was 9.7, in good agreement with the findings of Klee and Krinks (33). Filtration of the inhibitor on a calibrated Sephacryl S-200 column indicated that the protein had a Stokes radius of 39 Å (Fig. 3, inset). Assuming that the protein is globular, the activity peak corresponds to a molecular weight of 80,000, a value identical to that observed earlier using a less pure preparation (34). Isolelectric focusing with ampholine in polyacrylamide gel indicated a pI of 6.0 (data not shown).

To determine the stability of the inhibitor, a sample containing 0.36 mg of protein/ml was stored in Buffer A under various conditions. At 4°C, the inhibitor lost 50% of its activity in 28 h while the sample fortified with 1% bovine serum albumin retained full activity. At −90°C, the inhibitor was fully active over a period of 16 days; full inhibitor activity was also conserved at −20°C in 1% bovine serum albumin and 50% glycerol.
An Inhibitor Protein of cAMP Metabolic Enzymes

The thermal stability of the inhibitor was further examined at 68°C (Fig. 4). In the presence of the activator and EGTA, all inhibitor activity was lost after 5 min; in contrast, in the presence of the activator and Ca²⁺, the inhibitor retained 60% of its activity. Even after 40 min, some 20% of the activity remained. In the controls containing EGTA or Ca²⁺, all the inhibitor activity was lost within 5 min, indicating that Ca²⁺ or EGTA did not affect the thermal stability. The activator is stable under this condition (35). Since the inhibitor-activator complex occurred in Ca²⁺ and not in EGTA, the inhibitor appeared more thermally stable in the complex form, probably because of a conformational change when it interacted with the activator. Brain adenylate cyclase was also more thermally stable in the presence of the activator and Ca²⁺ (40); in contrast, brain phosphodiesterase was less thermally stable (7).

Subunit Structure—To examine the subunit structure of the inhibitor, a sample was subjected to electrophoresis in a Laemmli sodium dodecyl sulfate-polyacrylamide gel (38). Fig. 5 shows that the sample was resolved into two bands. A densitometry scanning of the sodium dodecyl sulfate gel pattern indicated that the ratio of the area under the slower peak to that under the faster peak was approximately 3:1. A comparison of their mobilities relative to those of marker proteins indicated that the two bands corresponded to molecular weights of 60,000 and 18,500 (Fig. 5, inset). Since the molecular weight of the native inhibitor was 80,000, this suggests that the protein consists of two polypeptides at 1:1 ratio.

The inhibitor protein of phosphodiesterase purified from bovine brain by Klee and Krinks (33) also displayed two subunits in a sodium dodecyl sulfate-polyacrylamide gel; the molecular weights were 61,000 and 15,000, but the relative proportion of the two polypeptides was not determined.

Order of Inhibitor Addition Affects Phosphodiesterase Activity—As shown elsewhere (32–34), the inhibitor interacted with the activator in the presence of Ca²⁺ to form an inhibitor-activator complex. It is believed that the inhibitor exerted its effect on phosphodiesterase by combining with the activator, making the latter unavailable to the enzyme. In early studies, as well as in the present investigation described up to now, the assay of the inhibitor was initiated by the addition of phosphodiesterase into a reaction mixture containing the activator and the inhibitor. The observed enzyme activity was a measure of the extent of excess of activator or inhibitor. For example, if the inhibitor was present in excess, the resultant activity would be equal to the basal activity; in contrast, if the activator was present in excess, the resultant activity would fall in the range between the basal and maximal activities, depending on the extent of excess.

To determine the effect of the sequence of inhibitor addition on the activity of phosphodiesterase, one of the three proteins, phosphodiesterase (E), the inhibitor (I), or the activator (A), was added last to the reaction mixture. In this experiment, the activator was slightly in excess of the inhibitor. As shown in Fig. 6, phosphodiesterase activity was slightly above the basal level when E was added last; presumably the majority of the activator was in the form of I·A complex. When A was added last, the activity was considerably higher than the basal level, but was much lower than the activity expected if E and I competed with equal avidity for A. This implies that the affinity of I for A was greater than that of E for A under this condition.

**Fig. 5.** Electrophoresis of inhibitor protein on sodium dodecyl sulfate-polyacrylamide gel. A slab gel with a 5 to 15% linear polyacrylamide gradient was prepared according to Laemmli (38). Protein was stained with Comassie brilliant blue. The amount of protein was 20 μg. The gel was scanned with an Ortec 4130 densitometer. The ratio of the area under the two peaks was 2.8. Calibration with P-galactosidase (β-GAL, M₆ = 140,000), bovine serum albumin (BSA, M₆ = 67,000), ovalbumin (OVAL, M₆ = 42,000), and soybean trypsin inhibitor (T.I., M₆ = 21,000) indicated that the molecular weights of the two peaks were 60,000 and 18,500. These values suggest that the inhibitor consists of two subunits at 1:1 ratio.

**Fig. 6.** Effect of the order of inhibitor addition on phosphodiesterase activity. The reaction mixture contained 40 mM Tris-HCl (pH 8.0), 5 mM MgCl₂, 50 μM CaCl₂, 1 mM [³H]cAMP, 6.5 milliunits of phosphodiesterase (E), and where indicated, 2 units of activator (A) and 1.8 units of inhibitor (I). After the introduction of the third protein component (E, A, or I), the reaction was immediately started by the addition of the substrate. Phosphodiesterase was assayed at 30°C for 5 min as described under "Experimental Procedures."
An Inhibitor Protein of cAMP Metabolic Enzymes

The present work describes a simple procedure for the purification of an inhibitor protein from bovine brain to apparent homogeneity in two column steps. The scheme takes advantage of the fact that the inhibitor in the crude extract could be resolved from the Ca\textsuperscript{2+}-dependent phosphodiesterase on Affi-Gel blue column (1, 34). This made it possible to remove the remaining contaminating proteins on a subsequent activator-agarose affinity column. Although the latter binds both the inhibitor and the enzyme, the prior removal of phosphodiesterase allows it to bind the inhibitor selectively.

This procedure is attractive in several aspects: the protocol is simple and could be completed within 3 days; the yield is considerably higher than a currently available method (33); the product is completely free of phosphodiesterase, and the two columns could be easily regenerated for repetitive use.

Because the inhibitor could not be determined accurately in a tissue extract containing phosphodiesterase and the activator, it was not possible to determine the inhibitor level in the brain. The yield of 6 to 10 mg of inhibitor/kg of bovine brain cerebra may be a gross underestimate of its tissue content.

During the preparation of this manuscript, Klee and Krinks (33) reported the purification of an inhibitor protein of phosphodiesterase from bovine brain to apparent homogeneity. The protein was heat-labile, consisted of two polypeptides with molecular weights of 61,000 and 15,000, and formed a Ca\textsuperscript{2+}-dependent complex with the activator. Wang and Desai (32) recently purified an inhibitory factor of phosphodiesterase results from a competition of the inhibitor and the enzyme. The relative affinity of A for E and I cannot be rigorously studied without the availability of a pure phosphodiesterase.

DISCUSSION

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REFERENCES

Purification and characterization of an inhibitor protein of brain adenylate cyclase and cyclic nucleotide phosphodiesterase.  
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