Purification and Subunit Structure of Bovine Brain Modulator Binding Protein*

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The Ca"+-dependent modulator protein, which was originally discovered as an activator protein of cyclic nucleotide phosphodiesterase in mammalian tissues (1–8), has recently been found to mediate the Ca"+-dependent association of the modulator protein with the highly acidic modulator protein. The purified modulator binding protein is shown to be a globular protein of molecular weight 85,000. It contains two distinct subunits, subunits A and B, of molecular weights about 60,000 and 14,500, respectively. The mass ratio of subunits A/B is determined to be about 2.3:1. From these results, the subunit structure of modulator binding protein is suggested to be AB2. In the presence of 6 M urea, modulator binding protein dissociates into its constituent subunits, and subunit A and B can be separated on a G-100 Sephadex column. The isolated subunit A exhibits inhibitory activity against the Ca"+-activatable cyclic nucleotide phosphodiesterase. Subunit B has no phosphodiesterase inhibitory activity, nor does it affect the activity of subunit A in the enzyme reaction. These results suggest that subunit A is responsible for the association of modulator binding protein to the modulator protein. The function of subunit B is not known at present.

The Ca"+-dependent modulator protein, which was originally discovered as an activator protein of cyclic nucleotide phosphodiesterase in mammalian tissues (1–8), has recently been found to mediate the Ca"+ activation of several other reactions. These include reactions catalyzed by a brain adenylate cyclase (9), erythrocyte membrane (Ca"+/Mg"+)-ATPase (10, 11), a membrane-bound protein kinase (12), and a cytosolic protein kinase which has potent activity toward myosin light chain phosphorylation (13, 14). In addition, this protein has been shown to possess considerable sequence homology with troponin C (15–18) and is a protein which can associate with the Ca"+-dependent modulator protein in the inhibition of the Ca"+-activatable cyclic nucleotide phosphodiesterase reaction (27, 28) by the specific association with the modulator protein in the presence of Ca"+ (29, 30). One of the proteins, designated as the modulator binding protein, is heat-labile (29) and the other, a heat-stable protein, is designated the heat-stable inhibitor protein (29). The heat-stable protein inhibitor has been purified to close to homogeneity (30). Independently, Klee and Krinks (31) have discovered and purified an inhibitor protein of cyclic nucleotide phosphodiesterase from bovine brain. From its thermal stability and molecular weight, the inhibitor protein purified by Klee and Krinks (31) appear to be the same as the modulator binding protein.

In the present report, modulator binding protein is purified to homogeneity using a procedure based on its Ca"+-dependent specific association with the modulator protein. The procedure is adopted from that used previously for the purification of the Ca"+-activatable cyclic nucleotide phosphodiesterase from bovine heart (32). Physical characterizations of the modulator binding protein indicate that it is indeed identical to the inhibitor protein purified by Klee and Krinks (31). In addition to providing an alternate purification procedure for this protein, the present report provides a description of the subunit structure of the protein, and identifies the subunit responsible for the association with the modulator protein.

EXPERIMENTAL PROCEDURES

Acrylamide Gel Electrophoresis—Electrophoresis at pH 8.3 was performed essentially as described by Davis (33). Electrophoresis were run at 4°C at 4.0 mA/gel and stopped when the bromophenol blue marker had migrated 2.0 cm from the bottom of the gel. The gel was either stained for protein with Coomassie blue or sliced into 2.0-mm segments and each slice was extracted with 500 μl of 20 mM Tris-HCl, 1 mM magnesium acetate (Buffer A), pH 7.0, containing 10 mM 2-mercaptoethanol and 0.1 mM Ca"+. The extracts were assayed for modulator binding protein.

Sodium dodecyl sulfate-polyacylamide gel electrophoresis was carried out by the method of Weber and Osborn (34). The dye front was marked by a piece of wire. Shrinkage of the gel during staining was always corrected for the calculation of electrophoretic mobility of the polypeptide.

Analytical Ultracentrifugation—Analytical ultracentrifugation was carried out with a Beckman Spinco model E analytical ultracentrifuge. Sedimentation velocity experiments were run at 65,000 rpm at 20°C using schlieren optics. The meniscus depletion run was carried out at 20°C at a rotor speed of 19,848 rpm. The sample solution of modulator binding protein was dialyzed against Buffer A containing 0.1 mM EGTA and 0.1 M KCl prior to use. Density of the solvent was determined using an Oswald viscometer. Molecular weight and sedimentation constants were all corrected for the solvent density and viscosity.

Heat-stable Inhibitor Protein—Heat-stable inhibitor protein was

* The abbreviations used are EGTA, ethylene glycol bis(β-aminoethyl ether)N,N'-tetraacetic acid; SDS, sodium dodecyl sulfate.
purified from bovine brain according to the procedure described by Sharma et al. (30).

**Purification of Modulator Binding Protein**—Procedures for the purification of modulator binding protein and related techniques, such as assays of cyclic nucleotide phosphodiesterase, modulator protein, and modulator binding protein, are presented in the attached miniprint supplement.2

**RESULTS**

**Summary of Purification**—The purification procedure is based primarily on the significantly different affinity of the modulator binding protein in its free state and in its modulator-bound state to DEAE-cellulose. Thus, by successive chromatography on DEAE-cellulose column first in the modulator-bound form and then in the free form, the modulator binding protein can be extensively purified.

Table I summarizes the data of a typical preparation from 2 kg of frozen bovine brain of the modulator binding protein. Judging from its specific activity, measured as the inhibitory activity in the Ca"+-activated phosphodiesterase reaction, the modulator binding protein can be purified about 400-fold from the bovine brain extract (the main fraction from the Sephadex G-200 column). The fractions with the highest specific activity were used for experiments in this study. Other fractions (the side peak fractions), however, can be pooled together and further purified by gel filtration on the Sephadex G-200 column. The purified modulator binding protein can be stored at -20°C for several months without losing its inhibitory activity in the phosphodiesterase reaction.

**Purity of Sample**—The purified modulator binding protein shows a single Coomassie blue staining band on the polyacrylamide gel after disc gel electrophoresis at pH 8.3 with 7.5, 12, or 15% polyacrylamide gels. Fig. 1 shows the electrophoretic pattern of the protein sample on a 7.5% gel, the single protein staining band is seen to correlate with the phosphodiesterase inhibitory activity as determined from an unstained and sliced gel. The homogeneity of the sample is also supported by the analytical ultracentrifugal analysis. The schlieren pattern of the modulator-binding protein sample in the sedimentation velocity experiment showed a single symmetrical peak with a sedimentation constant of about 5 s.

Fig. 2 shows the gel electrophoretic patterns of the purified modulator binding protein in the presence of various concentrations of modulator protein. At lower modulator protein concentrations, two protein bands having slightly different mobility can be observed on the gels (Fig. 2, Gels B to F). The slow moving band has a mobility similar to that of

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2 Portions of this paper (including Fig. 1) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full-size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 78U-1615, cite author(s), and include a check or money order for $1.00 per set of photocopies.

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**TABLE I**

| Step                  | Volume (ml) | Protein concentration (mg/ml) | Activity (units/ml) | Total units | Specific activity (units/mg) | Recovery (%) | Purification (fold) |
|-----------------------|-------------|-------------------------------|--------------------|-------------|-----------------------------|--------------|---------------------|
| Homogenate supernatant| 3,100.0     | 12.3                          | 173.0              | 536,300     | 14.0                        | 100.0        | 1.0                 |
| (NH4)2SO4, 0-55%      | 1,060.0     | 20.0                          | 400.0              | 420,000     | 20.0                        | 78.0         | 1.4                 |
| First DEAE-cellulose  | 1,240.0     | 2.1                           | 204.8              | 253,952     | 98.0                        | 47.0         | 7.0                 |
| Second DEAE-cellulose | 780.0       | 1.0                           | 277.8              | 216,684     | 278.0                       | 40.0         | 20.0                |
| Third DEAE-cellulose  | 300.0       | 0.12                          | 258.4              | 85,272      | 2,150.0                     | 16.0         | 154.0               |
| Sephadex G-200        |             |                               |                    |             |                             |              |                     |
| Left side (A)         | 23.5        | 0.153                         | 534.0              | 12,549      | 3,490.0                     | 2.3          | 249.0               |
| Main peak (B)         | 34.0        | 0.281                         | 1,600.0            | 54,400      | 5,694.0                     | 10.1         | 407.0               |
| Right side (C)        | 23.5        | 0.154                         | 572.0              | 13,442      | 3,714.0                     | 2.5          | 265.0               |
| Total                 | 81.0        | 0.21                          | 984.0              | 80,514      | 4,733.0                     | 15.0         | 338.0               |

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FIG. 2. Electrophoretic analysis of the interaction between modulator protein and modulator binding protein (MBP). Modulator binding protein samples (4 nmol in 200 μl) were incubated at 30°C for 30 min with varying amounts of modulator protein in Buffer A containing 0.1 mM CaCl<sub>2</sub>. Amounts of modulator protein used in the incubation were: Gel A, 0; Gel B, 0.2 nmol; Gel C, 0.4 nmol; Gel D, 0.6 nmol; Gel E, 0.8 nmol; Gel F, 1.0 nmol; Gel G, 1.2 nmol; Gel H, 1.6 nmol; and Gel I, 2.4 nmol. After the incubation, aliquots (170 μl) of samples were analyzed by polyacrylamide disc gel electrophoresis on 7.5% gels.

TABLE II

| Physical properties of modulator binding protein |
|-----------------------------------------------|
| Molecular weight                              |
| From sedimentation and gel filtration         | 84,000  |
| From sedimentation equilibrium                | 86,000  |
| Sedimentation constant, s<sub>i</sub>,         | 4.96    |
| Stokes radius (Å)                             | 40.5    |
| Absorbance (A<sub>278, 1% solutions</sub>)   | 9.8     |

The constancy of proportionality of the two protein bands in different preparations suggested that the two bands represented two types of subunits of modulator binding protein, rather than contaminations. To further test this suggestion, three different fractions of modulator binding protein from the G-200 Sephadex column (the last step of the purification) were analyzed by SDS-gel electrophoresis. Fig. 5 shows that all three fractions contained both A and B staining bands, the relative intensities of the two bands were about the same for the three fractions.

Using various molecular weight markers, the molecular weights of the two subunits of the modulator binding protein were determined by the SDS-gel electrophoresis method to be 60,000 and 14,500. The determinations were carried out on gel filtration on a calibrated G-200 Sephadex column (35). From the sedimentation constant and Stokes radius and using a partial specific volume ϑ of 0.73 ml/g, the molecular weight of modulator binding protein was calculated to be 86,000 (35). The molecular weight of the protein was also determined by the sedimentation equilibrium experiments using the meniscus depletion method. Three separate experiments were carried out and the molecular weight obtained was 84,000 ± 1,200. A typical equilibrium experiment is presented in Fig. 3; the linear plot further suggests the homogeneity of the protein and indicates that the protein does not undergo self-association under the experimental conditions.

The ultraviolet spectrum of the purified protein is typical of a protein without prosthetic groups with its absorption maximum at 278 nm. Using the method of Babul and Stellwagen (36) to determine the protein mass, the absorbancy of the purified protein was calculated to be 9.8 for a 1% solution at 278 nm.

Modulator Binding Protein Contains Two Different Subunits—Modulator binding protein which appeared homogeneous by the criteria of disc gel electrophoresis, ultracentrifugation, and the interaction with modulator protein showed two main protein bands on SDS-gel electrophoresis. Additional faint protein bands were seen in some preparations. These bands however, represented no more than 5% of the total protein as judged from the densitometric tracings of the gels. A typical SDS-gel electrophoretic pattern of the protein along with its densitometric tracing is presented in Fig. 4. The two main protein bands are termed A and B bands as indicated in the figure. From the densitometric tracing, the relative amounts of protein in bands A and B were determined and the ratio of amounts of A/B was estimated to be 2.3:1. This proportionality appeared constant, it was found to be in the range of 2.1:1 to 2.4:1 for four different preparations with an average value of 2.32:1. All these determinations were carried out with gels stained by Coomassie brilliant blue.

FIG. 3. Sedimentation equilibrium run for modulator binding protein. Ultracentrifugation was carried out at 19,848 rpm and 20°C. Modulator binding protein, 0.5 mg/ml, was in Buffer A containing 0.1 M NaCl and 10 mM 2-mercaptoethanol.

FIG. 4. Analysis of purified modulator binding protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Electrophoresis was carried out with 15.0 μg of modulator binding protein as described under “Experimental Procedures.” Densitometric analysis of the gel was carried out at 660 nm. Bromphenol blue represent the position of the tracking dye.
concentrations of the pooled samples were determined by the Coomassie Brilliant blue G-250 binding assay (37) using bovine serum albumin as the standard reference protein. When fractions under the two peaks were pooled and protein subunits was approximately 2.3. Identical ratio was obtained when subunits A/B samples (Fig. 7) were concentrated by Diaflo ultrafiltration using PM 10 and UM 2 membranes, respectively. The concentrated samples were then exhaustively dialysed against 0.01 M ammonium bicarbonate. Protein concentrations of the samples were then determined by both the dye binding method and refractometric measurements using the interference optics as a differential refractometer (36). For both subunits, the dye binding methods appeared to have underestimated the protein concentration. Correction factors of 1.14 and 1.19 were needed to correct for the concentration values of subunits A and B, respectively. Using these correction factors, mass ratio of subunits A to B of modulator binding protein was calculated to be 2.22:1.

Table III summarizes the data on the subunit structure of modulator binding protein. The molar ratios of subunits to the whole protein were calculated by using the value 85,000 as the molecular weight of the protein (Table II). From these ratios, the molar ratio of subunits A/B can be calculated to be 1.85. The result suggests that modulator binding protein has both 7.5 and 15% gels. The molecular weight standards used in these determinations were phosphorylase b, bovine serum albumin, ovalbumin, chymotrypsinogen, and cytochrome c.

Separation of the Subunits—The two subunits of the brain modulator binding protein could be separated on a preparative scale by Sephadex G-100 gel filtration in the presence of SDS. The protein was dissociated by incubation at 100°C for 5 min in a Tris-HCl buffer, pH 7.5, containing 1% SDS and 1% 2-mercaptoethanol. The dissociated protein was then chromatographed on the gel filtration column as indicated in Fig. 6. The column eluents were analyzed for the protein content by the Coomassie blue binding assay (37). Fig. 6 shows that two protein peaks were obtained from the Sephadex G-100 column. Analysis by SDS-gel electrophoresis indicates that these two fractions corresponded to the two subunits of the modulator binding protein (Fig. 6, inset). The gel filtration elution profile shows that the ratio of area under A to that under B subunits was approximately 2.3. Identical ratio was obtained when fractions under the two peaks were pooled and protein concentrations of the pooled samples were determined by the Coomassie Brilliant blue G-250 binding assay (37) using bovine serum albumin as the standard reference protein.

In addition to SDS, urea at 6 M causes the dissociation of modulator binding protein. Subunits A and B of the protein had also been separated on G-100 Sephadex column in the presence of 6 M urea as shown in Fig. 7. Protein concentration of the separately pooled subunits were determined by the dye binding method and again, the mass ratio of subunits A/B was found to be 2.3:1. Since different proteins, on unit weight basis, bind different amounts of the dye, concentrations of the subunits determined by the dye binding method (37) were corrected by the refractometric measurement of the protein solutions (36). The pooled subunits A and B samples (Fig. 7) were concentrated by Diaflo ultrafiltration using PM 10 and UM 2 membranes, respectively. The concentrated samples were then exhaustively dialysed against 0.01 M ammonium bicarbonate. Protein concentrations of the samples were then determined by both the dye binding method and refractometric measurements using the interference optics as a differential refractometer (36). For both subunits, the dye binding methods appeared to underestimate the protein concentration. Correction factors of 1.14 and 1.19 were needed to correct for the concentration values of subunits A and B, respectively. Using these correction factors, mass ratio of subunits A to B of modulator binding protein was calculated to be 2.22:1.

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a subunit structure of AB₂.

Subunit A Is the Modulator Binding Unit—Although 6 M urea causes modulator binding protein to dissociate, the protein does not irreversibly lose its inhibitory activity against cyclic nucleotide phosphodiesterase. After a sample of modulator binding protein had been incubated at 4°C in buffer A containing 6 M urea for 18 h, it was found to have retained all its inhibitory activity against phosphodiesterase. For this experiment the assay of modulator binding protein involved an overall dilution of the sample by 90 times so that the urea concentration carried over by the protein sample was about 0.067 M. Control experiments showed that up to 0.5 M urea had no effect on the modulator protein activation of cyclic nucleotide phosphodiesterase nor on the inhibitory activity of modulator binding protein.

Since modulator binding protein retains its phosphodiesterase inhibitory activity after prolonged incubation in 6 M urea, the possibility that this activity resides in one of its subunits has been examined. The gel filtration profile of Fig. 7 shows that the phosphodiesterase inhibitory activity was associated with the first protein peak which corresponded to subunit A of modulator binding protein (Fig. 7, inset). When the pooled fraction of subunit A was assayed for the amount of modulator binding protein, it was found that 50% of the original inhibitory activity was recovered in this fraction. None of the activity was found using the pooled fraction of subunit B.

When mixtures of subunits A and B were used for the inhibition of phosphodiesterase reaction, the activities were found to be identical to those of subunit A alone. Thus, subunit B neither inhibits the Ca²⁺-activatable cyclic nucleotide phosphodiesterase nor affects the inhibition of the enzyme by subunit A.

Comparison of Inhibitory Activities of Modulator Binding Protein and Heat-stable Inhibitor Protein—In addition to modulator binding protein, bovine brain contains a heat-stable inhibitor protein which is also specific for the modulator protein (28). The relative potencies of the two proteins in the phosphodiesterase reaction are compared in Fig. 8. In terms of molar concentration, it may be seen that the heat-stable inhibitor protein is at least 10 times more potent than the modulator binding protein in the inhibition of the Ca²⁺-modulator-dependent phosphodiesterase reaction.

Relative Amounts of Modulator Protein and Modulator-regulated Proteins in Bovine Brain—Although the heat-stable inhibitor protein is much more potent than the modulator binding protein in the phosphodiesterase inhibiting activity, the concentration of this protein in bovine brain is much lower than that of the modulator binding protein. Table IV shows the relative amounts of the two proteins along with those of the modulator protein and the Ca²⁺-dependent phosphodies- terase in bovine brain. The concentrations of the various proteins were calculated from their respective specific activities of crude bovine brain extract and those of the pure proteins. Since the Ca²⁺-activated phosphodiesterase has not yet been obtained in its homogeneous state, the specific activity of the pure enzyme was estimated from the value of an 80% pure bovine heart enzyme preparation (32). Results in Table IV show that modulator binding protein is by far the most abundant among bovine brain proteins which show specific Ca²⁺-dependent association with modulator protein. However, modulator binding protein still exists in the brain in much lower molar concentration than modulator protein.

Discussion

Modulator binding protein was discovered in bovine brain originally as an inhibitor factor of the Ca²⁺-activatable cyclic nucleotide phosphodiesterase (27). It was later found that the protein inhibited the enzyme reaction by reversibly associating with modulator protein and was therefore given the present designation (29). Independently, Klee and Krinks (31) discovered the existence in bovine brain of an inhibitor protein of cyclic nucleotide phosphodiesterase and suggested that this inhibitor protein was the same as the modulator binding protein.

Klee and Krinks (31) have purified their inhibitor protein

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TABLE IV

| Protein                  | Pure sample | Total activity/kg | Amount* |
|--------------------------|-------------|-------------------|---------|
|                          | Specific activity | Molecular weight | Unit     | mg/kg | μmol/kg |
| Modulator binding protein| 4,000-6,000 (5,000) | 85,000            | 250,000-270,000 (260,000) | 52 | 0.61 |
| Modulator protein        | 80,000-120,000 (100,000) | 17,500         | 9.0 x 10⁶ | 90 | 5.1  |
| Phosphodiesterase        | ~150         | 120,000           | 1,400-1,700 (1,550) | 10 | 0.07 |
| Heat-stable inhibitor protein | 83,375     | 70,000            | 15,000-19,000 (17,000) | 0.2 | 0.003 |

* Calculated by using the mean values.

Mean values.

Estimated from a partially purified Ca²⁺-activatable phosphodiesterase from bovine heart (32).

† From Sharma et al. (30).
to homogeneity using a procedure which depended in part on an affinity chromatography step using a modulator protein-Sepharose 4B conjugate. In the present study, the modulator binding protein is purified to homogeneity from bovine brain by a different procedure which, however, also depends on the Ca\(^{2+}\)-dependent and reversible association between modulator binding protein and modulator protein. Physical characterizations of the pure modulator binding protein further substantiate the suggestion that this protein and the inhibitor protein discovered by Klee and Krinks (31) are the same. Both protein preparations have similar molecular weight and show similar SDS-gel electrophoretic patterns.

Upon gel electrophoresis in the presence of SDS, modulator binding protein sample shows predominantly two protein bands on the gel corresponding to polypeptides of molecular weights 60,000 and 14,500. Although the possibility that the smaller polypeptide is a contaminant of the protein preparation cannot be ruled out completely, a suggestion that the two polypeptides represent subunits of modulator binding protein is supported by the following observations: (a) the sample of modulator binding protein shows single band upon gel electrophoresis under non-denaturing conditions, (b) the relative amounts of the two peptides in the protein sample are about the same in different preparations and in different fractions of a purification step (Fig. 5), and (c) the molecular weight of the native protein is greater than that of the large polypeptide but smaller than twice that of the polypeptide. Furthermore, the suggestion is compatible with the observation of Klee and Krinks (31) that chemically cross-linked modulator binding protein exhibits an additional protein band with a molecular weight of about 80,000 on SDS-electrophoretic gel.

Thus, modulator binding protein appears to contain two dissimilar subunits, subunits A and B, which have molecular weight of about 60,000 and 14,500, respectively. These subunits have been isolated and the molar ratio of the subunits in modulator binding protein has been determined. Results from these studies along with the value of molecular weight of modulator binding protein determined by analytical ultracentrifugation have led to the suggestion that this protein has a subunit structure of AB.

The existence of distinct subunits in modulator binding protein has raised the question as to the functional roles of the different subunits. The observation that subunit B isolated by gel filtration in the presence of 6 M urea possesses the inhibitory activity against the Ca\(^{2+}\)-activated phosphodiesterase suggests that subunit A is responsible for the association of modulator binding protein to modulator protein. The functional role of subunit B, however, is not known.

Since several proteins and enzymes have been found to undergo specific association with the modulator protein, the possibility that they contain a common subunit which is responsible for their binding to the modulator protein has been suggested. However, results from recent studies do not support such a suggestion. Three of these proteins, modulator binding protein, heat-stable inhibitor protein (30), and the myosin light chain kinase (13), have been purified to near homogeneity. These proteins do not contain common subunits. Furthermore, unlike modulator binding protein, myosin light chain kinase (13) and heat-stable inhibitor protein (30) do not contain dissimilar subunits. Therefore, it seems more likely that the proteins which are capable of specific association with modulator protein contain common protein structural domain rather than common subunit which is specific for modulator protein.

Since all the modulator-regulated enzymes studied so far can undergo Ca\(^{2+}\)-dependent association with modulator protein (38), modulator binding protein, which also exhibits such property, has been suggested to represent an additional modulator-regulated protein (29). The existence of distinct subunits of modulator binding protein and the observation that only one of them is required for the binding to modulator protein appear to further support such a suggestion. However, to date the biological activity of modulator binding protein has not been found in spite of various attempts (29).

The inhibitory activity of modulator binding protein as measured in vitro appears to be specific against the action of modulator protein. In addition to the modulator-dependent phosphodiesterase reaction, the activation of the erythrocyte membranous (Ca\(^{2+}\)/Mg\(^{2+}\))-ATPase and that of brain adenylate cyclase by the modulator protein have been shown to be abolished by modulator binding protein (39). It should be noted that such specific inhibitory activity of this protein by no means indicates that modulator binding protein functions as an inhibitor against modulator protein in the cells. Myosin light chain kinase which functions as a regulator for smooth muscle contraction also exhibits specific inhibitory activity against the Ca\(^{2+}\)-activated phosphodiesterase reaction (13).

Among the known modulator-regulated enzymes and proteins, modulator binding protein is by far the most abundant in bovine brain (Table IV). On the molar basis, it is at least 10 times higher in concentration than any of the other known modulator-regulated proteins. However, since the concentration of modulator protein is much higher than modulator binding protein, modulator protein still appears to exist in excess over the regulated proteins. Thus, in bovine brain, the limiting factor for the various modulator-dependent enzyme reactions is probably free calcium ions rather than modulator protein.

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