

Resolution of adenylate cyclase sensitive and insensitive to Ca^{2+} and calcium-dependent regulatory protein (CDR) by CDR-Sepharose affinity chromatography

(brain cortex/cyclic AMP/modulator binding protein)

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ABSTRACT Partially purified adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] from bovine brain cortex was fractionated into two separate forms by calcium-dependent regulatory protein (CDR)-Sepharose affinity chromatography. The major form of the enzyme, comprising $\approx 80\%$ of the applied activity, did not bind to the affinity column in the presence of Ca^{2+} and was insensitive to the CDR. Approximately 20% of adenylate cyclase activity was absorbed to CDR-Sepharose in the presence of Ca^{2+} . This activity was stimulated by Ca^{2+} and CDR. This study directly demonstrates that brain cortex contains Ca^{2+} -CDR-sensitive and -insensitive forms of adenylate cyclase and indicates that CDR-Sepharose may be a useful tool for purification of adenylate cyclase. The Ca^{2+} -stimulated adenylate cyclase was purified at least 55-fold with a 13% yield.

Brain adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] exhibits a biphasic response to Ca^{2+} concentration, with stimulation occurring at low Ca^{2+} and inhibition at higher Ca^{2+} concentrations (1-5). The calcium-dependent regulatory protein (CDR) mediates Ca^{2+} stimulation of adenylate cyclase (3, 5-8) and the Ca^{2+} -sensitive phosphodiesterase (9-13). Both enzymes apparently form a complex with CDR in the presence of Ca^{2+} .

CDR covalently coupled to Sepharose has been effectively used for the partial purification of the Ca^{2+} -sensitive phosphodiesterase (14) and modulator binding protein (15). Because it has been proposed that CDR forms a complex with brain adenylate cyclase in the presence of Ca^{2+} , the efficacy of CDR-Sepharose as a tool for the purification of bovine brain adenylate cyclase was evaluated. In this study, it was discovered that partially purified adenylate cyclase can be separated into two fractions by CDR-Sepharose chromatography. The first fraction is insensitive to CDR and is only inhibited by Ca^{2+} . The second fraction is stimulated by Ca^{2+} and CDR. These observations directly support the proposal by Brostrom *et al.* (8) that brain cortex contains a mixture of CDR-dependent and CDR-independent adenylate cyclase activities. A scheme for the partial purification of the Ca^{2+} -CDR-sensitive adenylate cyclase is presented.

MATERIALS AND METHODS

Materials. All reagents were the finest grade obtainable. Affi-Gel Blue was purchased from Bio-Rad and CNBr-activated Sepharose 4B was obtained from Pharmacia.

Adenylate Cyclase Assay. The enzyme was assayed by the method of Salomon *et al.* (16) by using [α - ^{32}P]ATP as a substrate and cyclic [^3H]AMP to monitor recovery. ATP used in assays was purified by DEAE-Sephadex A-25 chromatography

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followed by Dowex AG-50 chromatography. For routine determinations, assays contained 1 mM ATP, 10 mM MnCl_2 , and 1 mM EDTA. When the Ca^{2+} sensitivity was examined, 0.2 mM ATP, 5 mM MgCl_2 , and 0.2 mM ethylene glycol-bis(β -aminoethylether)- N,N,N',N' -tetraacetic acid (EGTA) was present in assays. All assays were performed at 30°C for 10 min in triplicate. Protein was determined by the method of Peterson (17), with bovine serum albumin as a standard.

Solubilization of Adenylate Cyclase. The enzyme was solubilized by the method of Johnson and Sutherland (18), modified as outlined below. Fresh bovine cortex was suspended in 2 vol of homogenization buffer (20 mM glycylglycine, pH 7.2/1 mM MgCl_2 /1 mM EDTA/0.25 M sucrose/3 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride). The cortex was disrupted in a blender and the homogenate was centrifuged at 4000 $\times g$ for 30 min at 4°C. The supernatant was discarded and the pellet was suspended in 2 vol of homogenization buffer, Dounce homogenized, and passed through four layers of cheesecloth. The centrifugation step was repeated twice; the final pellet was suspended in a minimal amount of homogenization buffer and frozen in aliquots at -60°C. Membrane preparations were solubilized with 20 mM Tris-HCl, pH 7.35/5 mM MgCl_2 /5 mM NaF/1 mM EDTA/0.25 M sucrose/1.0% Lubrol PX, with a detergent-to-protein ratio of 2.5:1. This mixture was allowed to incubate, with occasional mixing, for 1.5 hr at 0°C. The solubilization mixture was centrifuged at 100,000 $\times g$ for 1 hr at 4°C and the supernatant was recovered.

Affi-Gel Blue Chromatography. The standard elution buffer for this column contained 20 mM Tris-HCl (pH 7.35), 0.1% Lubrol PX, 5 mM NaF, 0.25 M sucrose, and 2.5 mM dithiothreitol. Solubilized adenylate cyclase (150 ml) was slowly applied to a 30-ml Affi-Gel Blue column that had been pre-equilibrated with the standard elution buffer containing 5 mM MgCl_2 and 1 mM EDTA. The loaded column was then washed with two column volumes of standard elution buffer containing 1 mM MgCl_2 , 1 mM EDTA, and 1 mM EGTA, followed by one column volume of the standard elution buffer containing 5 mM MgCl_2 and 1 mM EDTA. Adenylate cyclase activity was step eluted with a buffer containing 1 M KCl, 8 mM ATP, 16 mM MgCl_2 , and 1 mM EDTA. The pooled material was desalted on a Bio-Gel P-2 column which was equilibrated and run with standard elution buffer containing 5 mM MgCl_2 and 1 mM EDTA. The desalted sample was concentrated 8-fold by ultrafiltration with an Amicon XM 50 membrane. All of the manipulations described above were carried out at 4°C. The concentrated preparation was frozen in liquid nitrogen and stored at -60°C.

Preparation of CDR-Sepharose. CNBr-activated Sepharose

Abbreviations: CDR, calcium-dependent regulatory protein; EGTA, ethylene glycol-bis(β -aminoethyl ether)- N,N,N',N' -tetraacetic acid.

4B was swollen for 15 min in 1 mM HCl and the excess HCl was removed by filtration. The wet resin was suspended in 0.1 M NaHCO₃, pH 8.0/0.5 M NaCl/50 μM CaCl₂ containing purified bovine brain CDR (19) at a ratio of 1 mg of CDR per 3 ml of packed resin. The mixture was incubated for 12 hr at 4°C with gentle mixing. The resin was then washed with ten column volumes of 1 M KCl.

CDR-Sepharose Chromatography. The standard elution buffer for this column contained 20 mM Tris-HCl (pH 7.35), 0.1% Lubrol PX, 1 mM MgCl₂, 0.25 M sucrose, and 2.5 mM dithiothreitol. Ten milliliters of solubilized adenylate cyclase or the concentrated fraction from the Affi-Gel Blue column was applied to a 10-ml CDR-Sepharose column that was preequilibrated with standard elution buffer containing 2 mM CaCl₂, 1 mM EDTA, and 0.5 mM EGTA. The column was then washed with the same buffer until no further protein or adenylate cyclase activity was eluted. The column was then eluted with standard elution buffer containing 2 mM EGTA.

Sodium Dodecyl Sulfate/Polyacrylamide Gel Electrophoresis. The electrophoresis was performed on 1-mm slab gels by the method of Laemmli (20). CDR levels were quantitated by monitoring stimulation of the CDR-depleted phosphodiesterase as described (19).

RESULTS

Interactions between Adenylate Cyclase Preparations and CDR-Sepharose. Solubilized adenylate cyclase was applied to a CDR-Sepharose column; the elution profile is shown in Fig. 1A. None of the applied adenylate cyclase activity was absorbed by CDR-Sepharose in the presence of Ca²⁺. Similar results were obtained when the amount of applied solubilized material was varied over a wide range, indicating that the column was not

simply overloaded. It seemed more likely that adenylate cyclase was not absorbed because of the presence of endogenous CDR or other CDR-binding components in the solubilized preparation.

Because of the observations described above, an attempt was made to remove endogenous CDR from solubilized preparations by Affi-Gel Blue chromatography. This resin was used because preliminary experiments indicated that Affi-Gel Blue could be used to separate adenylate cyclase from CDR. We were unable to repeat the results of Stellwagen and Baker (21), who reported that brain adenylate cyclase is strongly absorbed to Blue Dextran Sepharose. However, the enzyme was absorbed by Affi-Gel Blue and endogenous CDR was removed by washing with EGTA-containing buffers. Adenylate cyclase was eluted from Affi-Gel Blue by a buffer system containing 8 mM ATP, 16 mM MgCl₂, 1 M KCl, and 0.1% Lubrol PX (Fig. 2). This chromatographic procedure resulted in a 3.3-fold purification of adenylate cyclase with a yield of 41%. More importantly, the CDR content was reduced by a factor of 1/450 (Table 1).

The CDR-depleted adenylate cyclase preparation obtained by Affi-Gel Blue chromatography was applied to CDR-Sepharose (Fig. 1B). CDR-Sepharose fractionated adenylate cyclase into two forms based upon their affinity for the resin in the presence or absence of free Ca²⁺. Fraction I was not retained by the column and accounted for 77% of the removed activity. Fraction II was specifically bound to CDR-Sepharose in the presence of Ca²⁺; it was eluted with excess EGTA and represented 23% of the recovered adenylate cyclase activity. These percentages are based upon adenylate cyclase assays carried out in the presence of 10 mM MnCl₂ and 1 mM ATP. When fraction I was reapplied to the same CDR-Sepharose column,

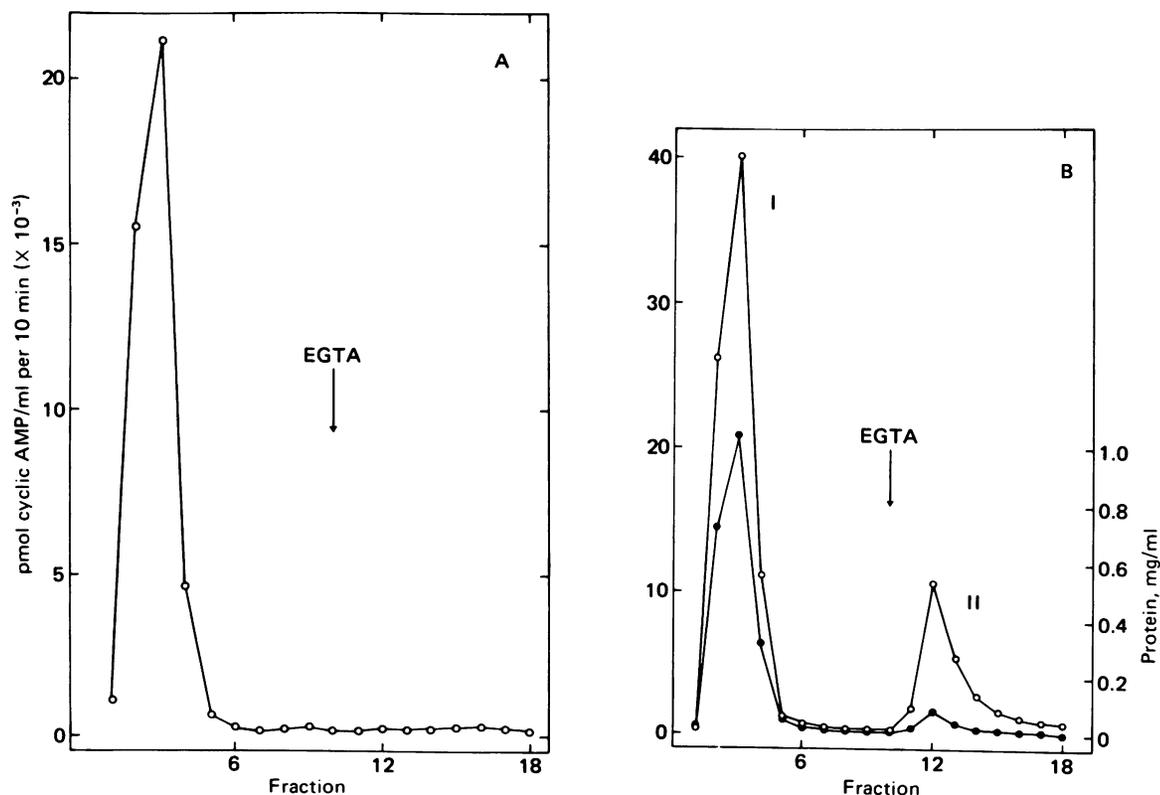


FIG. 1. Elution profiles for interaction of adenylate cyclase preparations with CDR-Sepharose. (A) Application of solubilized adenylate cyclase to CDR-Sepharose. Fraction size, 4.8 ml. (B) CDR-depleted adenylate cyclase obtained from Affi-Gel Blue chromatography. Fraction size, 4.8 ml. O, Adenylate cyclase activity; ●, protein. Adenylate cyclase was assayed with 1 mM ATP and 10 mM MnCl₂ with a standard error ≤5% for all determinations.

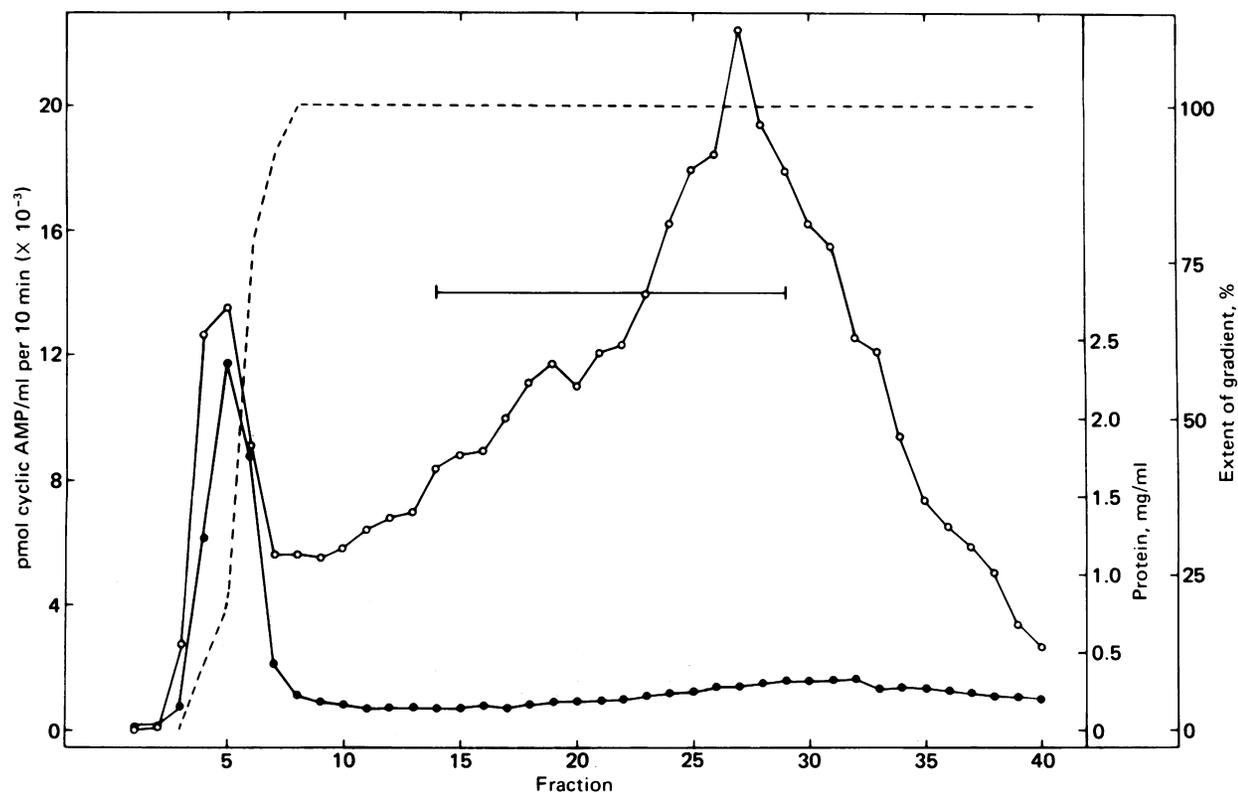


FIG. 2. Affi-Gel Blue chromatography. Solubilized adenylate cyclase was applied to an Affi-Gel Blue column and chromatographed. Fraction size, 6 ml. O, Adenylate cyclase activity; ●, protein concentration; - - -, extent of ATP/KCl gradient. The fractions identified by the horizontal bar were pooled, desalted, and concentrated. Those fractions exhibiting a 2.5-fold or greater purification of adenylate cyclase activity were pooled. Adenylate cyclase was assayed, with 1 mM ATP and 10 mM $MnCl_2$ with a standard error $\leq 5\%$. Since some column fractions contained significantly higher ATP concentrations because of the eluting gradient, data are corrected for the variation in the specific activity of $[\alpha\text{-}^{32}P]ATP$ used in assays. The fractions obtained in the flow-through and preliminary wash are not shown; however, they contained less than 20% of the applied protein and less than 15% of the applied adenylate cyclase activity. The gradient was complete (extent = 100%) when ATP, $MgCl_2$, and KCl were at 8 mM, 16 mM, and 1 M, respectively. The extent of gradient was monitored by conductance and absorbance at 259 nm.

only 2% of the activity was absorbed by the resin and 98% of the activity was not retained. Reapplication of fraction II to CDR-Sepharose resulted in specific absorption of 85% of the applied activity. This result is consistent with the observation that fraction II contained $0.16 \mu\text{g}$ of CDR per mg of protein compared to $0.02 \mu\text{g}$ of CDR per mg of protein present in the fraction obtained from Affi-Gel Blue chromatography (Table 1). The higher level of CDR present in fraction II was most likely due to slow release of CDR from the resin since the

CNBr-activated linkage is not absolutely stable. Even after extensive washing of the column, low levels of CDR were still detectable in the effluent. The CDR-Sepharose used in these experiments also absorbed the CDR-sensitive phosphodiesterase.

Ca²⁺ and CDR Sensitivity. Because fractions I and II obtained by CDR-Sepharose chromatography exhibited different affinities for the resin, the Ca²⁺ and CDR sensitivities of these two samples were examined. These two fractions were eluted

Table 1. Partial purification of CDR-sensitive adenylate cyclase

| Fraction | Protein, mg | Adenylate cyclase | | Purification* | CDR content,† $\mu\text{g}/\text{mg}$ protein |
|-----------------------------------|-------------|-----------------------------|---|---------------|--|
| | | Total activity, pmol/10 min | Specific activity,‡ pmol/mg per 10 min | | |
| Particulate | 590 | 1,618,500 | 2,743 | — | ND |
| Crude solubilized | 178 | 3,293,500§ | 18,500 | 6.7 | 9 |
| Concentrated Affi-Gel Blue eluant | 22.3 | 1,356,500 | 60,800 | 22 | 0.02 |
| CDR-Sepharose | | | | | |
| Fraction I | 15.3 | 703,500 | 45,700 | 17 | 0.04 |
| Fraction II | 1.45 | 218,400 | 151,000 | 55 | 0.16 |

* Purifications are expressed relative to total adenylate cyclase activity in membranes.

† CDR was assayed by monitoring stimulation of CDR-depleted phosphodiesterase. ND, not determined.

‡ Adenylate cyclase was assayed by using 1 mM ATP and 10 mM $MnCl_2$.

§ Solubilization of brain adenylate cyclase resulted in an increase in total activity. This could reflect permanent stimulation by Lubrol PX or NaF, unmasking of more active sites by solubilization, or separation of an inhibitory factor upon solubilization.

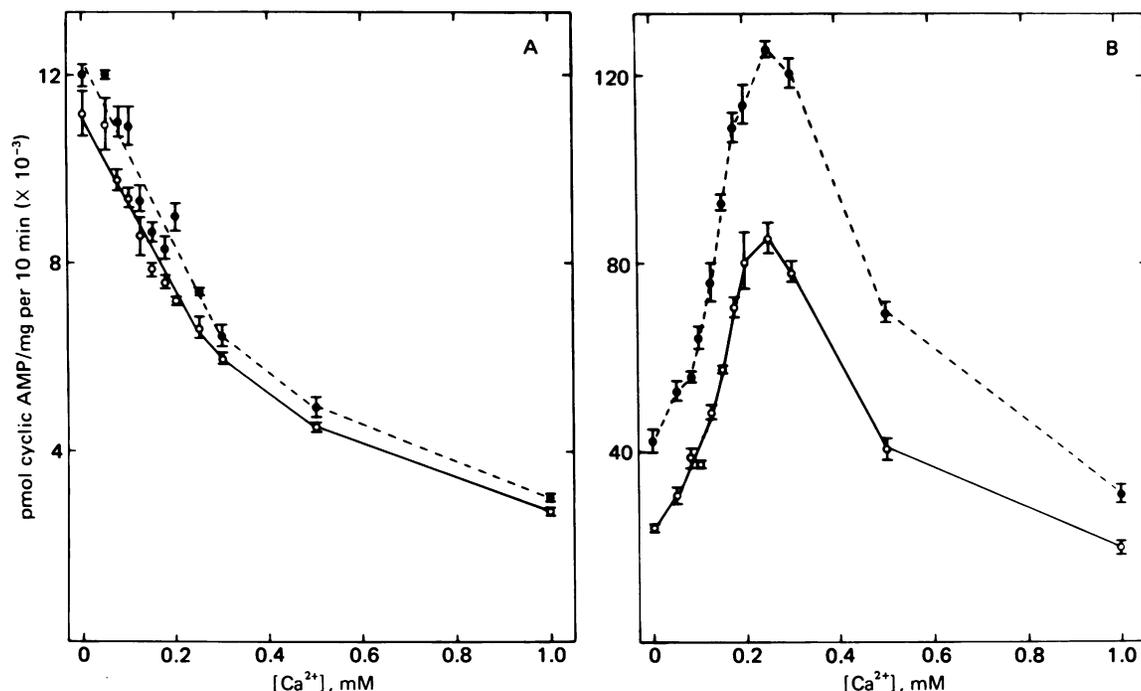


FIG. 3. Calcium sensitivity of adenylate cyclase fractions obtained by CDR-Sepharose chromatography. (A) Pooled fraction I from the CDR-Sepharose column (Fig. 1B) was adjusted to 2 mM EGTA and desalted into 20 mM Tris, pH 7.35/2.5 mM dithiothreitol/250 mM sucrose/500 μ M EGTA on a Bio-Gel P-2 column. The desalted sample was then assayed for adenylate cyclase activity as a function of added Ca^{2+} concentration. (B) Pooled fraction II from the CDR-Sepharose column was concentrated 4-fold and desalted on a Bio-Gel P-2 column in the same buffer described above. Adenylate cyclase was assayed with 0.2 mM ATP and 5 mM MgCl_2 . The final concentration of EGTA during assays was 200 μ M. Adenylate cyclase activity in the absence (O) and presence (●) of 4.4×10^{-3} mg of added CDR per ml.

from CDR-Sepharose with buffer systems containing different concentrations of EGTA and CaCl_2 . Therefore, fraction I was adjusted to 2 mM EGTA and both samples were desalted on Bio-Gel P-2 with an elution buffer containing 500 μ M EGTA. In the presence of 200 μ M EGTA, adenylate cyclase activity in fraction I was progressively inhibited by added Ca^{2+} in the range of 0 to 1 mM (Fig. 3A). The addition of purified bovine brain CDR had little or no effect on this activity at any Ca^{2+} concentration. Under identical conditions, adenylate cyclase activity in fraction II was stimulated 4-fold upon addition of Ca^{2+} between 0 and 0.25 mM (Fig. 3B). Higher Ca^{2+} concentrations inhibited the activity. In addition, CDR stimulated the activity of all Ca^{2+} concentrations. The fact that Ca^{2+} stimulation was observed in the absence of added CDR was attributable to CDR contamination in fraction II (Table 1).

In order to demonstrate that the adenylate cyclase activity isolated in fraction II was partially stimulated by endogenous CDR, assays were carried out in the presence of the modulator binding protein (Fig. 4). The modulator binding protein inhibits the Ca^{2+} -dependent phosphodiesterase (15) and brain adenylate cyclase by complexing CDR (22). As illustrated in Fig. 4, the modulator binding protein inhibited adenylate cyclase activity in fraction II to the same extent in the presence or absence of added CDR. Adenylate cyclase activity in fraction II was also assayed in the presence of Mn^{2+} . In the presence of 1 mM ATP and 10 mM MnCl_2 , adenylate cyclase was stimulated 2.5-fold by exogenous CDR.

Partial Purification of Ca^{2+} -CDR-Sensitive Adenylate Cyclase. The primary objective of this study was to separate the Ca^{2+} -CDR-sensitive and -insensitive forms of adenylate cyclase by using CDR-Sepharose chromatography. However, it is also apparent that CDR-Sepharose offers some promise for purification of the CDR-sensitive enzyme (Table 1). Using total adenylate cyclase activity in the membrane preparations as a

reference point, we purified the Ca^{2+} -CDR-sensitive adenylate cyclase 55-fold with a 13% yield (Table 1). If it is assumed that

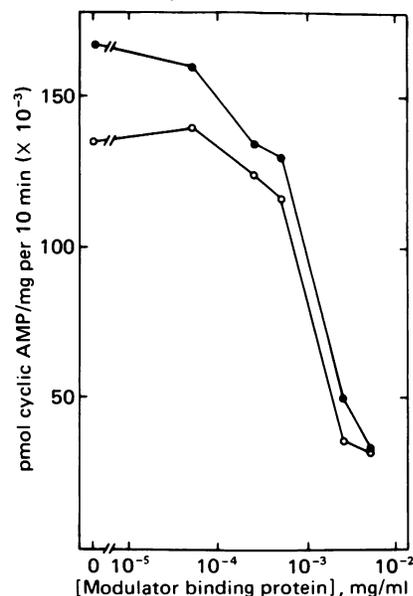


FIG. 4. Inhibition of the Ca^{2+} -sensitive adenylate cyclase by modulator binding protein. Adenylate cyclase activity in a pooled fraction II sample was desalted into 20 mM Tris, pH 7.35/2 mM dithiothreitol/250 mM sucrose/500 μ M EGTA as described in the legend for Fig. 3. The specific activity of this particular preparation was 93,700 and 235,000 pmol of cAMP/mg per 10 min in the absence and presence, respectively, of 4.4×10^{-2} mg of CDR per ml. Adenylate cyclase activity was assayed with 1.0 mM ATP, 5 mM MgCl_2 , and 250 μ M added Ca^{2+} as a function of modulator binding protein concentration. The final concentration of EGTA during assays was 200 μ M. Assays performed in the absence (O) and presence (●) of 1.1×10^{-2} mg of CDR per ml.

the two forms of adenylate cyclase are present in the membranes at the ratio expressed by the CDR-Sepharose elution profile (23% Ca^{2+} -CDR-sensitive adenylate cyclase), the extent of purification was 237-fold with a 56% yield. No firm estimate for purification can be given at this time because of uncertainties concerning the relative amounts of the two enzyme activities in membranes. Sodium dodecyl sulfate gels of fraction II revealed eight protein bands exhibiting molecular weights ranging from 20,000 to 200,000, indicating that the preparation was certainly not homogeneous. It is interesting to compare this preparation to other published purifications of adenylate cyclase. Adenylate cyclase purified to homogeneity from *Brevibacterium liquifaciens* has a basal turnover number of 139 min^{-1} (23). If a molecular weight for adenylate cyclase of 220,000 is assumed (24), the partially purified adenylate cyclase from canine heart has an apparent turnover number of 18 min^{-1} (25) and the partially purified enzyme described in this study has a turnover number of 3 min^{-1} . Adenylate cyclase activity in fraction II was not stimulated by norepinephrine or F^- . It was, however, quite stable and was stored frozen for periods up to 1 month with no loss of activity.

DISCUSSION

Brain adenylate cyclase shows a biphasic response to Ca^{2+} (1–5) and CDR mediates Ca^{2+} stimulation of the enzyme (3, 5–8). Brostrom *et al.* (8) recently proposed that brain cortex contains separate CDR-sensitive and -insensitive forms of adenylate cyclase. The present study directly corroborates this proposal and provides a convenient method for separation of the two activities. This technology now makes it possible to study independently the CDR-sensitive and -insensitive adenylate cyclase activities.

CDR-Sepharose was successfully used to separate partially purified adenylate cyclase into two fractions. Fraction I was not absorbed to CDR-Sepharose; it was inhibited by Ca^{2+} and exogenous CDR had little or no effect on the activity. In contrast, fraction II was absorbed to CDR-Sepharose, it was eluted with EGTA, and the enzyme was stimulated by Ca^{2+} and CDR. The latter activity was inhibited by the modulator binding protein.

The existence of two forms of adenylate cyclase resolvable by CDR-Sepharose chromatography may reflect one of several different possibilities. Brain cortex may actually contain two distinct adenylate cyclase species. Alternatively, there is considerable evidence that the adenylate cyclase system is comprised of two or more nonidentical subunits (26–30). It is possible that solubilization and Affi-Gel Blue chromatography may have separated one or more subunits responsible for CDR sensitivity. However, the former interpretation is favored since Brostrom *et al.* (8) have provided convincing evidence for the existence of CDR-dependent and -independent forms of the enzyme in native membranes. The physiological significance of these observations is not obvious; however, these two activities may be localized in different areas of the brain or differ in subcellular compartmentalization. By analogy, it is interesting that the phosphodiesterase activity in crude tissue homogenates is a mixture of isozymes, only one of which is sensitive to Ca^{2+} and CDR (13).

The purification of the Ca^{2+} -CDR-sensitive adenylate cyclase afforded by solubilization, Affi-Gel Blue chromatography, and CDR-Sepharose chromatography was modest. However, CDR-Sepharose shows considerable promise for purification of the enzyme, and the partially purified enzyme was quite stable, suggesting that further purification of the enzyme is technically feasible.

In summary, this study has provided a method for separation

of the CDR-sensitive and CDR-insensitive adenylate cyclase activities from brain cortex and provides direct evidence that the Ca^{2+} -stimulated adenylate cyclase forms a complex with CDR in the presence of Ca^{2+} .

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1. Bradham, L. S., Holt, D. A. & Samo, M. (1970) *Biochim. Biophys. Acta* **201**, 250–260.
2. Von Hungen, K. & Roberts, S. (1973) *Nature (London) New Biol.* **242**, 58–60.
3. Brostrom, C. O., Huang, Y. C., Breckenridge, B. M. & Wolff, D. J. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 64–68.
4. Macdonald, I. A. (1975) *Biochim. Biophys. Acta* **397**, 244–253.
5. Brostrom, M. A., Brostrom, C. O., Breckenridge, B. M. & Wolff, D. J. (1976) *J. Biol. Chem.* **251**, 4744–4750.
6. Cheung, W. Y., Bradham, L. S., Lynch, T. J., Lin, Y. M. & Tallant, E. A. (1975) *Biochem. Biophys. Res. Commun.* **66**, 1055–1062.
7. Lynch, T. J., Tallant, E. A. & Cheung, W. Y. (1976) *Biochem. Biophys. Res. Commun.* **68**, 616–625.
8. Brostrom, C. O., Brostrom, M. A. & Wolff, D. J. (1977) *J. Biol. Chem.* **252**, 5677–5685.
9. Wolff, D. J. & Brostrom, C. O. (1974) *Arch. Biochem. Biophys.* **163**, 349–358.
10. Lin, Y. M., Liu, Y. P. & Cheung, W. Y. (1974) *J. Biol. Chem.* **249**, 4943–4954.
11. Kakiuchi, S., Yamazaki, R., Teshima, Y. & Uenishi, K. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 3526–3530.
12. Wang, J. H., Two, T. S., Ho, H. C. & Stevens, F. C. (1975) *Adv. Cyclic Nucleotide Res.* **5**, 179–194.
13. Ho, H. C., Wirch, E., Stevens, F. C. & Wang, J. H. (1977) *J. Biol. Chem.* **252**, 43–50.
14. Watterson, D. M. & Vanaman, T. C. (1976) *Biochem. Biophys. Res. Commun.* **73**, 40–46.
15. Klee, C. B. & Krinks, M. H. (1978) *Biochemistry* **17**, 120–126.
16. Salomon, Y., Londos, C. & Rodbell, M. (1974) *Anal. Biochem.* **58**, 541–548.
17. Peterson, G. L. (1977) *Anal. Biochem.* **83**, 346–356.
18. Johnson, R. A. & Sutherland, E. W. (1973) *J. Biol. Chem.* **248**, 5114–5121.
19. LaPorte, D. C. & Storm, D. R. (1978) *J. Biol. Chem.* **253**, 3374–3377.
20. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
21. Stellwagen, E. & Baker, B. (1976) *Nature (London)* **26**, 719–720.
22. Wallace, R. W., Lynch, T. J., Tallant, E. A. & Cheung, W. Y. (1978) *Arch. Biochem. Biophys.* **187**, 328–334.
23. Takai, K., Kurashina, Y. & Hayaishi, O. (1974) *Methods Enzymol.* **38**, 160–169.
24. Neer, E. J. (1978) *J. Biol. Chem.* **253**, 1498–1502.
25. Homcy, C., Wrenn, S. & Haber, E. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 59–63.
26. Pfeuffer, T. & Helmreich, E. J. M. (1975) *J. Biol. Chem.* **250**, 867–876.
27. Orly, J. & Schramm, M. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4410–4414.
28. Limbird, L. E. & Lefkowitz, R. J. (1977) *J. Biol. Chem.* **252**, 799–802.
29. Ross, E. M. & Gilman, A. G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3715–3719.
30. Naya-Vigne, J., Johnson, G. L., Bourne, H. R. & Coffino, P. (1978) *Nature (London)* **272**, 720–722.