Isolation and Properties of Brain α -Actinin

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(Received 5 December 1977)

α -Actinin isolated from bovine brain migrated on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis like muscle α -actinin with an apparent mol.wt. of 100 000 and cross-reacted with antibodies to muscle α -actinin. Brain α -actinin modulated actin-myosin Mg^{2+} -activated adenosine triphosphatase activity and, when bound by polystyrene particles, was found to bind muscle actin and tropomyosin from solution. Brain α -actinin, in conjunction with the other components of the contractile and relaxing complex, may play a role in the release of neurotransmitters from synaptic vesicles.

The presence of contractile proteins in non-muscle cells has been well documented in recent years (Perry, 1976; Goldman *et al.*, 1976). In brain, actin (Puszkin *et al.*, 1972; Bray & Thomas, 1976; Puszkin & Kochwa, 1974), myosin (Berl & Puszkin, 1970; Burridge & Bray, 1975), tropomyosin (Fine *et al.*, 1973) and troponin components (Puszkin *et al.*, 1975; Fine & Blitz, 1976), all elements of the contractile and relaxing systems, have been isolated and partially characterized. Furthermore, subcellular-fractionation and immunological studies have demonstrated the presence of contractile proteins in synaptic endings. Synaptic endings contain the sites of storage and release of chemical transmitters (Hall, 1972), and this led to the proposal that neurotransmitter release was in part mediated and modulated by the interplay of the various components of the microfilamentous contractile apparatus (Puszkin & Kochwa, 1974; Berl *et al.*, 1973). It is believed that release of neurotransmitters stored in synaptic vesicles occurs when a given number of vesicles approach the synaptosomal membrane and, either by fusion (Heuser & Reese, 1973) or some other mode, release their contents into the synaptic cleft. Since the mechanism producing migration of vesicles to their proper position at the synaptic membrane is not understood, studies were initiated to determine if vesicles contained anchoring material suitable for their attachment to a microfilamentous matrix. These studies were in line with a continuing attempt to further delineate the composition and function of all the elements of the contractile system present in brain tissue.

In the present paper we report the isolation and properties of a new contractile protein from enriched fractions of coated vesicles. This protein appears to be

the counterpart of α -actinin from the Z-bands of muscle tissue, where the anchoring site for thin filaments (actin, tropomyosin and troponin) resides.

Experimental

Materials

Polystyrene (Lytron) particles were prepared commercially (Boundy & Boyer, 1952). A single batch of Lytron 615 (kindly supplied by Dr. E. Rosen, Monsanto Chemical Co., Springfield, MA, U.S.A.) was treated for experimental use as described elsewhere (Puszkin *et al.*, 1975). GTP, ATP and dithiothreitol were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). DEAE-cellulose (DE-11) was purchased from Whatman, Clifton, NJ, U.S.A. [γ - ^{32}P]ATP was purchased from New England Nuclear, Boston, MA, U.S.A. All other reagents used were of analytical grade I.

Protein preparation

Myosin (Richards *et al.*, 1967) and actin (Spudich & Watt, 1971) were prepared from rabbit muscle. α -Actinin was prepared from dog muscle essentially as described previously (Robson *et al.*, 1970).

Preparations containing vesicles

Coated vesicles were prepared from bovine brain by a slight modification of published procedures (Pearse, 1975). The brains were cleansed of meninges and surrounding blood vessels and the grey matter was separated from the white matter by suction into a flask at 0°C. The mince obtained by suction was then extracted with an equal volume of 0.1 M-Tris/HCl buffer (pH 7.0) containing 1 mM-EGTA, 0.5 mM- Mg^{2+} and 1 mM-ATP by using three 10 s bursts of a Waring blender at top speed. The homogenate was centrifuged at 20 000g for 30 min and the supernatant

Abbreviations used: SDS, sodium dodecyl sulphate; Mg^{2+} -ATPase, Mg^{2+} -activated adenosine triphosphatase.

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centrifuged at 100000 g for 1 h to obtain a somewhat less enriched but otherwise larger vesicle fraction (P_{100}), which was used in most extraction experiments to maximize the yield of α -actinin. Alternatively, the 20000g supernatant was centrifuged at 55000g for 1 h to obtain a crude coated-vesicle fraction for application to sucrose gradients as described previously (Pearse, 1975).

Preparation of α -actinin

The P_{100} fraction prepared above was extracted with 50ml of 20mM-Tris/HCl buffer (pH 7.5) containing 0.5mM-dithiothreitol and 1mM-EDTA per ox brain. The pellet was suspended in buffer and homogenized by using 10–12 strokes of a motor-driven Teflon/glass homogenizer, followed by centrifugation at 100000g for 1 h to recover the vesicle fraction. The second extraction was carried out almost like the first, except that it was performed with stirring for 16h. The homogenate was again centrifuged at 100000g for 1 h and the supernatant stored in the cold while the pellet was extracted a third time. This extraction was performed overnight with a similar volume of 10mM-Tris/HCl buffer (pH 8.5) containing 0.5mM-dithiothreitol and 1mM-EDTA at 4°C with stirring. The homogenate was again centrifuged at 100000g for 1 h and the supernatant fractionated by addition of solid $(\text{NH}_4)_2\text{SO}_4$ to 30% saturation. The other two extracts were treated similarly. All the pellets were dissolved in a minimal volume of 20mM-Tris/HCl buffer (pH 7.5) containing 0.5mM-dithiothreitol and dialysed against at least 20 vol. of buffer overnight. The α -actinin preparation was chromatographed on DEAE-cellulose with a discontinuous KCl gradient of 10ml steps of 0.1, 0.2 and 0.5M in 20mM-Tris/HCl (pH 7.5). When a purified coated-vesicle fraction was used, the extraction volumes were decreased to 10ml. The extracts were concentrated as described above and tested for reaction with antibody by immunodiffusion. The small amount of protein obtained during these procedures precluded further purification and was the reason for utilizing the more heterogeneous starting fraction described above. Electron microscopy showed that the P_{100} fraction contains a large number of coated vesicles along with various other membranous structures (result not shown).

Polyacrylamide-gel electrophoresis

Gel electrophoresis was carried out in cylindrical 7.5% polyacrylamide gels containing 0.1% SDS in 10mM-sodium phosphate buffer as described previously (Weber & Osborn, 1969). The samples were mixed with a dye solution containing 30% (v/v) glycerol and 0.1% 2-mercaptoethanol and heated for 2–3 min at 100°C before loading on the gels. Electrophoresis was performed overnight at a constant current of 3mA/gel. The samples were removed when Bromophenol Blue tracking dye reached the bottom

of the gel. After electrophoresis, the gels were fixed, stained with Coomassie Blue and destained by diffusion.

Determination of Mg^{2+} -ATPase activity

Mg^{2+} -ATPase activity of proteins was determined by the release of P_i from ATP containing $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (Puszkín *et al.*, 1977).

Immunization of rabbits

Unselected white New Zealand-strain male rabbits weighing 2–3 kg were immunized over a period of 4–6 weeks by biweekly intradermal injections of muscle α -actinin solution emulsified in an equal volume of complete Freund's adjuvant. Each injection contained 1 mg of α -actinin in a volume of 1–2 ml. After 6 weeks, the rabbits were bled and tested for antibody production. The sera were collected and stored at -20°C . Individual sera were not pooled.

Double gel diffusion

Double gel diffusion was performed in 0.85% agarose dissolved in 0.02M-Tris/HCl buffer (pH 7.5). Proteins were allowed to diffuse at 20°C for 24–72 h, washed in 0.9% NaCl, then in water and dried. For immunoelectrophoresis, 0.85% agarose was dissolved in 0.05M-barbital buffer (pH 8.6) and electrophoresis was performed without cooling for 4 h at 150V on $7.5\text{ cm} \times 7.5\text{ cm}$ glass plates with Bromophenol Blue as a marker dye.

Coating of Lytron particles with protein

Protein concentrations were adjusted with 0.02M-Tris/HCl buffer (pH 7.5) to 0.6–1.2mg/ml, and adsorption by Lytron particles was carried out at room temperature as described previously (Puszkín *et al.*, 1975).

Protein concentration

Protein concentrations were measured by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

Results

Preparation of coated vesicles

The appearance of the final coated vesicle preparation following the method of Pearse (1975) is shown in Plate 1. This fraction is largely enriched with coated vesicles. A suspension of purified vesicles and the initial crude vesicle fraction were compared by polyacrylamide-gel electrophoresis in Fig. 1. The polypeptide band of 180000-mol.wt. 'coated protein' is enriched after various sucrose-gradient centrifugations needed to obtain purified vesicles. Other prominent bands are also observed. The presence of a significant band in the 100000-mol.wt. region, which

EXPLANATION OF PLATE I

Electron micrograph of a coated-vesicle fraction obtained by sucrose-gradient centrifugation

The vesicle pellet was fixed in 1.5% glutaraldehyde in 0.1 M-phosphate buffer (pH 7.4) for 1 h and washed with 0.1 M-phosphate buffer (pH 7.4) overnight. It was stained with 1% OsO₄ in 0.1 M-phosphate buffer (pH 7.4) for 1 h, washed with buffer and dehydrated with graded ethanol solutions followed by embedding in Epon. Thin sections were cut, placed on grids and stained with uranyl acetate and lead citrate, and observed with a Joel 100-B electron microscope at 80 kV (magnification 132 000 ×).

Fig. 1. *SDS/polyacrylamide-gel electrophoresis of crude and purified coated-vesicle fraction proteins*

Electrophoresis was performed in single gels of 7.5% polyacrylamide. Gel (1) contains the proteins present in the initial vesicle pellet before application to sucrose gradients as described by Pearse (1975). Gel (2) contains the proteins present in the coated-vesicle fraction obtained after the sucrose-gradient centrifugation steps. Note the enrichment of the band at 180000 as well as that at 100000 in the purified vesicle fraction compared with the initial fraction. Gel (3) is a standard loaded with muscle α -actinin + actin.

co-migrated with muscle α -actinin, as shown in Fig. 2, enhanced the possibility that a protein comparable with α -actinin could be extracted from this vesicle preparation.

Purification of brain α -actinin

The individual steps for the isolation and purification of α -actinin from brain are summarized in Scheme 1. The 0–30%-satd.-(NH₄)₂SO₄ fractions obtained after extracting the P₁₀₀ fraction were run on SDS/polyacrylamide gels to determine their protein

Fig. 2. *Co-migration of muscle α -actinin with a protein present in a purified coated-vesicle fraction*

Gel (1) was loaded with 3 μ g of muscle α -actinin + 15 μ g of muscle actin; gel (2) was loaded with coated-vesicle fraction alone (50 μ g); gel (3) was loaded with coated-vesicle fraction (50 μ g) + 3 μ g of muscle α -actinin; gel (4) was loaded with coated-vesicle fraction (50 μ g) + 6 μ g of muscle α -actinin.

composition. Generally, the third low-ionic-strength and higher-pH extraction medium (pH 8.5) contained the largest quantity of α -actinin, but occasionally the

Scheme 1. Summary of the purification procedure used to obtain brain α -actinin

low-ionic-strength extraction medium (pH 7.5) used for the second extraction showed a significant enrichment in this protein (Fig. 3). The fraction containing the highest concentration of the α -actinin-like protein band was applied to a DEAE-cellulose column (0.5cm $\times$ 5cm) prepared in 20mM-Tris/HCl buffer (pH 7.5) containing 0.5mM-dithiothreitol and eluted with a discontinuous KCl gradient consisting of 10ml steps of 0.1, 0.2 and 0.5M. The 0.1M-KCl fractions that contained most of the α -actinin-like protein band as determined by SDS/polyacrylamide-gel electro-

phoresis were dialysed against 0.02M-Tris/HCl (pH 7.5) to remove salt and stored frozen in 10% sucrose until needed.

As illustrated in Fig. 3, the extraction procedure itself results in an extensive enrichment of the α -actinin compared with its initial proportion in the P_{100} fraction (gel 1). Chromatography on a DEAE-cellulose column further enhances purity to approx. 90% as determined by densitometric gel scanning. Table 1 summarizes the yield of protein obtained in the various 30%-satd.-(NH₄)₂SO₄ precipitates of a

Fig. 3. Protein composition at different steps in the purification procedure for brain α -actinin

Gel (1) was loaded with starting synaptic-vesicle fraction (77 μ g). The band labelled 180 represents the protein migrating in the 180000-mol.wt. region of the gel; 100, 55 and 45 represent proteins migrating in the 100000-, 55000- and 45000-mol.wt. regions respectively. Gel (2) was loaded with first synaptic-vesicle extract after 30%-satd.-(NH₄)₂SO₄ fractionation (80 μ g). Gel (3) was loaded with supernatant of the first synaptic-vesicle extract after 30%-satd.-(NH₄)₂SO₄ fractionation (70 μ g). Gel (4) was loaded with a second synaptic-vesicle extract after 30%-satd.-(NH₄)₂SO₄ fractionation (83 μ g). Gel (5) was loaded with a third synaptic-vesicle extract after 30%-satd.-(NH₄)₂SO₄ fractionation (95 μ g). Gel (6) was loaded with a DEAE-cellulose-purified brain α -actinin (110 μ g). Gel (7) was loaded with α -actinin (8 μ g); tubulin (10 μ g) and actin (15 μ g).

typical experiment. These data and those of the relative amounts of the α -actinin band present in this fraction (Fig. 3, gel 5) indicate that the α -actinin-like protein represents 1.5–2% of the protein present in the crude vesicle fraction.

Table 1. Recoveries of a typical purification of brain α -actinin from two ox brains

The protein concentration of all fractions was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Purification stage	Volume (ml)	Concentration (mg/ml)	Total protein (mg)
(1) P ₁₀₀ fraction	83	7.2	605
(2) 30%-satd.-(NH ₄) ₂ SO ₄ of first extract	11.8	12.5	147
(3) 30%-satd.-(NH ₄) ₂ SO ₄ of second extract	10.8	11.2	121
(4) 30%-satd.-(NH ₄) ₂ SO ₄ of third extract	7	3	21
(5) Extracted P ₁₀₀ fraction	24	3.5	84
(6) DEAE-cellulose column effluent	5	2	10

Double gel diffusion

Brain protein extracts were routinely screened by double gel diffusion to detect the presence of brain α -actinin. Monospecific antisera induced in rabbits to the dog muscle protein cross-reacted with the brain protein, as illustrated in Fig. 4(a). Immunological identity between the muscle α -actinin and its brain counterpart of three different preparations is typical of that observed in all other α -actinin preparations in our laboratory. The additional precipitin bands observed closer to wells 3 and 5 of Fig. 4(a) in some cases probably are formed by aggregates of α -actinin that migrate at a slower rate through the gel. A similar explanation applies to bands observed on immunoelectrophoresis (Fig. 4c). Antigenic reactivity correlated well with the SDS/polyacrylamide-gel-electrophoresis patterns of the various extracts. No precipitin band was observed with either the first or second brain extracts of the P₁₀₀ fractions, which were shown to have a relatively small quantity of protein migrating in the 100000-mol.wt. region (see Fig. 4b). Immunoelectrophoresis was performed to verify the specificity of the antisera to the muscle protein and to show that the reaction of the antisera with the brain extract was due to the presence of α -actinin.

Fig. 4(c) illustrates the antigenic similarity of brain and muscle proteins. The precipitin bands observed in Fig. 4(c) are apparently produced by complexes of α -actinin that migrate through the agarose at different rates, the size and position of the bands being virtually identical for both the brain and muscle preparations. The anti-(α -actinin) antibodies have been previously reported to react specifically with the 100000-mol.wt. band resolved by SDS/polyacrylamide-gel electrophoresis (Puszkin *et al.*, 1977); this, coupled with the fact that serum from four different rabbits immunized

Fig. 4. Immunodiffusion and immunoelectrophoresis of muscle and brain α -actinin

(a) The left-hand system contained antibodies to muscle α -actinin in the centre well. The peripheral wells contained: muscle α -actinin (1.7 mg/ml) (wells 1, 3 and 5); DEAE-cellulose-purified brain α -actinin (3.6 mg/ml) (well 2); brain α -actinin I (0.5 mg/ml) (well 4); brain α -actinin II (0.8 mg/ml) (well 6). The right-hand system contained antibodies to muscle α -actinin in the centre well and in the peripheral wells contained muscle α -actinin (1.7 mg/ml) (wells 1, 3 and 5) and DEAE-cellulose-purified brain α -actinin (3.6 mg/ml) (wells 2, 4 and 6). (b) Immunodiffusion of the various extracts from a typical preparation of brain α -actinin against antibody to dog muscle α -actinin is shown. Wells (1) and (6) contain the 30%-satd. $(\text{NH}_4)_2\text{SO}_4$ fraction from the first brain vesicle extract. Wells (2) and (7) contain the 30%-satd. $(\text{NH}_4)_2\text{SO}_4$ fraction from the second brain vesicle extract. Wells (3) and (8) contain the 30%-satd. $(\text{NH}_4)_2\text{SO}_4$ fraction from the third brain vesicle extract. Wells (4) and (9) contain a purified brain α -actinin fraction. Wells (5) and (10) contain dog muscle α -actinin. The centre wells contain the antibody to muscle α -actinin. (c) Immunoelectrophoresis of brain and muscle α -actinin is shown. Well (1) contains brain α -actinin, well (2) contains dog muscle α -actinin and the trough contains anti-(muscle α -actinin).

Fig. 5. SDS/polyacrylamide-gel electrophoresis of brain and muscle proteins

Gel (1) was loaded with brain α -actinin (4 μg); gel (2) was loaded with a combination of dog muscle and brain α -actinin; gel (3) was loaded with a standard mixture of muscle α -actinin (2 μg) and rabbit muscle tropomyosin (5 μg); gel (4) was loaded with a standard of rabbit muscle myosin (3 μg) and actin (5 μg).

with dog muscle α -actinin all reacted with the brain protein, is consistent with the suggestion that the antigen-antibody reaction in double gel diffusion with brain extracts is due to the presence of an antigenically similar α -actinin.

Co-migration of purified brain and muscle α -actinins

The brain and muscle proteins were electrophoresed at concentrations of 5 and 2 μg respectively, both separately and combined in single polyacrylamide gels. Fig. 5 illustrates the similarity of the two proteins.

Table 2. *Interaction of brain α -actinin with muscle tropomyosin and actin as determined by binding to polystyrene particles*
 Protein concentrations were adjusted to 0.5–1.10 mg/ml. Small volumes of polystyrene particles were added to protein solution in appropriate buffers and the mixtures gently agitated at room temperature for the formation of the first protein coat. Polystyrene particles were sedimented by centrifugation at 35000g for 20 min and the pellet was resuspended in the appropriate second protein solution for 30 min. The polystyrene particles were sedimented as before. The amount of protein adsorbed in every instance was estimated from the difference between the initial protein concentration and the remainder in solution after adsorption. In all cases, care was taken to produce polystyrene particles with the first coat of protein completely saturating the surfaces of polystyrene particles. Correction for impurities was performed by densitometric scanning of the proteins resolved by SDS/polyacrylamide-gel electrophoresis. The corrected data are given in the Table. The following molecular weights were used to obtain the molecular ratios reported: α -actinin, 200000 (Suzuki *et al.*, 1976); actin monomer, 42000 (Perry, 1976); tropomyosin, 68000 (Woods, 1967). The numbers in parentheses are the average molar ratios observed for muscle α -actinin and are taken from Puszkín *et al.* (1977).

First protein coat	Second protein coat	Molecular ratio F-actin or tropomyosin/ α -actinin
Brain α -actinin (0.126 mg)	F-actin (0.220 mg)	8.3
(0.112 mg)	(0.180 mg)	7.7 (8.0)
(0.123 mg)	(0.210 mg)	8.1
Brain α -actinin (0.119 mg)	Tropomyosin (0.175 mg)	4.3
(0.109 mg)	(0.150 mg)	4.0 (4.8)
(0.116 mg)	(0.175 mg)	4.4
F-actin (0.210 mg)	Brain α -actinin (0.131 mg)	7.6
(0.205 mg)	(0.122 mg)	8.0 (7.9)
(0.175 mg)	(0.108 mg)	7.7
Tropomyosin (0.140 mg)	Brain α -actinin (0.065 mg)	6.2
(0.135 mg)	(0.070 mg)	5.7 (5.0)
(0.120 mg)	(0.050 mg)	7.1

Fig. 6. *Effect of brain α -actinin on actin-activated Mg-ATPase of muscle myosin*

Conditions were: 80 mM-KCl, 0.5 mM-MgCl₂, 50 mM-Tris/HCl (pH 7.5) and 100 μ g of muscle myosin. Muscle actin was present at 63 μ g and brain α -actinin was added in the following amounts: 7.3 μ g (1); 14.5 μ g (2), 72.5 μ g (3), and 145 μ g (4). Proteins and reagents were mixed in a volume of 900 μ l at room temperature for 10 min and then diluted to 5 ml with 50 mM-Tris/HCl (pH 7.5), followed by incubation at 37°C for 10 min. The ³²P-labelled ATP solution (0.1 mM) was added to initiate the reaction. Incubation was carried out at 37°C for 10 min and 1 ml portions were removed at each time point. Brain α -actinin did not affect the rate of muscle myosin ATPase activity. The rate of myosin ATPase illustrated in the Figure can be considered as from either myosin alone or myosin/ α -actinin mixture.

Interaction of brain α -actinin with muscle actin and tropomyosin

Lytron particles were utilized to determine if an interaction exists between brain α -actinin and actin and tropomyosin of muscle. The particles were coated with α -actinin, F-actin or tropomyosin as described previously (Puszkín *et al.*, 1977), and a second coat of the complementary protein was applied. Table 2 lists the results of such experiments and compares them with those previously obtained with dog muscle α -actinin (Puszkín *et al.*, 1977). SDS/polyacrylamide-gel electrophoresis of proteins bound by, and then eluted from, Lytron particles confirmed the results obtained by the protein determinations. The interactions were not only qualitatively similar, but the molar ratios of the bound proteins were similar to those observed with muscle α -actinin.

Effect of brain α -actinin on muscle actomyosin Mg²⁺-ATPase activity

Muscle α -actinin was shown (Arakawa *et al.*, 1970) to have a stimulatory effect on actomyosin Mg²⁺-ATPase activity (Arakawa *et al.*, 1970). Subsequently, this effect has been used as a measure of the biological activity of α -actinin. As shown in Fig. 6, the brain α -actinin preparation was found to have a stimulatory effect on a reconstituted muscle actomyosin system.

Myosin Mg^{2+} -ATPase alone was not stimulated by either muscle or brain α -actinin.

Discussion

The isolation procedure described here for bovine brain α -actinin resulted in a significant purification of this protein. The proportion of P_{100} protein that migrates in the 100000-mol.wt. region is about 1.5–2%. The extraction procedure yielded a fraction that was approx. 70% pure by gel electrophoresis, i.e. better than a 10-fold enrichment. The purity was further increased by passage through a DEAE-cellulose column, to approx. 90%, as shown by gel scanning.

The choice of an enriched vesicle fraction to start the purification of brain α -actinin was dictated by preliminary results from experiments utilizing whole brain extracts or extracts from various other sub-cellular fractions. The whole brain extract yielded a large quantity of a protein population that also contained a polypeptide band migrating in the 100000-mol.wt. region. Preparations of synaptosomes and synaptic membranes also showed a 100000-mol.wt. protein. None of these extracts produced a precipitin band when tested with antibody to the dog muscle α -actinin, probably owing to its relatively lower concentration. The most enriched fraction in 100000-mol.wt. protein was the purified coated-vesicle fraction, and because of its unambiguous 'neuronal' origin it was chosen as the source of brain α -actinin. The purified vesicle extract has the added advantage of displaying a good antigenic reactivity with antibodies to muscle α -actinin (Puszkin *et al.*, 1976). The presence of an α -actinin-like protein in this fraction tacitly implies a role in synaptosomal functions.

The requirement for several extractions to release the α -actinin from the vesicle fraction may be explained in part by the presence of Mg^{2+} in the initial homogenization medium. In muscle Arakawa *et al.* (1970) found that a long period of extraction (3–4 days) at low ionic strength was required to release α -actinin from the myofibrils. However, if they added as little as 1 mM- Mg^{2+} to the extracted fibrils, no α -actinin could be recovered from the extract. It suggested that α -actinin may re-bind tightly to myofibrils in the presence of Mg^{2+} . The length of extraction time required to obtain α -actinin from myofibrils may be due to slow swelling of fibrils to admit the extracting medium and disruption of the hydrophobic binding of proteins present in the Z-band to release α -actinin. An alternative procedure for purification of α -actinin from muscle utilizes the solubilizing power of deoxycholate (Etlinger & Fischman, 1972), lending support to the hypothesis that the α -actinin of muscle is bound by hydrophobic interactions. The brain protein may be attached tightly to membranes by similar hydrophobic interaction.

The presence of a reticular protein coat around vesicles, as well as the implied location of α -actinin in the vesicle membrane, may explain why several extraction procedures are needed for the release of α -actinin. If solubilization of the protein from the membrane requires a combination of hypo-osmotic shock and exposure of both sides of the membrane to solvent, then removal of the coat might be required to allow rupture of the membrane. Kadota *et al.* (1976) have studied the effect of pH 7.5, which is the pH used for the first two extractions. The extracts obtained from the first two such extractions are enriched with the 180000-mol.wt. protein (Fig. 5), reportedly the protein comprising the vesicle coat (Pearse, 1975). Here again, removal of the vesicle coat could facilitate the extractions of α -actinin.

The properties of the brain preparation were shown to be consistent with its being the brain counterpart to muscle α -actinin. The brain protein cross-reacted with antibodies induced in rabbits by the muscle protein, interacted with a reconstituted muscle actomyosin system, resulting in a stimulation of Mg^{2+} -ATPase activity, bound muscle actin and tropomyosin, and had a similar molecular weight to the muscle protein as determined by SDS/polyacrylamide-gel electrophoresis.

The activation of actomyosin Mg^{2+} -ATPase activity by the brain α -actinin was similar to that obtained with the muscle protein. The maximal activation was about 100%, which is in line with those recorded for rabbit muscle (Goll *et al.*, 1972) and for dog muscle α -actinin (Puszkin *et al.*, 1977). The interaction of a non-muscle α -actinin with a muscle actomyosin system was not unexpected, since we have shown that muscle α -actinin is capable of interacting with a hybrid actomyosin system prepared from brain actin (Puszkin *et al.*, 1976) and erythrocyte actin (Puszkin *et al.*, 1977) with muscle myosin.

Utilizing a protein-binding technique developed in our laboratories (Puszkin *et al.*, 1975), it was found that α -actinin could bind tropomyosin or actin from solution. By use of Lytron particles coated with a given protein, the interaction between α -actinin and muscle tropomyosin or actin was determined. The surfaces of the Lytron particles adsorbed brain α -actinin with an affinity similar to that reported previously for muscle α -actinin (Puszkin *et al.*, 1977).

Calculations of the number of molecules of actin or tropomyosin bound by brain α -actinin agree favourably with those found for muscle α -actinin, as did the amount of brain α -actinin bound to actin or tropomyosin of muscle.

The isolation of α -actinin from a coated-vesicle-enriched fraction of bovine brain leaves open the question of its location in other areas of the brain. The preparative procedure described here results in the loss of all cytoplasmic and much of the membrane-bound α -actinin present in the tissue. A method of

purification with a better yield of brain α -actinin from unfractionated cortex eventually will be developed when further properties of α -actinin are known.

The association of the brain α -actinin with vesicular membranes as well as the moderately stringent conditions required for its extraction are consistent with earlier findings (Mooseker, 1976), that demonstrated the presence of α -actinin on the inner surfaces of membranes of the intestinal microvilli. Actin filaments were shown attached to these membranes at the position where the α -actinin was localized, a finding consistent with its apparent role in the Z-disc of muscle. The observed cross-linking of the actin strands by α -actinin is in agreement with results of Lazarides & Burridge (1975), who demonstrated the presence of α -actinin in several non-muscle cells by immunofluorescent techniques. These authors observed a variable spacing of α -actinin along the cytoplasmic actin filaments, presumably representing sites of cross-linking between various filaments. Furthermore, α -actinin was also localized in areas of cell-to-cell contacts, and it was suggested that these membranes contain α -actinin as an attachment site for the actin filaments observed in these regions.

The ultrastructural identification (LeBeux & Willemot, 1975) of actin filaments in brain by arrow-head decoration with a fragment of the myosin molecule from muscle confirmed their presence in the cytoplasm as well as in the nerve endings of neurons (Puszkin *et al.*, 1972; Berl *et al.*, 1973). The attachment of these filaments to synaptic vesicles and membranes was also observed (LeBeux & Willemot, 1975). All these observations support the existence of a membrane-bound attachment site for actin filaments. The nature of this attachment site requires study, and the properties exhibited by α -actinin suggest that this protein might fulfil anchoring and/or regulatory functions. Proteins that can bind actin have been reported in the literature for a variety of tissues. Spectrin (Marchesi & Steers, 1968; Fairbanks *et al.*, 1971), a high-molecular-weight protein found in erythrocyte membranes, has been shown to interact with erythrocyte actin (Puszkin, *et al.*, 1978) and muscle actin (Tilney & Detmers, 1975; Sheetz *et al.*, 1976) in a way that suggests it serves as an anchoring point for actin filaments to those membranes. Proteins with spectrin-like properties have been isolated from several disparate sources (Pollard & Korn, 1973; Hartwig & Stossel, 1975; Stossel & Hartwig, 1976), and these authors have presented evidence for the interaction of F-actin filaments with these spectrin-like proteins all located in the cell membranes.

The association of α -actinin with a coated-vesicle fraction also suggests that it may play an important role in the migration of vesicles to and from the synaptosomal membranes. This may be achieved if α -actinin acts as an anchoring point for actin filaments

that may ultimately mediate release of the vesicles contents.

This work was supported, in part, by N.I.H. grants NS-12467, HL-20718 and GM-07036, and Grant-In-Aid 75-811 of the American Heart Association. We thank Dr. S. Kochwa and Dr. R. E. Rosenfield for their critical evaluations of the manuscript. S. P. is an Established Investigator of the American Heart Association.

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