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ACTOMYOSIN-LIKE PROTEIN FROM BRAIN

SEPARATION AND CHARACTERIZATION OF THE ACTIN-LIKE COMPONENT

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SUMMARY

1. Actin-like protein (neurin) has been separated from actomyosin-like protein (neurostenin) isolated from bovine brain. This was accomplished by gel filtration chromatography (Sephadex G-200) and by ultracentrifugation in a continuous sucrose gradient containing 0.6 M KI.

2. The actin-like protein stimulated the Mg^{2+}-ATPase activity of muscle myosin.

3. It contained bound nucleotide which exchanged with free [^{14}C]ATP.

4. It polymerized in the presence of 0.1 M KCl and 0.1 mM Mg^{2+} with release of P_i; increase in viscosity occurred upon dilution of the 0.6 M KI to 0.1 M.

5. The neurin reacted immunologically to form a single band with antiserum to neurostenin.

6. The neurin, similar to muscle actin, contained 3-methylhistidine.

7. The sedimentation constant of the protein was 2.8 S.

INTRODUCTION

The isolation from mammalian brain of actomyosin-like, actin-like and myosin-like proteins has been recently described[1,2]. These proteins were named neurostenin, neurin and stenin respectively to distinguish them from similar proteins from other sources[2].

Pursuing further our studies of this system, we were able to isolate the actin-like moiety directly from the actomyosin-like complex by chromatography on Sephadex G-200 or by ultracentrifugation on sucrose gradients containing 0.6 M KI. This procedure is based on the method described by SZENT-GYORGYI[3] for the purification of actin from muscle actomyosin substituting KI for KCl. We are reporting also the presence of 3-methylhistidine, as well as other properties. The results lend support to the actin-like nature of the neurin.

EXPERIMENTAL PROCEDURE

Materials

The Na_2ATP was obtained from Pabst Laboratories. The [8-^{14}C]adenosine 5'-triphosphate tetrasodium salt (41.9 μC/μmole) was obtained from New England Nuclear. The AG 1-X4, Cl^- 200–400 mesh (purified Dowex, Bio-Rad Laboratories) was obtained from Calbiochem. The Agar (Noble) was obtained from Difco. Sephadex G-200 and Blue dextran 2000 were purchased from Pharmacia Co. Acrylamide and Bis were obtained from Canalco and were recrystallized from acetone before use. All reagents were of analytical grade except Na_2ATP used in the buffers for the Sephadex columns and sucrose gradients which was grade II (97% purity). Crystallized bovine serum albumin was obtained from Pentex, Ill. 3-Methylhistidine and ε-*N*-monomethyllysine were obtained from Calbiochem, Calif.. Beckman's custom spherical resin type PA 35 was used for the detection of 3-methylhistidine.

Methods

Preparation of the proteins. Actomyosin was prepared from the fresh back muscles of the cat by the procedure of SZENT-GYORGYI[3]. A similar procedure was used to prepare actomyosin from fresh bovine aorta. Rat, cat and bovine brain actomyosin-like protein (neurostenin) was prepared from fresh tissue by extraction with 0.6 M KCl in 0.01 M bicarbonate buffer (pH 9.2) (Weber-Edsall Solution), as described previously[1].

Actin was prepared from the long back muscles freshly obtained from the cat or rabbit. The procedure used was essentially that described by CARSTEN AND MOMMAERTS[4]. Actin-like brain protein (neurin) was prepared by adaptation of the same method to bovine brain[2]. Muscle (cat) myosin was usually prepared simultaneously with the brain proteins for comparison purposes[5]. The colchicine-binding protein was prepared from bovine brain using the method of WEISENBERG *et al.*[6].

Sephadex chromatography. The Sephadex G-200 columns (2.5 cm × 45 cm) were equilibrated with 0.05 M Tris–1 mM ATP–0.6 M KCl (pH 7.8) (Buffer A) or with buffer in which the KCl was replaced by 0.6 M KI and 0.04 M sodium thiosulfate (Buffer B). The columns were allowed to flow at a rate of about 15 ml/h maintained by a peristaltic pump (Buchler Co.), and run at 4°. The column was calibrated from the elution volume of Blue dextran. All other protein markers were chromatographed with a volume equal to that used for the protein samples. Samples of neurostenin to be chromatographed were twice precipitated and either solubilized from a precipate state into the same buffer used to equilibrate the columns or dialyzed against the buffer used for the column 2–4 h prior to use. The sample to volume bed ratio was less than 1:20.

Sucrose gradient centrifugation. Centrifugations were carried out at 4° using Spinco equipment. For zone sedimentation, linear 3–30% (w/v) sucrose gradients were prepared in nitrocellulose tubes; the gradient device (Buchler Co.) was designed to prepare three simultaneous and equal gradients of 30 ml each. The linearity of sucrose gradients was verified occasionally with ATP dissolved in the diluted sucrose solution or with Blue dextran 2000 dissolved in the higher sucrose concentration solution and read at 260 nm or 620 nm, respectively. Each gradient was overlaid with 2 ml of protein solution in Buffer A or B and centrifuged at 4° for 18 h, at

25000 rev./min in a SW 25.1 rotor which was decelerated without breaking. Samples were collected from the bottom of the gradient in 1.5-ml fractions and assayed for protein and ATPase activity. Protein markers were run under identical conditions using similar volume samples.

Viscometry. Viscometric determinations were performed in a Cannon-Ubbelohde dilution viscometer, size 50 at 22 ± 1° with solvent outflow times of 254–258 sec.

Protein bound nucleotides. The bound nucleotides were determined spectrophotometrically after release by deproteinization with 5 % $HClO_4$ at 4°. A value of ε (260 nm) of $13.9 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ was used. The values were corrected for absorption at 280 nm. Free ATP in the solution was removed with AG 1-X4 prior to deproteinization[7,8].

Protein determination. Protein was determined by the procedure of LOWRY *et al.*[9]. The standard curve was prepared from bovine serum albumin.

[^{14}C]ATP exchange. The method is essentially that of KUEHL AND GERGELY[10] and described with its modification elsewhere[2].

Antisera. Antibodies to bovine brain neurostenin were raised in individual rabbits by weekly intramuscular injections. The first injection consisted of 4 mg of protein homogenized in 2.5-ml adjuvant, complete. The animals were boosted with intramuscular injection of 2–4 mg of protein and bled after 3 weeks.

Immunodiffusion studies. These were carried out with the Gilman apparatus by the Ouchterlony technique. The plates were prepared with 1 % agar, 0.2 M KCl, and 0.04 M sodium pyrophosphate buffer (pH 8.0) and 0.1 % NaN_3. The bands were allowed to develop for 2–4 days at room temperature and then for 6–8 days in the cold room.

Disc electrophoresis. Gel electrophoresis was performed on single 7.5 % acrylamide gels containing deionized 8 M urea. The samples were run at 4° on a Canalco electrophoresis apparatus. First a current of 1 mA/gel was applied for 45 min to layer the protein at the top surface of the gel followed by a current of 4–5 mA/gel until 15 min after the tracking dye passed out of the gels (total 65–70 min). The buffer was 5 mM Tris glycine (pH 8.2). The gels were stained for 45–60 min in 1 % Amido Schwartz in 7 % acetic acid and destained by diffusion overnight in 7 % acetic acid. 75–100 μg of protein per gel was used. Recrystallization from acetone of the acrylamide and Bis improved the resolution of the bands.

ATPase assays. The assays were carried out at 37° in 0.03 M imidazole–HCl (pH 6.8)–0.06 M KCl–$5 \cdot 10^{-4}$ M ATP–$1 \cdot 10^{-4}$ M ouabain–$1 \cdot 10^{-3}$ M Mg^{2+} (unless otherwise indicated); 0.05–0.1 mg of protein in a final volume of 1 ml was used. The assays were terminated by addition of 0.4 ml 20 % trichloroacetic acid. The released P_i was determined by the method of MARSH[11] as previously modified[2].

Amino acid analysis. The proteins were dialyzed for 48 h against several changes of distilled water containing thymol and lyophilized. 2–3 mg of dried protein were suspended in 2 ml of 6 M HCl, frozen at −40°, the tubes flushed with N_2 and sealed. Hydrolysis was carried out at 118° for 22 h. The hydrolysates were dried under reduced pressure and taken up in 0.32 M $HClO_4$ solution from which suitable aliquots were analyzed. The amino acids were determined in a Beckman amino acid analyzer Model 120C using a two column system; one for the acidic and neutral amino acids and the other for the basic amino acids. The use of a range expansion card permitted a 10-fold increase in the sensitivity of the instrument. The position of the 3-methylhistidine was verified by standards to which the authentic compound had been added.

The elution time was 210 min. The buffer used was 0.2 M sodium citrate (pH 4.32).

Boundary sedimentation. Sedimentation velocity of the neurin isolated from whole bovine brain was determined in a Spinco Model E analytical ultracentrifuge. The protein was dissolved in 0.2 mM ascorbate–0.2 mM ATP (pH 7.2); concentration of protein was 4 mg/ml. It was run in a 12 mm, 2° sector Kel-F cell. Sedimentation coefficient was measured from the displacement of the peak of the Schlieren pattern.

P_i release during polymerization. The neurin separated from neurostenin by zone centrifugation was polymerized by diluting the KI content from 0.6 M to 0.1 M. The protein was pelleted by ultracentrifugation at 100000 × *g* for 3 h and depolymerized in 0.2 mM ATP–0.2 mM ascorbate (pH 7.2). KCl and $MgCl_2$ was added to a final concentration of 0.1 M and 1 mM, respectively, and samples were taken at 1 h and 16 h for determination of P_i release.

RESULTS

The preparations of neurostenin are somewhat unstable; the proteins tend to aggregate and sediment. The larger aggregates were usually removed by centrifugation at 35000 × *g* for 15 min. The stability of the enzyme activity of neurostenin obtained from rat and cat brain stored at 4° in 0.05 M Tris–0.6 M KCl (pH 7.2) or at −20° in 50 % (v/v) glycerin was evaluated. The ATPase activity measured over a period of 2 weeks fell about 20–30 % under both conditions. Consequently all experiments were usually conducted within one week of preparation of protein and storage was maintained at 4° in the original buffer (0.05 M Tris–0.6 M KCl). The yield of neurostenin varied from preparation to preparation. The final amount of protein obtained

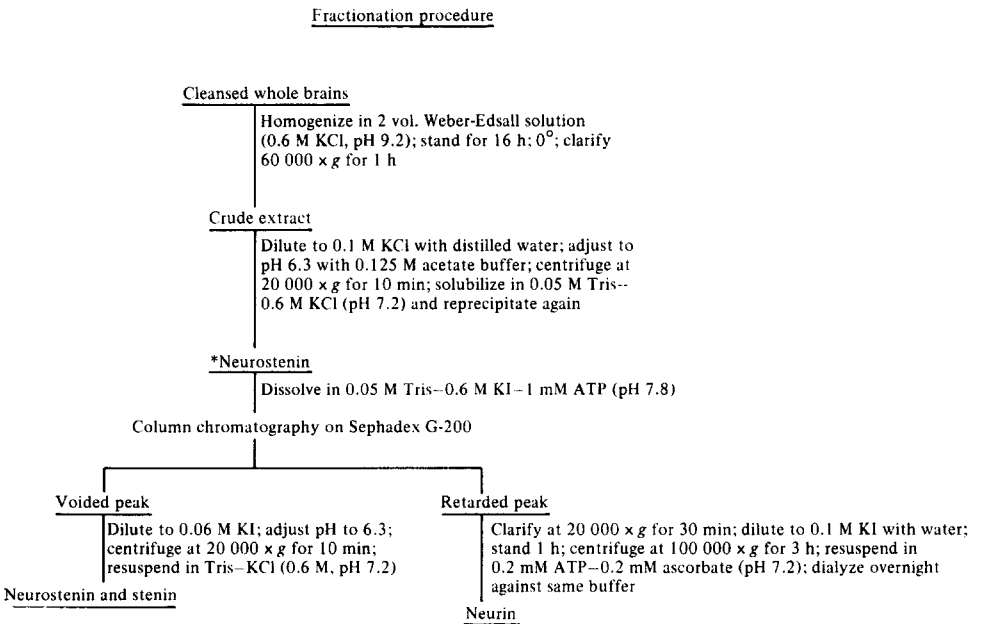

Fig. 1. Outline of procedure for purification of neurostenin and neurin. The protein at this step was also used to separate neurin from stenin on sucrose gradients.

from bovine brain usually represented about 1 % of the total brain proteins; the yield obtained from cat or rat brain was somewhat higher and ranged between 1 to 2 % of the total brain proteins.

Sephadex G-200 chromatography. Fig. 1 presents an outline of the procedure used for the preparation of neurostenin and neurin. These separations provided neurin for the experiments described below. A typical elution pattern is represented in Fig. 2. Between 70 and 80 % of the protein applied to the G-200 column was recovered. Because neurostenin is insoluble at low ionic strength, gel filtration was always performed with high ionic strength buffers. When 0.6 M KCl was used in the presence of ATP only one protein peak emerged from the column. This peak always emerged in the void volume and no retarded proteins were found. The protein retained its (Mg^{2+}–Ca^{2+})-stimulated ATPase activity. It was reasoned that if the separation of the stenin and neurin components is achieved, the neurin would be polymerized and excluded from the gel emerging with stenin in the void volume. Attempts were made to separate these two proteins using KI instead of KCl. It is known that KI at a concentration of 0.6 M would depolymerize muscle actin forming G-actin[12] and

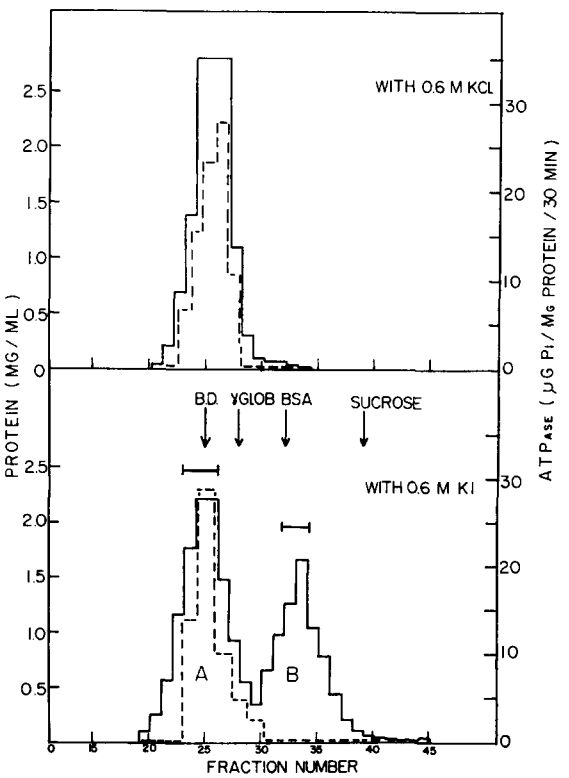

Fig. 2. Gel filtration of neurostenin on Sephadex G-200. The columns (2.5 cm × 45 cm) were equilibrated and eluted with buffer A (upper half) or with Buffer B (lower half). 10–20 mg of protein in 5–10 ml of Buffer A or B were applied to the columns. Temp., 4°; flow rate approx. 15 ml/h; fractions of 2 ml were collected. The arrows indicate the elution positions of blue dextran (B.D.), γ-globulin (γGLOB), bovine serum albumin (BSA) and sucrose. The solid line indicates protein concentration. The broken line indicates Mg^{2+}-stimulated ATPase activity. The fractions were dialyzed against 0.6 M KCl–0.05 M Tris (pH 7.2) prior to assay. The fractions between the horizontal bars were pooled and used for further studies.

furthermore, in the presence of 1 mM ATP will preserve its property to repolymerize[13]. G-actin would therefore separate from myosin on Sephadex G-200. Under these conditions, neurostenin did form two protein peaks. One peak, which emerged in the void volume, still contained both Mg^{2+}- and Ca^{2+}-stimulated ATPase activity. A second protein peak, however, emerged behind the position of the bovine albumin marker. This second peak was devoid of ATPase activity and when tested with muscle myosin presented typical actin-like characteristics (neurin) (Tables I–IV).

Sucrose gradient centrifugation. With this method the separation correlated with the results obtained with chromatography on Sephadex G-200. When the ultracentrifugation was carried out on 3–30 % sucrose gradients buffered with 0.6 M KCl only one band moved into the gradient with a shoulder that extended to the top of the gradient (Fig. 3). This protein band contained Mg^{2+}- and Ca^{2+}-activated ATPase activity. The peak moved down to a position calculated to be 15 % sucrose (0.44 M). If 0.6 M KI was used instead of KCl, neurostenin dissociated into two separate bands, of which only the heavy band possessed ATPase activity (Fig. 3, Band A). The peak

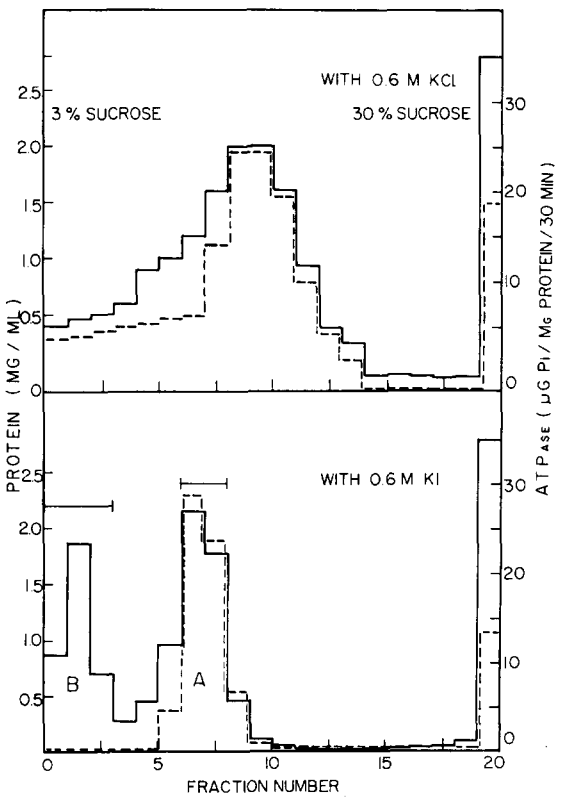

Fig. 3. Zone sedimentation of neurostenin in sucrose gradients. Sedimentation is from left to right. The gradients were prepared in Buffer A (upper half) or Buffer B (lower half). 5–10 mg of protein in 2 ml of Buffer A or B was applied to each 30 ml gradient. Sedimentation time was 18 h at 25000 rev./min in a SW 25.1 rotor (90000 × *g*), at 4°. Samples were collected from the bottom of the gradient in 1.5 ml fractions and dialyzed against 0.6 M KCl–0.05 M Tris (pH 7.2) prior to assay. The solid line indicates protein concentration. The broken line indicates Mg^{2+}-stimulated ATPase activity. The fractions between the horizontal bars were pooled for further studies.

of this band sedimented to a position calculated to be 0.35 M sucrose. It probably contained both stenin and undissociated neurostenin. The second lighter band (Fig. 3, Band B) equilibrated at 0.15 M sucrose. It was usually diluted to bring the concentration of KI to 0.1 M, conditions for actin polymerization. Ultracentrifugation resulted in the recovery of a protein pellet which was dissolved in 0.2 mM ascorbate and 0.2 mM ATP and clarified of aggregated material by centrifugation for 20 min at 75000 × *g*. This protein (neurin) was used in the studies described below. 75–85 % of the total proteins applied to the gradient were recovered in the two bands. The rest was found at the bottom of the tubes as the aggregated protein.

Activation of muscle myosin Mg^{2+}-ATPase by neurin. The neurin separated from neurostenin by either chromatography on Sephadex G-200 or sucrose gradients did activate the Mg^{2+}-ATPase of muscle myosin approx. 4–5 fold (Table I). The Ca^{2+}-activated ATPase was either increased or remained unaltered. In our hands varied responses were also obtained when muscle actin was mixed with muscle myosin.

Protein bound nucleotide content. Neurin prepared from neurostenin contained bound nucleotide which was released upon addition of perchloric acid. The values obtained varied from preparation to preparation due possibly to the presence of denatured protein or to contamination by other proteins. The highest value obtained

TABLE I

ACTIVATION OF MUSCLE MYOSIN Mg^{2+}-ATPase ACTIVITY BY BRAIN NEURIN

The incubation medium contained 0.03 M imidazole-HCl buffer (pH 6.8)–0.06 M KCl–$5 \cdot 10^{-4}$ M ATP–10^{-3} M Mg^{2+}–10^{-4} M ouabain and 0.01–0.05 mg of each protein, final volume 1 ml. Incubation time was 30 min at 37°. The ATPase activity was calculated on the basis of the amount of myosin in the assay medium. The neurin had no ATPase activity. (a) Neurin separated from neurostenin by gel filtration on Sephadex G-200. (b) Neurin separated from neurostenin by zone centrifugation on sucrose gradients. *n* = number of preparations.

Expt.	(*μg P_i released per mg protein per 30 min* (± *S.D.*))		*Activation factor*	*n*
	Myosin	*Myosin + neurin*		
a	22.8 ± 8.0	99.2 ± 9.1	4.3	5
b	20.3 ± 5.8	96.6 ± 14.6	4.7	4

TABLE II

NUCLEOTIDE BOUND TO BRAIN NEURIN SEPARATED FROM NEUROSTENIN BY ZONE SEDIMENTATION ON SUCROSE GRADIENTS

The protein bound nucleotide was released by deproteinization with 5 % $HClO_4$ at 4°. The nucleotide concentration in the supernatant was determined spectrophotometrically. ε (260 nm) = $13.9 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$. Free ATP was removed from the protein solution with AG 1-X4 Cl^- prior to deproteinization.

Protein prep.	*Moles of nucleotide per 50000 g of neurin*
1	0.78
2	0.54
3	0.98

was 0.98 mole per 50000 g of neurin (Table II). In muscle actin, the ratio of nucleotide to protein on a mole to mole basis is probably 1 (ref. 14) but considerable variation in this ratio has been reported[10, 14–18].

The absorption spectrum of the released nucleotide is presented in Fig. 4. It shows a maximum at 260 nm.

[^{14}C]ATP exchange of neurin isolated from neurostenin. The results obtained showed that ATP bound to neurin exchanges with free [^{14}C]ATP. The values of the three most active preparations were in the range of 0.5 mole of nucleotide per 50000 g of protein.

P_i release from neurin during polymerization. The neurin separated from neurostenin by zone centrifugation could be polymerized by diluting the KI of the solution from 0.6 to 0.1 M; upon centrifugation, 60–85 % of the protein was found to sediment and was solubilized in a low ionic strength buffer containing 0.2 mM ATP and 0.2 mM ascorbate. Upon addition of 0.1 M KCl and 1 mM Mg^{2+}, the protein was repolymerized as revealed by the release of P_i from bound ATP. After 1 h 0.4–0.5 mole

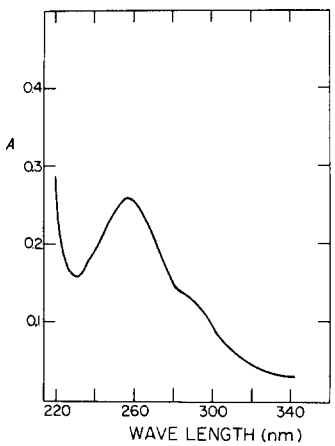

Fig. 4. Ultraviolet spectrum of the bound nucleotide released from neurin by 5 %$HClO_4$. The absorption spectrum was determined at pH 1 in a Bausch and Lomb Spectronic 505 instrument. The free ATP was removed from the protein solution by addition of AG 1-X_4 Cl^- resin prior to deproteinization.

TABLE III

RELEASE OF P_i FROM BRAIN NEURIN DURING POLYMERIZATION

The protein was separated from neurostenin by zone centrifugation on sucrose gradients. The reaction mixture contained 1–2 mg of protein per ml, 0.1 M KCl, 1 mM Mg^{2+}, 0.2 mM ATP and 0.2 mM ascorbate (pH 7.2). Incubation time 16 h at 4°.

Protein prep.	*Moles of P_i per 50000 g of neurin*
1	0.81
2	1.06
3	1.03
4	0.92

of P_i was released. After 16 h the amount of P_i released was 0.81–1.06 mole/50000 g of protein (Table III).

Viscosity of neurin during polymerization. The relative viscosity of the band of neurin separated on Sephadex G-200 increased with dilution of its KI content from 0.6 M to 0.1 M. As it emerged from the column, at a concentration of 1.5–2 mg of protein per ml, the relative viscosity was 1.08; dilution of the protein solution to obtain a KI concentration of 0.1 M raised the relative viscosity to 1.20 after 1 h. When the protein was pelleted by ultracentrifugation and dissolved in a low ionic strength buffer (0.2 mM ascorbate–ATP, pH 7.2) the relative viscosity was 1.04, a value similar to that observed with neurin as it emerged from the gel filtration columns.

Disc electrophoresis. The results achieved with disc electrophoresis performed on single polyacrylamide gels are displayed in Fig. 5. Bovine brain neurostenin (gel

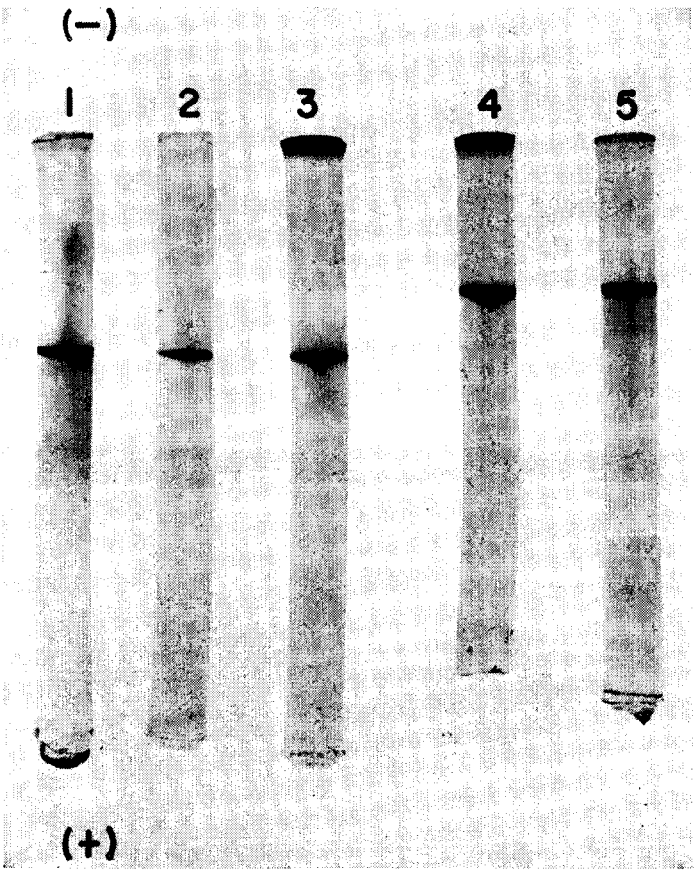

Fig. 5. Acrylamide gel electrophoresis of neurin and neurostenin. Electrophoresis was performed on 7.5% single acrylamide gels containing 8 M urea. 75–100 μg of protein in 10% sucrose was applied to the top of each gel. A current of 1 mA/gel was applied for 45 min followed by a current of 4–5 mA/gel. Total time 115 min at 4°. Gel 1, neurin separated from whole brain by the acetone procedure; Gel 2, neurin separated from neurostenin on a sucrose gradient; Gel 3, neurin separated from neurostenin on Sephadex G-200; Gel 4, cat brain neurostenin; Gel 5, bovine brain neurostenin.

No. 5) and cat brain neurostenin (gel No. 4) moved into the gel essentially as one band with some material sedimented on the top probably as a result of aggregation. Neurin obtained directly by the acetone method, when polymerized by KCl and Mg^{2+}, presented several bands (not shown). This protein, when depolymerized and dialyzed against 8 M urea and run in a gel containing 8 M urea, then moved as a

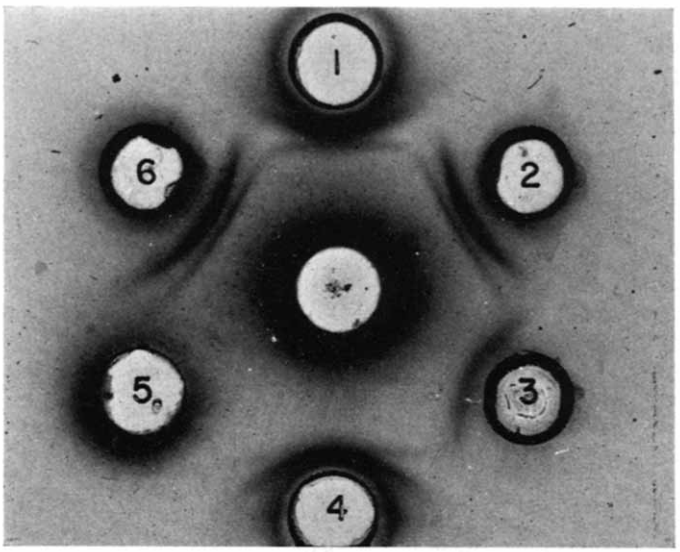

Fig. 6. Immunodiffusion reactions of neurostenin from bovine, rat and cat brain with antiserum to bovine brain neurostenin. Center well contained rabbit antiserum to bovine neurostenin. Wells 1 and 4 contained bovine neurostenin. Wells 2 and 6 contained cat neurostenin. Well 3 contained rat neurostenin. Well 5 contained cat muscle actomyosin.

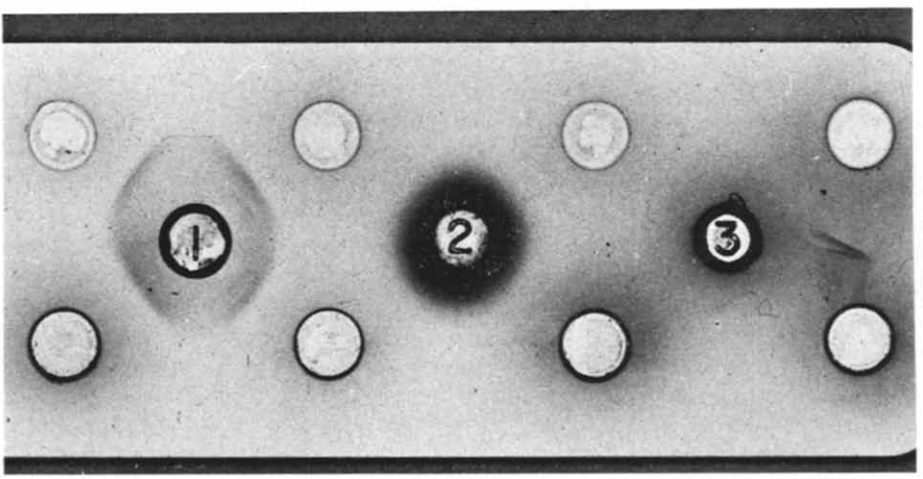

Fig. 7. Immunodiffusion reaction of antibodies against bovine brain neurostenin with cat brain stenin, cat muscle myosin and bovine aorta actomyosin. The top and bottom rows of wells all contained rabbit antiserum to bovine brain neurostenin. Well 1 contained cat brain stenin. Well 2 contained cat muscle myosin. Well 3 contained bovine aorta actomyosin.

single band (gel No. 1) with a depth of penetration similar to that obtained by neurin separated from neurostenin either by gel filtration on Sephadex G-200 (gel No. 3) or on sucrose gradients (gel No. 2).

Immunodiffusion studies. The diffusion of neurostenin into the agar gel was facilitated by the presence of 0.2 M KCl and 0.04 M pyrophosphate. Neurostenin from bovine brain reacted with its specific antibody giving two bands (Fig. 6). The antiserum cross reacted with neurostenin isolated from cat and rat brain. It did not react with cat muscle actomyosin. The antibodies against neurostenin from bovine brain formed a single band with cat stenin but did not cross-react with myosin from cat muscle or with actomyosin from bovine aorta (Fig. 7).

Neurin from bovine brain and also the colchicine-binding protein (microtubular protein) from bovine brain formed one band against the antiserum to bovine

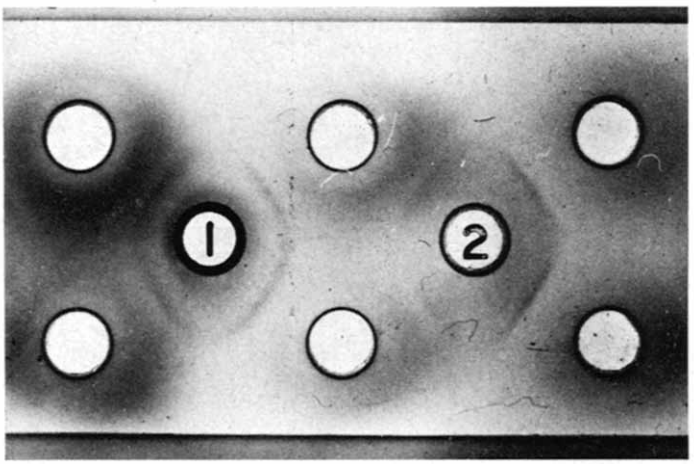

Fig. 8. Immunodiffusion reactions of antibodies to bovine brain neurostenin with bovine brain neurin and colchicine-binding protein. The top and bottom rows of wells contained rabbit antiserum to bovine brain neurostenin. Well 1 contained bovine brain neurin. Well 2 contained bovine brain colchicine-binding protein.

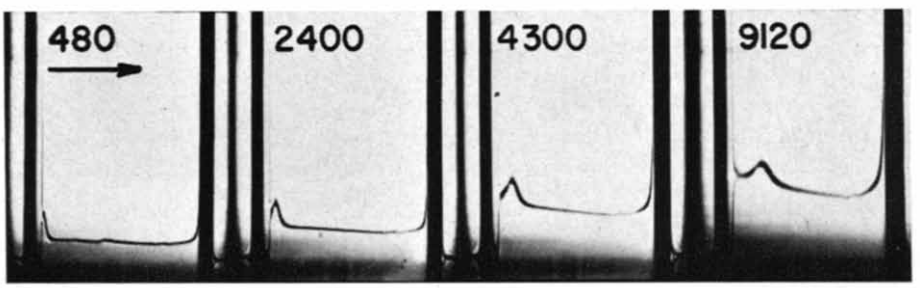

Fig. 9. Boundary sedimentation of bovine brain neurin. The protein was depolymerized in 0.2 mM ascorbate–0.2 mM ATP and aggregated protein removed by centrifugation at 105000 × *g* for 20 min. The protein concentration was 4 mg/ml. Pictures were taken at the time in seconds indicated in photographs after reaching maximum speed of 50740 rev./min. The phase-plate angles were 70°, 50°, 45° and 30°, respectively. Temperature was 9.15°. The arrow indicates direction of sedimentation. $s_{20,w} = 2.8$.

neurostenin (Fig. 8). This seems to indicate that common antigenic properties exist between the protein isolated from the microtubules and neurin.

3-Methylhistidine. The neurin contained 3-methylhistidine. The average of three determinations were 0.33 mole/50000 g of protein. Microtubular protein did not contain 3-methylhistidine.

Boundary sedimentation. The sedimentation velocity ultracentrifugation analysis of the purified brain neurin showed a single, major, symmetrical boundary with a sedimentation coefficient of 2.8 S (Fig. 9). A small component which sedimented at a much faster rate was also noted. It probably represents aggregated protein.

DISCUSSION

Neurin isolated from neurostenin preparations or obtained directly from acetone-dried brain tissue presented characteristics typical of muscle actin[17].

First, neurin can be polymerized to a fibrillar state, and sedimented by ultracentrifugation. During the process of polymerization, P_i is liberated in the medium. Second, neurin and muscle myosin combine at high ionic strength to form an actomyosin-like complex characterized by an increase in viscosity which is reversibly decreased in the presence of added ATP[2]. Third, neurin can stimulate the Mg^{2+}-ATPase activity of myosin when the two proteins are mixed at low ionic strength. Further similarities between neurin and actin from muscle include the exchange of bound nucleotide with free ATP, the elution characteristics of neurin by chromatography on Sephadex G-200, and the presence of 3-methylhistidine. The ability to isolate neurin from neurostenin when KI is substituted for KCl in a manner similar to that described for actomyosin[13] adds another parameter to the similarity between neurin and muscle actin.

3-Methylhistidine was repeatedly detected although its level was less than that reported for rabbit actin[19]. Our procedure, however, detected lesser amounts of this amino acid in rabbit actin than the highest amounts reported in the literature (1 mole/47000 g of protein)[20]. We could not detect evidence for the presence of ε-*N*-monomethyllysine which has been reported in amoeba actin[21]. We also could not detect any 3-methylhistidine in the colchicine-binding protein[6].

Neurin dissociated from neurostenin (Table I) or isolated directly from brain[2] stimulated the Mg^{2+}-ATPase activity of muscle myosin. Neurin in this respect is less effective than muscle actin in activating myosin. More neurin than myosin, on a mg basis, is required to achieve maximum Mg^{2+}-ATPase activity of myosin (Table IV). An excess of neurin over myosin for activation of myosin may be required as compared with actin from rabbit striated muscle where the reverse is true[3,22]. Such an actin to myosin relationship has been described for the analogous proteins isolated from slime mold. ADELMAN AND TAYLOR[23] estimated the myosin to actin raito (mg basis) of crude slime mold actomyosin to be between 1:1 and 1:3.

In preliminary studies, neurin, under conditions which favor polymerization, formed fibers similar to muscle actin as revealed by negative staining in the electron microscope.

The molecular weight of neurin requires further study. Its elution from Sephadex G-200 just following bovine serum albumin would place its molecular weight in the 50000 range. This assessment is supported by a comparison of its nucleotide content

and amino acid composition with that of muscle actin. Its sedimentation velocity in the ultracentrifuge suggests that its molecular weight may be lower than that of muscle actin. Its s value of 2.8 S is the same as that reported for amoeba actin (molecular weight 39500)[21].

TABLE IV

ACTIVATION OF MUSCLE MYOSIN ATPase BY INCREASING CONCENTRATIONS OF NEURIN

See Table I for incubation conditions.

Neurin (*mg/ml*)	(*μg P_i per mg myosin per 30 min*)	
	Mg^{2+}-ATPase	*Ca^{2+}-ATPase*
0.1	12.5	157
0.5	35	190
1.0	62	253
2.5	40	237
5.0	127	249
7.5	157	257
10.0	175	275

The neurin isolated by the present KI procedure stimulated the Mg^{2+}-activated ATPase activity of muscle myosin to a lesser degree than did neurin isolated directly from brain tissue[2]. The KI or thiosulfate may very well have partially inactivated this property. This may also explain the rather low exchange of bound ATP with [^{14}C]ATP. The ability to polymerize and to release P_i upon polymerization did not appear to be inhibited.

The immunodiffusion studies suggest that the neurostenin from bovine brain stimulates the production of antibodies which are less species specific than those stimulated by neurostenin from rat or cat brain[2]. In the previous study the antibodies to the latter two proteins did not cross react whereas in the present study the bovine brain protein antiserum did produce immunodiffusion bands with neurostenin from the three species. It is also possible that the bovine neurostenin has greater antigenic activity than the homologous protein from cat or rat brain. The antiserum to bovine neurostenin formed two bands with the complete antigen but only one band with neurin or stenin alone. This would suggest that antibodies to each protein may have been formed.

The presence in brain of actomyosin-like, actin-like and myosin-like proteins does not prove that these proteins perform a contractile function. However, since this system is capable of chemomechanical transduction it is very probable that they do function in a manner which does utilize this ability to convert chemical to mechanical energy. One such function would subserve the saltatory transport of materials and particles from the perikaryon down the axon to the nerve endings in association with the microtubules[24]. In support of this possibility is the demonstration that brain microtubular protein (tubulin, colchicine-binding protein) does possess actin-like properties[25]. It is also very probable, however, that microtubular protein and neurin are different proteins. It has been suggested that actin and colchicine-binding protein may be phylogenetically[24] related and this may also be suggested for neurin. This is supported by the demonstration of common antigenic properties between neurostenin

and colchicine-binding protein. Other evidence in this direction is the report that colchicine in low concentration reduces the rate of viscosity drop in mixtures of heart actomyosin and ATP[26].

The isolation of neurostenin from the nerve ending subfraction of brain tissue[27] raises the speculation that this protein may function in the movement of nerve endings in association with plasma membrane and neurofilaments[28]. Its presence in the synaptosomal fraction also suggests the speculation that this enzyme, which is activated by Ca^{2+}, functions in the release of putative transmitter agents stored in the vesicles in response to electrical stimuli. This speculation is prompted by the hypothesis of KATZ AND MILEDI[29] which proposes that depolarization of the presynaptic membranes results in an increase in its permeability to Ca^{2+} which then functions in transmitter release. It is further supported by the recent report by BLAUSTEIN[30] that preganglionic stimulation of sympathetic ganglia increases Ca^{2+} uptake by the ganglion.

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REFERENCES

1 S. PUSZKIN, E. PUSZKIN, D. D. CLARKE AND S. BERL, *Science*, 161 (1968) 170.
2 S. BERL AND S. PUSZKIN, *Biochemistry*, 9 (1970) 2058.
3 A. SZENT-GYORGYI, *Chemistry of Muscle Contraction*, New York, N.Y., Academic, 1951, p. 151.
4 M. E. CARSTEN AND W. F. H. M. MOMMAERTS, *Biochemistry*, 2 (1963) 28.
5 E. G. RICHARDS, C. S. CHUNG, D. B. MENZEL AND H. S. OLCOTT, *Biochemistry*, 6 (1967) 528.
6 R. C. WEISENBERG, G. G. BORISY AND E. W. TAYLOR, *Biochemistry*, 7 (1968) 4466.
7 T. HAYASHI AND K. TSUBOI, *Fed. Proc.*, 19 (1960) 256.
8 A. ASAKURA, *Arch. Biochem. Biophys.*, 92 (1961) 140.
9 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
10 W. M. KUEHL AND J. GERGELY, *J. Biol. Chem.*, 244 (1969) 4720.
11 B. B. MARSH, *Biochim. Biophys. Acta*, 32 (1959) 357.
12 F. B. STRAUB, *Studies Inst. Med. Chem. Univ. Szeged.*, 2 (1942) 3.
13 A. G. SZENT-GYORGYI, *Arch. Biochem. Biophys.*, 31 (1951) 97.
14 J. GERGELY, in J. GERGELY, *Biochemistry of Muscle Contraction*, Boston, Mass., Little Brown and Co., 1964, p. 119.
15 A. MARTONOSI AND M. A. GOUVEA, *J. Biol. Chem.*, 236 (1951) 1345.
16 A. MARTONOSI, M. A. GOUVEA AND J. GERGELY, *J. Biol. Chem.*, 235 (1960) 1700.
17 M. BARANY, B. NAGY, F. FINKELMAN AND A. CHRAMBACH, *J. Biol. Chem.*, 256 (1961) 2967.
18 R. C. STROHMAN AND A. J. SAMORODIN, *J. Biol. Chem.*, 237 (1962) 363.
19 M. ELZINGA, *Biochemistry*, 9 (1970) 1365.
20 A. M. ASATOOR AND M. D. ARMSTRONG, *Biochem. Biophys. Res. Commun.*, 26 (1967) 168.
21 R. R. WEIHING AND E. D. KORN, *Biochemistry*, 10 (1971) 590.
22 Y. TONOMURA, S. TOKURA AND K. SEKIYA, *J. Biol. Chem.*, 237 (1962) 1074.
23 M. R. ADELMAN AND E. W. TAYLOR, *Biochemistry*, 8 (1969) 4964.
24 F. O. SCHMITT AND F. E. SAMSON, *Neurosci. Res. Program Bull.*, 6 (1968) 157.

25 S. PUSZKIN AND S. BERL, *Nature*, 225 (1970) 558.
26 E. H. BARANY AND A. PALAIS, *Acta Physiol. Scand.*, 25 (1952) 174.
27 S. PUSZKIN, W. J. NICKLAS AND S. BERL, *Fed. Proc.*, 30, No. 2 (1971) 504.
28 N. K. WESSELS, B. S. SPOONER, J. F. ASH, M. O. BRADLEY, M. A. LUDVENA, E. L. TAYLOR, J. T. WRENN AND K. M. YAMADA, *Science*, 171 (1971) 135.
29 B. KATZ AND R. MILEDI, *Proc. R. Soc. London Ser. B (Biol. Sci.)*, 167 (1967) 23.
30 M. P. BLAUSTEIN, *Science*, 172 (1971) 391.