PHYSICAL AND ENZYMATIC PROPERTIES OF MYOSIN FROM PORCINE BRAIN

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ABSTRACT Porcine brain myosin is a cytoplasmic protein similar to, but distinct from, its muscle counterpart. It has a high K^+-ATPase activity at high ionic strength in EDTA and a low Mg^{+2}-ATPase activity that is activated fivefold by either porcine brain or rabbit skeletal muscle actin. The molecule consists of three classes of subunits, with molecular weights of ~195,000, 19,000, and 16,000. Brain myosin contains less glutamic acid, less lysine, and more threonine, serine, proline, and tyrosine than skeletal muscle myosin. The brain myosin extinction coefficient at 278 nm is 0.810 cm^2/mg. Hydrodynamic studies yield an $s^0_{20,w}$ of 4.95S, a $D^0_{20,w}$ of 1.07×10^{-7} cm^2/s for brain myosin, and indicate that the molecules aggregate at high ionic strength. The molecular weight of the molecule, as calculated from extrapolation of $D_{20,w}/S_{20,w}$ to zero concentration, is 444,000. The intrinsic viscosity of brain myosin is 0.191 ml/mg. These data are consistent with a highly asymmetric molecular species. Circular dichroism spectroscopy indicates that brain myosin is 58–60% α-helical in the presence of Ca^{+2} ions, and that removal of Ca^{+2} causes a small change in the spectrum.

An actomyosin-like protein was first discovered in brain tissue in 1968 by Puszkin et al., and since then its components have been isolated and examined. Chick brain myosin is similar to muscle myosin in its subunit composition, high ionic strength ATPase activity, and ability to interact with muscle F-actin (Burridge and Bray, 1975). However, when dialyzed extensively against buffers of low ionic strength, chick brain myosin forms paracrystalline aggregates, and upon partial proteolysis yields peptides distinct from those of skeletal myosin. Kuczmarski and Rosenbaum (1979) have reported that chick brain myosin antibodies will not crossreact with skeletal muscle myosin, nor will chick brain myosin form copolymers with skeletal muscle myosin. These properties identify avian brain myosin as similar to, but distinct from, vertebrate muscle myosin.

The unique properties that distinguish brain myosin are likely to be important in the mechanism by which the protein functions in the cell. Contractile proteins are postulated to play a role in such processes as nerve extension, axoplasmic transport, secretion, and cell surface receptor modulation (Trifaro, 1978). By analogy with muscle, the interaction of actin and myosin leading to hydrolysis of ATP and to the production of mechanical work is a likely basis for cellular movement. Details of the mechanism(s) involved are unknown. However, the intracellular state of myosin should be a crucial element in any proposed mechanism.

The size and shape of the myosin molecule and its hydrodynamic behavior will be important in determining how myosin is assembled within the cell. A knowledge of the structure of mammalian brain myosin is prerequisite to an understanding of the molecular basis for its

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action. The physicochemical properties of brain myosin have not been studied, nor have extensive physicochemical studies of any other nonmuscle myosin been reported.

The present study examines the biophysical characteristics and some of the enzymatic properties of myosin from porcine brain and reveals some interesting differences between brain and muscle myosins.

EXPERIMENTAL PROCEDURES

Reagents

DTT,[1] PMSF, EGTA, ATP, SDS, bovine plasma albumin, Fiske-Subbarow reducer, acid molybdate solution, and Tris were products of Sigma Chemical Company, St. Louis, Mo. Diethylpyrocarbonate crosslinked protein molecular weight markers for electrophoresis were from BDH Chemicals, Ltd., Poole, England. Electrophoresis purity grade Coomassie brilliant blue R-250, acrylamide, *bis*-acrylamide, ammonium persulfate, and *N,N,N',N'*-tetramethylethylenediamine were obtained from Bio-Rad. EDTA, trichloroacetic acid, Folin Ciocalteau 2N phenol reagent, and silicotungstic acid were obtained from Fisher Scientific Co., Newburgh, N. Y.; ultrapure ammonium sulfate was from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y. All other chemicals were reagent or enzyme grade. House deionized water was filtered through activated charcoal and through mixed bed ion-exchange columns (Continental Water Company, El Paso, Texas) immediately before use. The conductivity of water was less than that of a solution containing 0.1 ppm of KCl.

Porcine Brain Myosin

A procedure similar to that used by Pollard et al. (1974) for platelet myosin was used for purification. Brains were obtained from a local sausage company within 60 min of the death of the hogs and placed in ice-cold $(NaCl)_{150}(7.0)$.[2] All steps were performed at 4°–7°C. About 500 g of brain (from 4–6 animals) were dissected free of overlying membranes and blood vessels, finely chopped, and homogenized with four strokes of a motor-driven homogenizer in one liter of ice-cold extraction buffer, $(KCl)_{500}(EDTA)_{2.0}(streptomycin\text{-}SO_4)_{0.067}(DTT)_{3.0}(PMSF)_{1.0}(imidazole)_{20.0}(7.0)$. After stirring slowly for 15 min, the supernate was collected by centrifugation (24,000 *g*, 60 min, 5°C) and dialyzed against $(MgCl_2)_{25}(imidazole)_5(PMSF)_{1.0}(6.3)$ for 7 h. The precipitated actomyosin was collected by centrifugation (24,000 *g*, 90 min, 5°C) and suspended in $(KCl)_{600}(ATP)_{0.5}(MgCl_2)_{0.1}(DTT)_{1.0}(streptomycin\text{-}SO_4)_{0.067}(PMSF)_{1.0}(imidazole)_{20}(7.0)$, buffer A.

An equal volume of 50% saturated ammonium sulfate in $(EDTA)_{10}(7.0)$ was added to the actomyosin, and after stirring 10 min the precipitate was removed by centrifugation (30,000 *g*, 25 min, 5°C). Solid ammonium sulfate was added to the supernate to 60% saturation, and after stirring 20 min the precipitate was collected by centrifugation (30,000 *g*, 25 min, 5°C). This precipitate was suspended in $(KCl)_{600}(ATP)_{25}(MgCl_2)_{1.0}(DTT)_{1.0}(PMSF)_{1.0}(imidazole)_{20}(7.0)$, buffer B, and clarified by centrifugation (100,000 *g*, 30 min, 5°C). The supernate was then chromatographed on a 2.5 × 90 cm Sepharose 4B column immediately after application of 60 ml of buffer B. Elution was carried out at 30 ml/h with either buffer A or $(K_4P_2O_7)_{40}(7.5)$, depending on whether ion-exchange chromatography was to be performed immediately. The K^+(EDTA)-ATPase-containing fractions from the Sepharose column were pooled, and applied to a 1.6 × 15-cm Whatman DE-52 column (Whatman Inc., Clifton, N. J.)

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[1] *Abbreviations used in this paper:* ATPase, adenosinetriphosphatase; DTT, dithiothreitol; EGTA, ethylene glycol *bis*(β-aminoethyl ether)-N, N'-tetraacetic acid; n and r, the number of data points and the correlation coefficient for linear regression, respectively; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; Tris, *tris*(hydroxymethyl)amino methane.

[2] A shorthand notation is desirable for reference to complex aqueous solvent media (Frederiksen and Holtzer, 1968). Such a solvent is designated here by writing the chemical formula (or name) of each component (except water) with its millimolarity as a subscript, followed by parenthetical specification of the pH.

equilibrated with the pyrophosphate buffer. Protein was eluted at 60–70 ml/h in 4-ml fractions with a buffer containing $(EDTA)_{2.0}(streptomycin\text{-}SO_4)_{0.067}(Tris\text{-}Cl)_{40}(7.5)$, and an additional linear salt gradient of 0.0–0.4 M KCl. This procedure, which yields myosin in solution without ATP or P_i, was followed so that myosin $K^+(EDTA)$-ATPase activity could be assayed immediately after chromatography. Myosin was stored at −20°C, either before or after ion exchange chromatography, in $(KCl)_{600}(EDTA)_{40}(DTT)_{1.0}(KH_2PO_4)_{75}(7.2)$ diluted with an equal volume of cold de-aerated glycerol.

Porcine Brain Actin

Porcine brain actin prepared as described by Weir and Frederiksen (1980), was generously supplied by Jerry P. Weir.

Muscle Actin

Muscle actin was prepared from an acetone powder of rabbit skeletal muscle (Noelken, 1962) according to the method of Spudich and Watt (1971).

Gel Electrophoresis

Gel electrophoresis in SDS was performed essentially as described by Weber and Osborne (1969) with modifications as described by Frederiksen (1976). Diethylpyrocarbonate cross-linked protein molecular weight markers covering the ranges 14,300–71,500 and 53,000–265,000 were used as standards.

Protein Concentrations

Protein concentrations were routinely determined by an automated (Technicon autoanalyzer) modification of the method of Lowry et al. (1951) with bovine plasma albumin run concurrently as standard. Micro-Kjeldahl analyses (performed by Galbraith Laboratories, Knoxville, Tenn.) were used to determine the nitrogen in brain myosin solutions for measurement of the extinction coefficient. In these calculations, a nitrogen content of 17.6% for myosin (calculated from amino acid analysis) was used.

ATPase Assays

ATPase assays were performed in one of two ways. The first is essentially that described by Frederiksen (1976) using the Fiske-Subbarow (1925) method for determination of orthophosphate, except that volumes were scaled down by a factor of 2.5–3.0.

The second method is a modification of the Martin-Doty assay (1949) described by Pollard and Korn (1973). In this method, the assay was typically stopped by adding 0.250 ml of protein solution to a mixture of 0.550 ml 2-butanol:benzene (1:1)-0.125 ml $(silicotungstic\ acid)_{14.1}(H_2SO_4)_{1500}$-0.050 ml $(NH_4\text{-}molybdate)_{81}$. The sample was mixed vigorously for 10 s on a Vortex mixer, and the phases allowed to separate. Then, 0.34 ml of the organic phase was added to 0.64 ml of 0.365 M H_2SO_4 in ethanol. Freshly prepared $(SnCl_2)_{20}(HCl)_{462}(H_2SO_4)_{481}$ was added (0.032 ml), and the absorbance at 720 nm determined after mixing. Inorganic phosphate standards were run concurrently with each assay.

Low ionic strength assays were run at 25°C. High ionic strength assays were run in $K^+(EDTA)$ buffer $[(KCl)_{600}(EDTA)_{2.0}(DTT)_{1.0}(PMSF)_{1.0}(Tris\text{-}Cl)_{50}(8.0)]$ at 35°C unless otherwise stated. The ATP concentration in assays was always 1 mM.

Nucleic Acid Determination

Protein solutions were analyzed for the presence of DNA by the diphenylamine method, with type I calf thymus DNA as standard. Solutions were examined for RNA by the orcinol reaction with yeast RNA as standard (Schneider, 1957).

Analytical Ultracentrifugation

Sedimentation runs were made in the Beckman Spinco Model E ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) operated with schlieren optics. Protein solutions were exhaustively

dialyzed against K^+(EDTA) buffer. Dialysate was used for dilutions and as solvent in one compartment of the 12-mm double sector centrifuge cell. Runs were made in the An-H rotor at 20 ± 0.2°C, at 52,000 rpm. Sedimentation coefficients were calculated from plots of ln (distance) vs. time with the usual corrections for solvent viscosity and density. The intrinsic sedimentation coefficient, $s^0_{20,w}$, was found by extrapolation to zero concentration.

Diffusion Analysis

The diffusion coefficient of myosin was determined by the free diffusion method of Gosting (1956) as described by McIntyre et al. (1978). Diffusion proceeded approximately 24 h at 6°C, after which 24–30 fractions were removed from the top of each tube. The myosin concentration of each fraction was determined by assay for K^+(EDTA)-ATPase activity to obtain the distribution profile of the active enzyme. A computer analysis of the data for each particular initial protein concentration and diffusion time yields D at each point and obtains the best D for the entire curve by nonlinear regression. Diffusion coefficients for several standard proteins were determined under identical conditions to obtain a standard curve of D (observed) vs. known $D^0_{20,w}$. This standard curve was used to determine $D_{20,w}$ for each concentration of brain myosin by extrapolation, and $D^0_{20,w}$ was found by extrapolation of $D_{20,w}$ to zero concentration. Standards used, together with their intrinsic diffusion coefficients ($\times 10^{-7}$cm^2/s), were rabbit skeletal muscle myosin (1.19), rat gamma globulins (3.8), bovine plasma albumin (5.94), hen egg white lysozyme (11.2), ribonuclease (11.9), and horse cytochrome *c* (13.0). The distribution profiles were obtained using absorbance at 280 nm for all standard proteins except cytochrome *c*, which was determined by absorbance at 415 nm.

Viscosity

Myosin samples were dialyzed exhaustively and clarified (100,000 *g*, 5°C, 30 min) just before measuring viscosity. Measurements were made at 20°C in Cannon-Ubbelohde semi-micro dilution viscometers. Dialysate was used as solvent. Temperatures were maintained using a Brinkmann Lauda K-2R water bath (Brinkmann Instruments, Inc., Westbury, N. Y.) in conjunction with a Cannon Instruments constant temperature bath (Cannon Instrument Co., State College, Pa.). Protein concentrations were determined before and after dilutions. The protein solutions had flow times 500–75 s greater than solvent.

Circular Dichroism

Circular dichroism spectra were determined by means of a Cary 60 spectropolarimeter equipped with a Model 6002 CD attachment and calibrated with D-camphor-10-sulfonic acid. Myosin concentrations used were 0.5–0.7 mg/ml. Samples were clarified (100,000 *g*, 5°C, 30 min) before use. Solutions and solvents were scanned three times and averaged to obtain the results shown. Results are given in terms of mean residue ellipticity, $[\theta]$, in units of deg · cm^2/decimol. The mean residue weight for brain myosin is 112, as calculated from amino acid analysis. The concentration of EGTA needed to lower the free Ca^{+2} level to $<10^{-7}$ M without significantly changing the Mg^{+2} level was calculated using the EGTA dissociation constants given by Solaro and Shiner (1976). The percentage of α-helix in brain myosin under the various conditions used was calculated from $[\theta]_{222}$ by the method of Chen et al. (1974) and from $[\theta]_{208}$ by the method of Greenfield and Fasman (1969).

Ultraviolet Absorbance Spectra

UV absorbance spectra were obtained with a Hitachi Model 100-60 double-beam spectrophotometer equipped with an 057 X-Y recorder. Matched cuvettes with 1.0 cm path length were used for all measurements. Myosin concentrations were 0.5–1.0 mg/ml; solutions and solvents were clarified before use (100,000 *g*, 30 min, 4°C). Each protein solution, with dialysate as reference, was scanned two or three times at a rate of 60 nm/min over the 190–320 nm range. The extinction coefficient is reported in units of cm^2/mg.

Amino acid analyses were carried out with a Beckman-Spinco 120C amino acid analyzer equipped with an Infotronics Model CRS-12-AB integrator. The Spackman et al. (1958) and Moore and Stein

TABLE I
ANALYSIS OF BRAIN MYOSIN PREPARATION

Fraction	Protein	Specific activity‡	Total activity	Yield
	*(mg)**	*(nmol/min · mg)*	*(μmol/min)*	*(%)*
Extract supernate	7,789	1.0	7.703	100
Low ionic strength precipitate	2,584	1.2	3.080	40
$(NH_4)_2SO_4$ pellet	99	20.1	1.985	26
Sepharose-myosin	4	415.0	1.660	22
DEAE-myosin	2	700.0	1.120	15

*Mg protein/500 gm wet brain.
‡K^+(EDTA)-ATPase activity at 35°C.

(1963) analyzer system was used as described by Shih and Hash (1971). Protein was hydrolyzed in sealed tubes for varying periods in 6 M HCl at 110°C. Tryptophan was determined on the amino acid analyzer after hydrolysis of the protein in the presence of methanesulfonic acid (Moore, 1972).

The partial specific volume was calculated from the amino acid analysis (Cohn and Edsall, 1943).

RESULTS

Isolation of Brain Myosin

The purification of myosin (Table I) consists of extraction, low ionic strength precipitation, ammonium sulfate fractionation, and agarose and ion-exchange chromatography. Although agarose chromatography results in a substantial purification of brain myosin, the myosin peak contains DNA, RNA, and polypeptides with molecular weights corresponding to tubulin and actin. Ion exchange chromatography removes these contaminants. The Sepharose and DE-52 elution patterns are shown in Figs. 1 and 2.

Polyacrylamide gel electrophoresis of the purified protein shows several components (Fig. 3). The relative mobilities and Coomassie blue staining intensities of the heaviest chain and

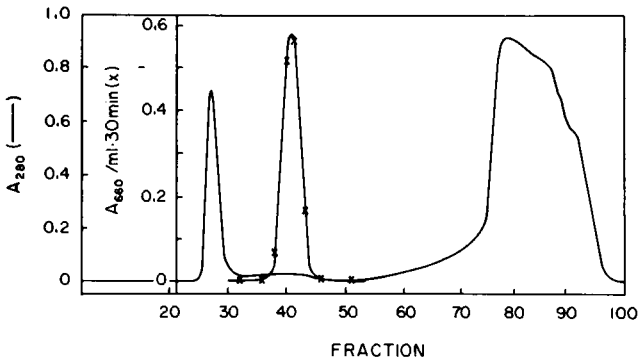

FIGURE 1 Agarose chromatography of the 25–60% ammonium sulfate fraction. 15 ml of the 25–60% ammonium sulfate fraction (c = 7 mg/ml) in buffer B was applied to a 2.5 × 90-cm Sepharose 4B column and eluted at 30 ml/h as described in Experimental Procedures. Fractions of 5 ml each were collected, and analyzed for protein concentration by absorbance at 280 nm (—). The elution position of myosin was determined by assaying aliquots (0.10 ml) of each fraction for K^+ (EDTA)-ATPase activity, represented here by A_{660}/30 min (**).

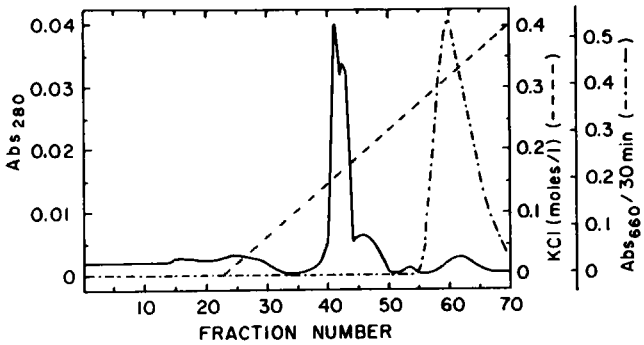

FIGURE 2 Ion exchange chromatography of Sepharose myosin. Sepharose myosin was applied to a 1.6 × 15-cm Whatman DE-52 column either directly after Sepharose chromatography or after storage in glycerol at −20°C as described in Experimental Procedures. Protein was eluted with a linear 0–0.4 M KCl gradient (- - -). Fractions of 4 ml were collected and assayed for concentration by absorbance at 280 nm (—). Aliquots of protein (0.10 ml) taken directly from the column fractions were assayed for K^+ (EDTA)-ATPase activity (·-·-) to determine the elution position of myosin.

the two light chains are constant from one preparation to another; therefore, these proteins are considered to be subunits of brain myosin. The small higher molecular weight peaks vary from one preparation to the next, and may be degradation products of the myosin heavy chain. Comparison of relative mobilities with protein standards yields molecular weights of ~195,000 for the heavy chain, and ~19,000 and 16,000 for the light chains. The stoichiometry

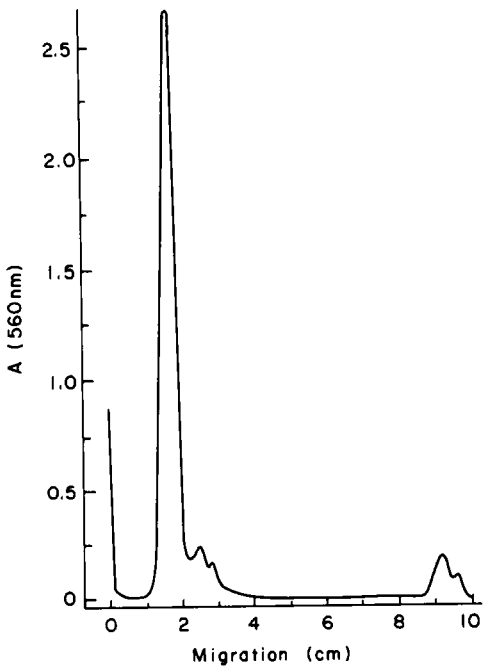

FIGURE 3 SDS-polyacrylamide gel electrophoresis of myosin from ion exchange chromatography. Myosin (50 μg) was applied to this 5% gel and electrophoresed as described in Experimental Procedures. After staining and destaining, the gel was scanned at 560 nm.

of the subunits is 2:2:1, respectively, as determined densitometrically from scans of the stained gels.

K^+(EDTA)-ATPase Activity

The K^+(EDTA)-ATPase activity of brain myosin was tested under a variety of conditions. Activity is maximal in 2 mM EDTA, and is depressed 68% by 10 mM $CaCl_2$ and at least 98% by 10 mM $MgCl_2$. K^+(EDTA)-ATPase activity is maximal between pH 9.0–9.5, and at KCl concentrations between 0.4 and 0.7 M. Activity rises sharply with increasing temperature in the 5°–35°C range. An Arrhenius analysis of the temperature data is linear, and yields an activation energy of 14.7 kcal/mol for the myosin ATPase ($n = 7$; $r = 0.996$). Brain myosin K^+(EDTA)-ATPase activity is a linear function of protein concentration, between 20 and 200 μg/ml myosin.

Low Ionic Strength Mg^{+2}-ATPase Activity

The physiological low ionic strength ATPase activity of brain myosin was assayed at 25°C in $(KCl)_{35}(MgSO_4)_{1.0}(imidazole)_{50}(DTT)_{1.0}(7.0)$. A Ca^{+2}-EGTA buffer was added to give a free Ca^{+2} concentration of 0.1 mM. EGTA dissociation constants used were those of Solaro and Shiner (1976). Myosin concentrations in these assays varied from 0.1 to 0.3 mg/ml.

The Mg^{+2}-ATPase activity of brain myosin alone is ~3 nmol P_i liberated/min · mg. The ability of porcine brain myosin to be activated by porcine brain or rabbit skeletal muscle actin is shown in Fig. 4. Actin activation refers to the ratio of the activity of brain myosin in the presence of actin to the activity of myosin alone. Neither actin preparation has ATPase activity. No significant difference was noted in the abilities of skeletal and brain actins to activate brain myosin at the various ratios of actin to myosin tested. Actin activation was not dependent on Ca^{+2}. At myosin concentrations of ~0.2 μM, the apparent K_m for actin activation is ~10 μM under these assay conditions. Both brain and skeletal muscle actins activate brain myosin almost fivefold at a molar ratio of 150:1, actin:myosin.

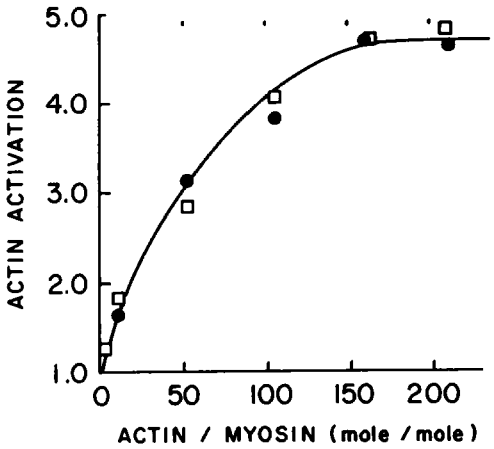

FIGURE 4 Actin activation of brain myosin. The actin activation of brain myosin Mg^{+2}-ATPase was determined at 25°C as described in Results. Skeletal (●) or brain (□) actin was included to the molar ratios of actin:myosin shown. Actin activation equals activity in the presence of actin/activity of myosin alone.

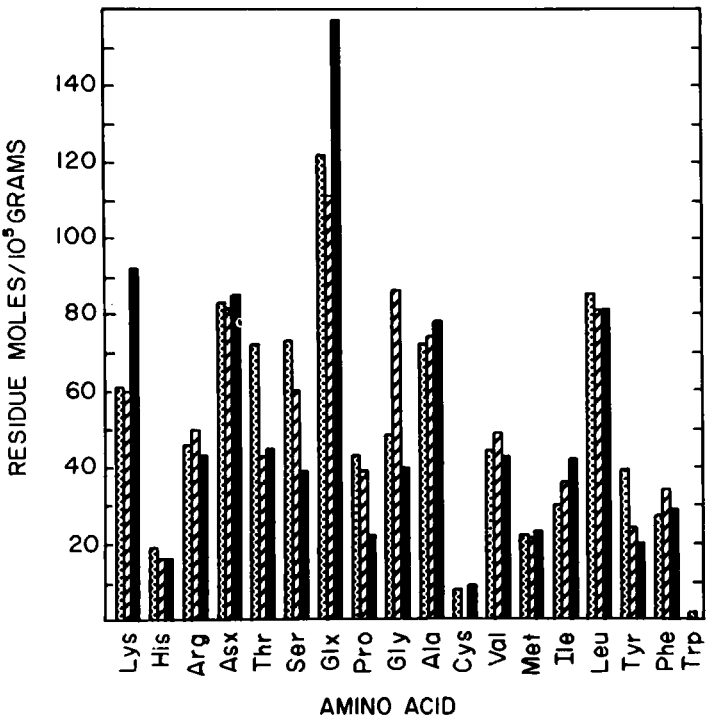

FIGURE 5 Amino acid analysis of brain myosin. Brain myosin was hydrolyzed for 24, 48, and 72 h and subjected to amino acid analysis as described in Experimental Procedures. Results are given in terms of residues/10^5 g. Brain myosin (□) is compared with bovine adrenal medulla myosin (□) and rabbit skeletal muscle myosin (■).

Amino Acid Analysis

The amino acid composition of brain myosin is shown in Fig. 5 and is compared with rabbit skeletal muscle (Lowey and Cohen, 1962), and cytoplasmic adrenal medulla myosins (Johnson et al., 1977). Results are expressed as residues/10^5 g. Brain myosin most closely resembles the myosin from bovine adrenal medulla. Porcine brain myosin has less lysine and glutamine, and more threonine, serine, proline, and tyrosine than skeletal muscle myosin. The percentages of acidic, basic, uncharged polar and nonpolar residues are, respectively, 14.5, 14.0, 35.2, and 36.3%. Of the acidic residues, 36% appear to be in the amide form. The mean residue weight for brain myosin is 112, and the nitrogen content is 17.6%.

Ultraviolet Absorbance and Circular Dichroism Spectra

The near ultraviolet absorption spectrum for porcine brain myosin was obtained in $(KCl)_{500}(KH_2PO_4)_{100}(8.0)$. The data show a typical protein spectrum, with a single broad peak centered at ~279 nm. The ratio of the absorbance at 280 nm to that at 260 nm is 1.65, indicating the absence of nucleic acid. The extinction coefficient for brain myosin is 0.810 cm^2/mg at 279 nm. This is much higher than that observed for smooth or skeletal muscle myosins (Frederiksen, 1979), as might be expected from the higher tyrosine content of brain myosin.

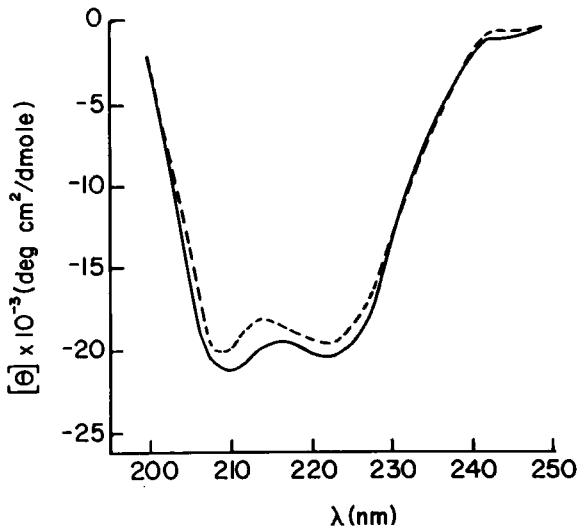

FIGURE 6 Circular dichroism of brain myosin. Spectra were obtained at 26°C, as described in Experimental Procedures. The solid line shows the spectrum of brain myosin in $(KCl)_{600}(CaCl_2)_{0.1}(MgCl_2)_{1.0}(Tris)_{50}(8.0)$. Addition of EGTA to 0.2 mM to the above myosin solution produces a somewhat more positive spectrum, shown by the dashed line.

Circular dichroism studies were undertaken to determine the secondary structure of brain myosin. The far ultraviolet circular dichroism spectrum of brain myosin is shown in Fig. 6. Neither DTT nor mercaptoethanol was used in these buffers because of the high background absorbance of these reagents below 240 nm. The spectrum was obtained in $(KCl)_{600}(CaCl_2)_{0.1}(MgCl_2)_{1.0}(Tris)_{50}(8.0)$; the fraction of α-helix in myosin, determined from $[\theta]_{222}$ and $[\theta]_{208}$ as described in Experimental Procedures is 58–60%. Addition of EGTA to 0.2 mM to the above solution lowers the Ca^{++} level to <20 nM while reducing the Mg^{+2} level only 2%, and produces a small but reproducible increase in $[\theta]$ that is particularly evident in the 205–220 nm wavelength range. Addition of EDTA to this solution, to 2 mM, alters the spectrum only slightly while essentially eliminating divalent cations. Therefore, the observed change in the spectrum appears to be caused by removal of Ca^{+2}.

Sedimentation Velocity

Brain myosin in K^+(EDTA) buffer from three separate preparations was subjected to analytical ultracentrifugation. The s_{app} increases linearly with concentration over the 0.2–0.5 mg/ml range. The points ($n = 5$) fall closely ($r = 0.983$) on the line: $S_{app} = 4.67S[1 + (0.375 \text{ ml/mg})c]$, determined by least squares. The intercept corresponds to an $s^0_{20,w}$ of 4.95S after corrections for solvent viscosity and density. The sedimentation coefficient increases with increasing myosin concentration, a phenomenon indicative of aggregation.

Diffusion

The standard curve for diffusion of a number of proteins is shown in Fig. 7, and a typical distribution profile for one of these standard proteins is shown in Fig. 8. The experimentally

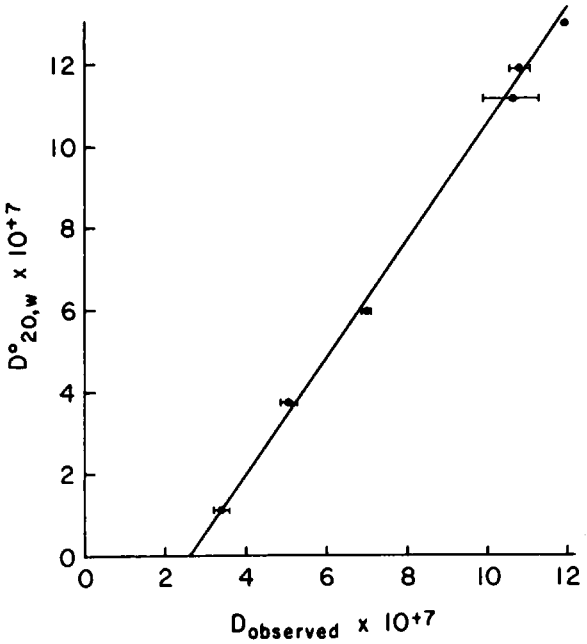

FIGURE 7 Diffusion standard curve. Diffusion coefficients for several standard proteins in K^+ (EDTA) buffer at 6°C were determined as described in Experimental Procedures and plotted against $D^{o}_{20,w}$. Standards used, in order of increasing diffusion coefficient, were rabbit skeletal muscle myosin, rat gamma globulins, bovine plasma albumin, hen egg white lysozyme, pancreatic ribonuclease, and horse cytochrome *c*. $D^{o}_{20,w} = -3.261 \times 10^{-7}(1 - 0.418D_{obs})$, where $n = 11$, $r^2 = 0.980$ from least squares.

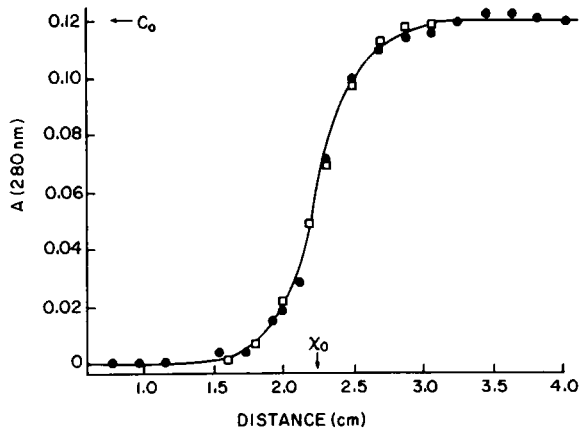

FIGURE 8 Diffusion of skeletal muscle myosin. The diffusion coefficient for skeletal muscle myosin was determined as described in Experimental Procedures. Protein was allowed to diffuse from a sharp layered boundary for 29.7 h at 6°C, then fractionated as described to obtain this distribution profile. The concentration of skeletal myosin in each fraction was determined by absorbance at 280 nm. Distance (cm) refers to the position of each fraction in the vertical diffusion cell, a cellulose nitrate tube. The plateau of the profile is taken as c_0, the initial protein concentration. The initial boundary, x_0, is the point at which the concentration equals 0.5 c_0. The experimental data points are given (●), as well as the points determined from the calculated diffusion coefficient (□), to show the accuracy of the analysis.

determined points are shown along with the points determined theoretically from the calculated diffusion coefficient.

Brain myosin in K^+(EDTA) buffer at various concentrations was subjected to similar analysis. $D_{20,w}$ is a linear function of concentration over the 0.3–0.7 mg/ml range, and the points (n = 5) fall closely (r = 0.998) on the line: $D_{20,w} = (1.07 \times 10^{-7}\text{cm}^2/\text{s})\ [1 - (1.22\ \text{ml/mg})c]$, determined by least squares.

Thus, the intrinsic diffusion coefficient of porcine brain myosin is $1.07 \times 10^{-7}\text{cm}^2/\text{s}$. The diffusion coefficient shows a negative concentration dependence, which again indicates aggregation. The diffusion experiments were performed at 6°C while the sedimentation was carried out at 20°C. Therefore, myosin aggregation is not strictly a temperature-dependent phenomenon.

Molecular Weight

The molecular weight of porcine brain myosin was determined by extrapolation of $D_{20,w}/s_{20,w}$ to zero protein concentration: $D_{20,w}/s_{20,w} = (2.01 \times 10^5\ \text{cm}^2/\text{s}^2)\ [1 - (1.29\ \text{ml/mg})c]$, as determined by least squares (n = 10, r = 0.997). The slope of the curve is negative. The second virial coefficient for brain myosin calculated from these data is $-1.37 \times 10^{-3}\ \text{cm}^3\text{mol/g}^2$. A negative virial coefficient also indicates self-association of protein. The reciprocal of the intercept corresponds to a molecular weight of 444,000 for brain myosin. This correlates well with the molecular weight determined from SDS-polyacrylamide gels, if the molecule were to consist of two heavy chains (195,000 daltons), two of the 19,000-mol wt light chains, and one or two of the 16,000-mol wt light chains. The frictional ratio, f/f_0, calculated from these data for porcine brain myosin, assuming no water is bound to the molecule, is 4.03.

Intrinsic Viscosity

The results of viscosity measurements of brain myosin solutions in K^+(EDTA) buffer at 20.0°C can be represented by $\eta_{sp}/c = 0.191\ \text{ml/mg} + (0.0328\ \text{ml}^2/\text{mg}^2)c$. Here, c is the protein concentration in mg/ml (range 0.5–2.5 mg/ml) and η_{sp} is the reduced specific viscosity (n = 7, r = 0.977). The intrinsic viscosity of brain myosin is 0.191 ml/mg and is slightly lower than that reported for aortic myosin (Frederiksen, 1979). The slope of the line gives a Huggins constant of 0.900, a value considerably higher than that of 0.306 for aortic myosin. This may also reflect the aggregation of brain myosin under these conditions.

DISCUSSION

Brain myosin is a 444,000-mol wt protein, consisting of 195,000-dalton heavy chains and two classes of light chains, of ~19,000 and 16,000 daltons. The stoichiometry of the subunits, as determined by densitometry from the scans of SDS-polyacrylamide gels, is 2:2:1, though the molecule could equally consist of two of each of the subunits. Potter (1974) has shown that, in skeletal muscle, light chains do not bind Coomassie blue dye equally on a weight basis. We have no evidence for a third light chain of higher molecular weight, as observed for chick brain myosin (Burridge and Bray, 1975; Kuczmarski and Rosenbaum, 1979).

The low ionic strength Mg^{+2}-ATPase of brain myosin can be activated about fivefold by brain or skeletal muscle actin, though high actin to myosin ratios are required to achieve maximal activation. The activation is not Ca^{+2}-sensitive. The high actin:myosin ratios necessary may be an intrinsic property of the system, since actin is present in much greater

abundance than myosin in brain. Our crude actomyosin has a molar ratio of 85:1 actin:myosin. Or, some element necessary for actin activation, such as a light chain kinase, may be absent from the purified myosin preparation. This may also account for the lack of Ca^{+2}-sensitivity and for the lower-fold activation than is seen with muscle.

Amino acid analysis of brain myosin shows it to be more similar to other cytoplasmic myosins than to skeletal muscle myosin. Bovine adrenal medulla myosin is similar to brain myosin in its lower lysine and glutamine content, and higher serine and proline contents. Brain myosin has a larger extinction coefficient, 0.810 cm/mg, than skeletal and aortic muscle myosins, 0.588 cm/mg and 0.558 cm/mg, respectively (Frederiksen, 1979), which can be explained by its higher tyrosine content.

Porcine brain myosin is 58–60% α-helical in the presence of micromolar Ca^{+2}. This is lower than the 65–70% helicities observed for skeletal and aortic myosins (Frederiksen, 1979), but is not totally unexpected since brain myosin has about twice as much proline as these myosins. A conformational change appears to occur in brain myosin upon reduction of Ca^{+2} from 0.1 mM to <1 nM, a phenomenon that has not been reported for any other myosin. However, we have shown here that 10 mM Ca^{+2} decreases the ATPase activity of brain myosin at high ionic strength, and Ca^{+2} has been reported to bind to smooth muscle myosins (Sobieszek and Small, 1976). In addition, Ca^{+2}-dependent regulator protein, which is homologous to the regulatory light chain of some invertebrate myosins, is known to undergo a marked conformational change in the presence of Ca^{+2}, resulting in a 20–25% increase in α-helix (Liu and Cheung, 1976). A similar conformational change in one class of brain myosin light chains could account for about one-half the observed effect of Ca^{+2}.

Thus cytoplasmic brain myosin resembles smooth and skeletal muscle myosins in many respects, but its properties are such that it is readily distinguishable from them. Kuczmarski and Rosenbaum (1979) showed that chick brain myosin antibodies do not crossreact with chicken smooth muscle myosin, nor with skeletal muscle myosin from chicken or rabbit. They also demonstrated that chick brain myosin does not form hybrid filaments with skeletal muscle myosin, when the two are dialyzed together against a low ionic strength buffer. Kaminer et al. (1976) reported formation of hybrid filaments between smooth and skeletal muscle myosins, and Pollard (1975) reported formation of platelet-skeletal muscle myosin filament hybrids. Thus brain myosin is, in at least some respects, distinct from myosin from other cytoplasmic sources as well as muscle sources.

Our work on porcine brain myosin supports the concept that it is a unique myosin. Although its molecular weight is similar to other myosins, its hydrodynamic properties are different. Porcine brain myosin aggregates at high ionic strength. Furthermore, at least the smaller aggregates form in such a manner that the enzymatic sites are not blocked, since ATPase activity under aggregating conditions increases linearly with myosin concentration. Smooth and skeletal muscle myosins are monomeric under these conditions (Frederiksen, 1979). One other myosin, that from bovine adrenal medulla, has been reported to aggregate at high ionic strength (Johnson et al., 1977). It is interesting that this myosin is also from a neurally-derived tissue, one that may have many of the same motility requirements as brain.

The hydrodynamic data presented here for brain myosin differ from that reported earlier for smooth and skeletal muscle myosins. The frictional ratio for brain myosin is 4.03 as compared with 3.57 for muscle myosins (Frederiksen, 1979). Although the intrinsic viscosity of this cytoplasmic myosin is less than that of the muscle myosins, it is quite large as compared

with $[\eta]$ for compact, globular proteins. Clearly, brain myosin is a highly asymmetric protein molecule.

The calculation of molecular dimensions from the hydrodynamic data presents somewhat of a problem since there is as yet no hydrodynamic model for a nonuniform rod. The rigid pearl necklace model of Kirkwood and his associates (Riseman and Kirkwood, 1950; Kirkwood and Auer, 1951) is the best available for estimation of the length and diameter of an asymmetric molecule. Substitution of $D^0_{20,w}$, $s^0_{20,w}$, and $\bar{\nu}$ into the equations for the model yields estimates for the axial ratio, diameter and length of 71, 2.5 nm and 174 nm, respectively. The use of $[\eta]$ and $\bar{\nu}$ yields an axial ratio of 64, a diameter of 2.5 nm, and a length of 162 nm. Other combinations of the data can be used in a similar way. This series of calculations gives the following average values: axial ratio, 67 ± 8; diameter, 2.6 ± 0.2 nm; length, 171 ± 10 nm. These calculations yield remarkably similar dimensions for brain and skeletal muscle myosins (Frederiksen, 1979). Because neither myosin fits the model ideally, the hydrodynamic data is a better base for comparison than these calculated dimensions. The work of Elliott et al. (1976), however, has shown chick brain and skeletal muscle myosins to be very similar in length when viewed in the electron microscope using a rotary shadowing technique. Thus the differences in hydrodynamic behavior between skeletal muscle and brain myosins cannot realistically be accounted for on the basis of size alone, and any shape differences must be subtle.

The size, shape, and in vitro aggregation properties of brain myosin may help to elucidate how it associates in the cell, since as yet myosin-containing thick filaments analogous to those in muscle have not been directly visualized in the cytoplasm of nonmuscle cells. Myosin filaments have been visualized by immunofluorescent techniques (Fujiwara and Pollard, 1976) but this does not reveal their mode of organization. The ability of myosin to associate in a specific manner is a crucial element in the mechanism of contraction in muscle, and thus is likely to be of key importance to its role in cytoplasmic cell motility as well.

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