Physical Properties of Myosin from Aortic Smooth Muscle†

Dixie W. Frederiksen

ABSTRACT: Porcine aortic myosin is a smooth muscle contractile protein similar to its striated muscle counterpart. Electrophoresis in sodium dodecyl sulfate indicates that the molecule consists of three classes of subunits with polypeptide chain molecular weights of 192 000, 19 000, and 15 000. At 277 nm the absorption spectrum gives an extinction coefficient for aortic myosin of 0.558 cm^2/mg; the circular dichroism spectrum of the protein indicates that aortic myosin contains about 70% of its residues in the α-helical configuration. Amino acid analysis shows that the smooth muscle myosin has significantly more arginine and leucine and significantly less valine and isoleucine than rabbit skeletal muscle myosin. Other studies yielded these data: $\bar{v}_{app}$ = 0.716 mL/g, $[\eta]$ = 0.213 mL/mg, $s_{20,w} = 5.84 \times 10^{-13}$ s. Similar studies with rabbit skeletal muscle myosin indicate that $\bar{v}_{app}$ = 0.711 mL/g and $s_{20,w} = 6.36 \times 10^{-13}$ s. These properties suggest that aortic myosin, like skeletal muscle myosin, behaves hydrodynamically like a rigid rod.

A number of structural and enzymatic distinctions separate the smooth muscle contractile process from that in striated muscle. Such differences are, in part, reflected in the properties of smooth and striated muscle myosins. Recent work on the biochemical properties of natural actomyosins from various smooth muscles has shown that these contractile protein complexes differ significantly from their striated muscle counterparts. Such differences are very likely important in the molecular mechanism of smooth muscle contraction. Although the interaction of actin and myosin coupled with the hydrolysis of ATP[1] to ADP and orthophosphate with the release of free energy undoubtedly forms the basis for contraction in all muscles, details of the molecular processes responsible for the conversion of chemical to mechanical energy are not yet clear. An understanding of the structure of smooth muscle myosin is prerequisite to elucidation of a precise molecular theory of smooth muscle contraction.

The physicochemical properties of the smooth muscle myosins have not been studied as intensely as those of skeletal muscle myosin. Sedimentation studies and viscometry of arterial myosin (Hamoir & Laszt, 1961) were undertaken before the observation that the protein readily aggregates in many otherwise benign solvents (Huriaux et al., 1965). The secondary structure of this particular myosin appears not to have been considered at all.

The present study of the physical properties of highly purified porcine aortic myosin in solution reveals considerable information about the structure of this protein. Aortic smooth muscle myosin may be somewhat smaller than skeletal muscle myosin, but the overall shapes and symmetries of the two proteins are very similar.

Experimental Procedure

Porcine Aortic Myosin. Thoracic aortas from 6–8 month old hogs were obtained within 30 min of the death of the animals and placed in ice-cold $(NaCl)_{150}(7.0)$.[2] The media were dissected and minced as described earlier (Frederiksen, 1976). The preparative procedure for myosin was modified from that of Sobieszek (1977). All steps in the procedure were carried out at 0–5 °C. About 200 g of minced media, pooled from 15 hogs, was suspended in 600 mL of $(KCl)_{60}(MgSO_4)_1(DTT)_1(imidazole)_{20}(6.8)$ and divided into six equal aliquots. Each aliquot was disrupted with three 10-s pulses of a high speed mechanical homogenizer (Brinkmann Polytron, 20-mm head) at maximum power. Suspensions were incubated at 0 °C for 3 min between pulses of the homogenizer. The residue was collected by centrifugation (10000*g*, 5 °C, 15 min), resuspended in an equal volume of $(KCl)_{600}(DTT)_1(EDTA)_2(EGTA)_1(ATP)_{10}(imidazole)_{20}(7.5)$ and subjected to a single 10-s pulse of the homogenizer. This supernatant was clarified by centrifugation (22000*g*, 5 °C, 45 min) and decanted. A second centrifugation (80000*g*, 5 °C, 4 h) resulted in the precipitation of a large part of the contaminating F-actin and other protein. The supernatant was decanted and brought to 30 mM in $MgSO_4$ by addition of $(MgSO_4)_{1000}(7.5)$. The solution was allowed to stand for 4–12 h at 5 °C. Precipitated myosin was collected by centrifugation (55000*g*, 5 °C, 60 min) and suspended in 10 mL of $(KCl)_{600}(DTT)_{0.5}(MgSO_4)_1(ATP)_5(imidazole)_{20}(7.0)$ with two strokes of a manual, Teflon pestle homogenizer. The solution was dialyzed against this solvent for 4 h, applied to a 450-mL bed volume agarose column (Sepharose 4B), and eluted with $(KCl)_{600}(DTT)_{0.5}(MgSO_4)_{0.1}(ATP)_{0.5}(imidazole)_{20}(7.0)$ at a flow rate of 30 mL/h. Myosin was eluted from the column at K_{av} = 0.33. The protein was used for experiments within 10 days of elution.

Rabbit skeletal muscle myosin was prepared from the back and hind legs of 3–4 month old male albino rabbits by the method of Holtzer & Lowey (1959) as modified by Schuster (1963).

Analytical Ultracentrifugation. All sedimentation runs were made in the Beckman Spinco Model E ultracentrifuge operated with schlieren optics or with a photoelectric scanning system. Protein solutions were dialyzed exhaustively against $(KCl)_{600}(EDTA)_1(DTT)_{0.5}(Tris\text{-}Cl)_{50}(8.0)$ and dialysate used for subsequent dilutions and as solvent in one compartment of the 12-nm double sector centrifuge cell. All runs were made at 52 000 rpm in the An-H rotor at 20.0 ± 0.2 °C. Sedimentation coefficients were calculated from plots of ln

† From the Department of Biochemistry, Vanderbilt University, Nashville, Tennessee 37232. *Received May 5, 1978; revised manuscript received November 29, 1978.* This work was supported by U.S. National Institutes of Health Grant HL 18516.

[1] Abbreviations used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; P_i, orthophosphate; PP_i, pyrophosphate; DTT, dithiothreitol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; CD, circular dichroism; UV, ultraviolet; UC, ultracentrifugation; HC, high molecular weight polypeptide chain of myosin; LC, low molecular weight polypeptide chain of myosin.

[2] A shorthand notation is desirable for reference to complex aqueous solvent media (Frederiksen & 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.

0006-2960/79/0418-1651$01.00/0

(distance) vs. time with the usual corrections for solvent viscosity and density.

Viscometry. Viscosity measurements were made with Ostwald-Fenske viscometers (Cannon Instrument Co.) which had water flow times of 265 to 565 s at 20 °C. Viscometry was performed at 20.0 ± 0.2 °C; the solutions and viscometers were thermostated with the aid of a Low-Temp Bath (Wilkens-Anderson Co.).

Stock protein solutions were dialyzed exhaustively against $(KCl)_{600}(EDTA)_1(DTT)_{0.5}(Tris\text{-}Cl)_{50}(8.0)$ before use in viscosity experiments; the dialysate for a particular protein solution was taken as the solvent. Solvents were cleaned by membrane filtration (Millipore, 0.25-μm pore size). Membranes were washed with 100 mL of $(KCl)_{600}$ and then with 100 mL of deionized water before use. Protein solutions were cleaned by centrifugation (22000g, 5 °C, 3 h). The upper one-third (excluding the meniscus) of the solutions was used. The most dilute protein solution used had a flow time 49 s greater than that of solvent; the most concentrated solution had a flow time 300 s greater than that of solvent.

Densitometry. Density measurements were performed with an oscillator system precision density meter (Mettler-Paar, Model DMA-02D). The glass U tube was maintained at constant temperature by means of a constant temperature circulator (Brinkmann, Lauda K-2/R). All experiments were done at 20.00 ± 0.02 °C. The instrument was calibrated with water, air, and Spectro-grade methanol; air times were remeasured after each determination in order to assure that the U tube had been properly cleaned. Times were determined for 40 000 cycles and were reproducible to within 60 μs.

Protein solutions and solvents were prepared and cleaned by the same procedures used to prepare solutions for viscometry. The most dilute protein solution had an oscillation time 17.5 ms greater than that of solvent; the most concentrated had an oscillation time 54.4 ms greater than solvent.

Gel Electrophoresis. Electrophoresis was carried out in sodium dodecyl sulfate by the method of Weber & Osborn (1969) with the modifications suggested by Frederiksen (1976). The diethyl pyrocarbonate cross-linked protein molecular weight markers used covered molecular weight ranges 14 100–70 500 and 53 900–269 500 in the buffer system used.

Samples were prepared for electrophoresis by overnight dialysis at 23–25 °C against $(NaDodSO_4)_{41}(HSCH_2\text{-}CH_2OH)_{128}(NaP_i)_{10}(7.0)$ followed by incubation at 100 °C for 10 min. The protein samples were routinely electrophoresed in 5-mm diameter disk gels consisting of 4.78% acrylamide–0.13% bisacrylamide polymerized in $(NaDodSO_4)_{4.1}(NaP_i)_{50}(7.0)$ at 5 mA per gel. Coomassie brilliant blue stained gels were scanned at 580 nm with a Gilford spectrophotometer equipped with a scanning attachment.

Protein concentrations were routinely determined by an automated (Technicon autoanalyzer) modification of the method of Lowry et al. (1951) with bovine plasma albumin as standard. Myosin concentrations were often determined from absorbance at 277 nm by using an extinction coefficient of 0.587 cm^2/mg (Frederiksen & Holtzer, 1968) for the skeletal muscle protein and 0.558 cm^2/mg for the smooth muscle protein. Micro-Kjeldahl analyses were used to determine the nitrogen content of aortic myosin solutions for measurement of the extinction coefficient. A nitrogen factor of 5.72 (calculated from the amino acid analyses) was used.

Circular dichroism spectra were measured by means of a Cary 60 spectropolarimeter equipped with a Model 6002 CD attachment and calibrated with *d*-camphor-10-sulfonic acid. Temperature was held at 20.0 ± 0.05 °C by a Lauda K-2/R constant-temperature circulator. The myosin concentrations used were 0.2–0.5 mg/mL; solvents and samples were cleaned by the procedures used to prepare solutions for viscometry. For the ultraviolet range (200–250 nm), the full scale range was 100 mdeg, and cells with a 0.5-mm pathlength were used. In the near-ultraviolet range (250–320 nm) the full scale deflection was 40 mdeg, and 2.0-cm pathlength cells were used. Each solution and each solvent were scanned three or four times to obtain the averages presented here. The circular dichroism results are reported in terms of the mean residue ellipticity [θ], in units of deg·cm^2/dmol. The mean residue weight used for aortic myosin was 114 calculated from amino acid analysis.

Ultraviolet absorption spectra were obtained at 22.1 ± 0.5 °C with a double beam spectrophotometer (Perkin-Elmer, Model 202). A pair of matched cuvettes with 1.00-cm pathlength was used for all scans. The myosin concentrations were 0.5–1.5 mg/mL; solutions and solvents were clarified by the procedures used to prepare samples for viscometry. Each protein solution, with dialysate as reference, was scanned three to four times at a rate of 50-nm wavelength per min over the 240–320-nm wavelength range. Extinction coefficients, ϵ, are reported in units of cm^2/mg.

Adenosine triphosphatase (ATPase) assays were carried out at 25.0 ± 0.1 °C by the method described previously (Frederiksen, 1976); orthophosphate was determined by the method of Fiske & SubbaRow (1925). High ionic strength K^+(EDTA)–ATPase activities were assayed in $(KCl)_{500}(EDTA)_5(DTT)_1(Tris\text{-}Cl)_{50}(8.0)$.

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 & Stein (1963) analyzer system was used as described by Shih & Hash (1971). Tryptophan was determined on the amino acid analyzer after hydrolysis of the protein in the presence of methanesulfonic acid (Moore, 1972).

Reagents. DTT, EGTA, ATP, $NaDodSO_4$, and bovine plasma albumin were obtained from Sigma. Diethyl pyrocarbonate cross-linked protein molecular weight markers for electrophoresis were from BDH Chemicals, Ltd., Poole, U.K. Distilled water was deionized and filtered through activated charcoal before use. All other materials were enzyme or reagent grade.

Results

Purity of Aortic Myosin. Analyses of protein fractions derived during the preparation are given in Table I and Figure 1. It can be seen that approximately half the K^+(EDTA)–ATPase activity is recovered in about 2% of the soluble protein. The procedure results in a 25-fold purification of the protein. Chromatography on agarose (Figure 2) results not only in a twofold purification of the aortic myosin but also in greatly stabilizing the protein to storage.

The protein obtained as described above was judged pure by several criteria. Myosin preparations subjected to electrophoresis in $NaDodSO_4$–polyacrylamide show several peaks (Figure 3). This protein profile is similar to that of myosins from other cell types. The relative intensities of the three major peaks in the myosin electrophoretograms do not change from one myosin preparation to another, nor do they vary from one agarose chromatography fraction to another. Each of the three polypeptide chains is considered to be a subunit of aortic myosin although the possibility remains that all or a portion of any one of these might be due to a contaminant. Figure

Table I: Analysis of Aortic Myosin Preparation

fraction	protein (mg)[a]	K^+-ATPase (μmol/min)	sp K^+-ATPase act. (nmol/(mg·min))	purification
soluble protein	1890.0	59.1	31.2	1.00
low μ extract	1049.0	13.0	12.4	
high μ extract (crude actomyosin)	840.0	45.9	54.7	1.75
UC supernatant (crude myosin)	491.0	47.5	96.7	3.10
UC precipitate (crude actin)	311.0	0.5	1.6	
Mg^{2+}-precipitated myosin	88.1	35.2	399.0	12.8
Mg^{2+}-supernatant	432.0	14.3	33.1	
myosin from Sepharose 4B	38.1	30.0	787.0	25.2

[a] Milligrams of protein per 100 g of wet medial layer from porcine aorta.

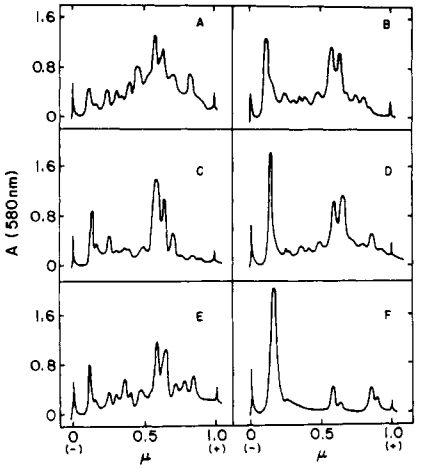

FIGURE 1: Polyacrylamide gel electrophoresis in $NaDodSO_4$ of fractions from preparation of myosin. Protein (15–25 μg) was applied to each gel for electrophoresis as described under Experimental Procedure. (A) Low ionic strength supernatant; (B) high ionic strength ATP supernatant; (C) high speed UC precipitate; (D) high speed UC supernatant; (E) supernatant from precipitation with Mg^{2+}; (F) Mg^{2+}-precipitated protein.

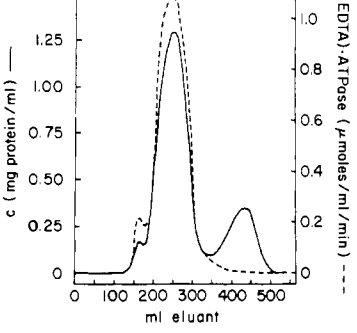

FIGURE 2: Agarose chromatography of Mg^{2+}-precipitated myosin. Twenty milliliters of Mg^{2+}-precipitated myosin (c = 6.59 mg/mL) in $(KCl)_{600}(DTT)_{0.5}(MgSO_4)_{1.0}(ATP)_{5.0}(imidazole)_{20.0}(7.0)$ was applied to a 450-mL bed volume Sepharose 4B column and eluted at 30 mL/h as described under Experimental Procedure.

3 indicates that the preparation does not contain significant contamination by other proteins nor is it affected by appreciable proteolysis after up to 10 days after chromatography. In addition, these myosin solutions exhibit a single schlieren peak in the centrifuge for up to 2 weeks at 5 °C after elution

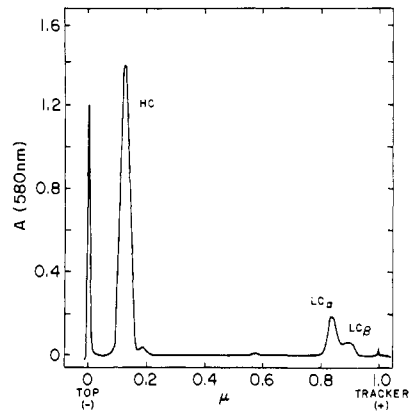

FIGURE 3: Polyacrylamide gel electrophoresis of purified myosin in $NaDodSO_4$. Chromatographed myosin was denatured in $NaDodSO_4$–$HSCH_2CH_2OH$ and electrophoresed as described under Experimental Procedure. Sixteen micrograms of protein was applied to this gel.

from agarose. Thus, polydispersity due to myosin aggregation is not a problem under the conditions used here. The proper selection and maintenance of the solvent system, however, are important. Fresh preparations of myosin dialyzed into low ionic strength (<0.2 M) buffers or into buffers without DTT [i.e., $(KCl)_{500}(KP_i)_{100}(7.5)$] are aggregated.

Attempts to purify aortic myosin by dissolution of the Mg^{2+}-precipitated protein in buffered, high ionic strength KI and subsequent chromatography (Pollard et al., 1974) met with only partial success. The myosin obtained was indistinguishable from that obtained by the procedure finally adopted except that the iodide-treated protein exhibited no actin-activated Mg^{2+}–ATPase activity.

Subunit Composition and Molecular Weight. Polyacrylamide gel electrophoresis of purified aortic myosin in $NaDodSO_4$–$HSCH_2CH_2OH$ indicates that the molecule consists of at least three distinct kinds of polypeptide chains (Figure 3). The molecular weight dependence of the relative electrophoretic mobility for the protein standards in this system is given by

$$M = (3.11 \times 10^5)10^{-1.312\mu}$$

Analysis of these data gives a regression coefficient (r) of 0.999 for n = 12. Comparison of the relative mobilities of the myosin subunits with those of the standards yields the following molecular weight estimates for these subunits: heavy chain (HC), 192 000; light chain α (LC_α), 19 000; and light chain β (LC_β) 16 000. Rabbit skeletal muscle myosin subjected to identical denaturing and electrophoretic conditions is found to have subunit molecular weights of 203 000, 27 000, 19 000 and 16 000.

If the aortic smooth muscle myosin molecule, like its skeletal and cardiac muscle counterparts, consists of two heavy and four light polypeptide chains, then the native smooth muscle myosin might be expected to have a molecular weight of approximately 454 000. The unequivocal assignment of a polypeptide chain stoichiometry to aortic myosin on the basis of the data here is not possible. Integration of the peaks corresponding to Coomassie blue stained bands in the scanned electrophoretograms shows that the peak areas are consistent with a stoichiometry for HC:LC_α:LC_β of 2:2.4:1. Potter (1974) has clearly demonstrated, however, that not all the contractile proteins bind dye to the same extent on a weight basis.

Ultraviolet Absorption and Circular Dichroism Spectra. The near-ultraviolet absorption spectra for porcine aortic smooth muscle myosin and for lapine skeletal muscle myosin were obtained for protein solutions in $(KCl)_{500}(EDTA)_5$-

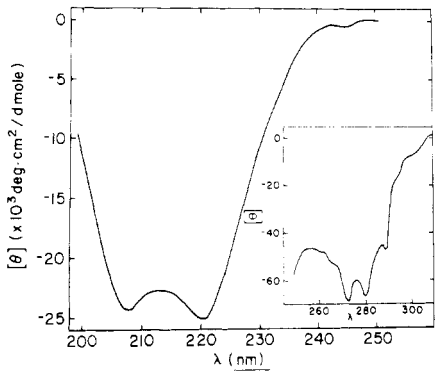

FIGURE 4: Circular dichroism spectrum of aortic myosin at 20 °C. The solvent is $(KCl)_{500}(KP_i)_{100}(7.5)$. The far- and near-UV wavelength ranges were scanned as described under Experimental Procedure.

$(DTT)_1(Tris\text{-}Cl)_{50}(8.0)$; the data show typical protein spectra for each myosin consisting of a single broad peak with maximum absorbance at 277–278 nm and a width at half the maximum absorbance of 25 nm. The extinction coefficient for aortic myosin at 277 nm is 0.558 cm^2/mg; that for skeletal muscle myosin is 0.589 cm^2/mg. In the wavelength range from 260 to 290 nm, the extinction coefficient of the smooth muscle protein is 0 to 5% less than that of skeletal muscle myosin.

Circular dichroism studies were undertaken to determine the secondary structure of aortic myosin in solution and to compare the relative α helicities of aortic smooth muscle and skeletal muscle myosins. The far-ultraviolet circular dichroism spectrum of aortic myosin in $(KCl)_{500}(KP_i)_{100}(7.5)$ is given in Figure 4, with the near-UV spectrum shown in the inset. Neither DTT nor $HSCH_2CH_2OH$ was used in buffers for CD experiments because such reagents provide excessively high background absorption of radiation at wavelengths below 240 nm. The fraction of α helix in aortic myosin as determined from $[\theta]_{208}$ by the method of Greenfield & Fasman (1969) or from $[\theta]_{222}$ by the method of Chen et al. (1972, 1974) is 69% or 72%, respectively. The fraction of β structure in the aortic protein is less than 10% as determined by either method.

Far-ultraviolet CD spectra determined for rabbit skeletal muscle myosin under the same conditions yielded α helicities of 63% and 67%. The circular dichroism spectra for the two myosins in the near-UV are quite similar with the mean residue ellipticity of the skeletal muscle protein in the 270–280-nm range approximately 13% more negative than that of aortic myosin.

Amino acid analysis of the aortic smooth muscle myosin, along with that for skeletal muscle myosin (Lowey & Cohen, 1962) and for bovine carotid myosin (Huriaux et al., 1965), is given in Table II. Apparent differences in the two arterial myosins most likely reflect differing degrees of homogeneity in the two preparations. Although the aortic protein has significantly less valine, less isoleucine, more leucine, and more arginine per 10^5 cm than skeletal muscle myosin, the amino acid profiles of the myosins are quite similar. Aortic myosin consists of 36% nonpolar, 17% polar, 18% basic and 29% amino acid residues. The mean residue weight for aortic myosin is 114, and the nitrogen content is 17.5%.

Sedimentation. Aortic myosin in $(KCl)_{600}(EDTA)_1(DTT)_{0.5}(Tris\text{-}Cl)_{50}(8.0)$ from three separate preparations was subjected to analytical ultracentrifugation. The points (n = 7) all fall closely (r = 0.994) on the line

$$s_{20,w} = (5.84 \times 10^{-13}\ s)[1 - (94.6\ mL/g)c]$$

determined by least squares. The intrinsic sedimentation coefficient is 5.84 S. A reciprocal plot of $s_{20,w}$ vs. concentration yields an intercept corresponding to an $s_{20,w}$ of 5.88 S. Determination of the concentration dependence of sedimentation for skeletal muscle myosin in this solvent results in an $s_{20,w}$ of 6.36 S. This is in keeping with the 6.41 S reported earlier by Holtzer & Lowey (1959) who used a partial specific volume of 0.728 mL/g for skeletal muscle myosin. The partial specific volume of aortic myosin is greater than that of skeletal muscle myosin; this, taken by itself, should result in a sedimentation coefficient only 1.9% less than that of the skeletal muscle protein.

Table II: Comparison of the Amino Acid Composition of Porcine Aortic Myosin with Bovine Carotid and Lapine Skeletal Muscle Myosins

	residue mol/10^5 g		
	porcine aortic	bovine carotid[a]	lapine skeletal[b]
Lys	87	84	92
His	16	12	16
Arg	55	49	43
Asx	88	89	85
Thr	45	40	44
Ser	40	38	39
Glx	167	183	157
Pro	20	15	22
Gly	41	34	40
Ala	78	76	78
Cys	7.4		8.8
Val	33	38	43
Met	24	23	23
Ile	28	33	42
Leu	92	93	81
Tyr	18	14	20
Phe	34	28	29
Trp	2.2		

[a] Huriaux et al. (1965). [b] Lowey & Cohen (1962).

Densitometry. The difference in densities of protein solution and dialysate, $\Delta\rho$, is related directly to the U-tube oscillation time

$$\Delta\rho = k(T_s^2 - T_0^2)$$

where T is the time required for a particular number of oscillations of the U tube, subscripts refer to solution and solvent, and k is an instrument constant. The density differences, determined as a function of concentration in $(KCl)_{600}(EDTA)_1(DTT)_{0.5}(Tris\text{-}Cl)_{50}(8.0)$, fall on the line given by

$$\Delta\rho = 0.2664c$$

where c is protein concentration in the same units as $\Delta\rho$ (n = 6; r = 0.9987). The slope of this line gives the buoyancy factor, $1 - \bar{v}_{app}\rho_0$. Here $\bar{v}_{app}$ is the apparent partial specific volume, equal to the partial derivative of the volume with respect to the mass of the macromolecular solute at constant temperature, pressure, and chemical potential of other components of the system, $\bar{v}_{app} = (\partial v/\partial m)_{T,P,\mu_i}$; ρ_0 is the dialysate density. The apparent partial specific volume is 0.716 mL/g.

Density measurements on solutions of rabbit skeletal muscle myosin under the same conditions as those used for the aortic myosin experiments give $\Delta\rho = 0.2712c$ (n = 4; r = 0.9992). The apparent partial specific volume of this protein is 0.711 mL/g.

Viscometry. The results of viscosity measurements of aortic myosin solutions from two different preparations can be represented by

$$\eta_{sp}/c = 0.213\ mL/mg + (0.0139\ mL^2/mg^2)c$$

Here η_{sp} is the reduced specific viscosity and c the protein concentration in mg/mL. The reduced specific viscosities of

aortic myosin solutions in $(KCl)_{600}(EDTA)_1(DTT)_{0.5}(Tris\text{-}Cl)_{50}(8.0)$ determined as described under Experimental Procedure fall closely on this line ($r = 0.998$; $n = 5$). The intrinsic viscosity of aortic myosin is 0.213 mL/mg, very close to that reported for skeletal myosin (Holtzer & Lowey, 1959; Schuster, 1963). The slope of this line gives a Huggins constant of 0.306, a value considerably greater than that reported for the skeletal muscle protein in a different solvent and at pH 6.7.

Discussion

Although reviewers have usually accepted the physical properties of smooth muscle myosins as similar to those of skeletal muscle myosin (Hartshorne & Askoy, 1977; Hamoir, 1973), others have pointed out that comparatively little is known about smooth muscle contractile proteins (Pollard & Weihing, 1974). The sedimentation coefficient has been determined for bovine carotid myosin (Hamoir & Laszt, 1962), lapine uterus myosin (Cohen et al., 1961; Wachsberger & Kaldor, 1971), gizzard myosin (Barany et al., 1966), and equine esophagus myosin (Kotera et al., 1969). With the exception of one of the uterine myosin reports (Wachsberger & Kaldor, 1971), these values have been consistently and significantly lower than that of skeletal muscle myosin. The earlier work was done before the advent of $NaDodSO_4$–polyacrylamide gel electrophoresis (Weber & Osborn, 1969) as an analytical criterion for protein homogeneity and before appreciation of the ready aggregation of smooth muscle myosin (Huriaux et al., 1965) under conditions that leave skeletal muscle myosin monodisperse. Previous determinations (Cohen et al., 1961; Wachsberger & Kaldor, 1971) of intrinsic viscosity for uterine myosin are disparate perhaps because of contaminating proteins or aggregated myosin. Before the molecular basis for contraction in smooth muscle can be elucidated, the structure of myosin must be more clearly defined.

The gross structures of rabbit skeletal muscle myosin and porcine aortic myosin are no doubt quite similar as evidenced by the data given here and by others (Hartshorne & Askoy, 1977; Hamoir, 1973; Pollard & Weihing, 1974). The myosin molecule is a complex, nonuniform particle. Differences in the physical properties of the two proteins cannot yet be neatly sorted.

Although many of the differences in the experimental quantities observed here might be explained in terms of the obvious disparity in the light chain complement of the two proteins, difficulties arise in reconciliation of the distinct aggregation properties of the two myosins. Since the helical tail of skeletal muscle myosin is, in large measure, responsible for aggregation and filament formation of this protein (Szent-Gyorgyi, 1953), light chains are not likely responsible for differences in the self-association of the two myosins.

On the other hand, a simplistic approach ascribing the varying hydrodynamic and optical properties of the myosins to the overall protein is not totally satisfactory. Consideration of the data presented here indicates that, hydrodynamically, both aortic and skeletal muscle myosins more closely approximate the rigid rod model than earlier data (Cohen et al., 1961; Wachsberger & Kaldor, 1971; Holtzer & Lowey, 1959) might suggest. The rigid pearl necklace model of Kirkwood and associates (Riseman & Kirkwood, 1950; Kirkwood & Auer, 1951) has been used with the properties reported here for the two myosins and with the molecular weight and intrinsic viscosity reported by Holtzer & Lowey (1959) for skeletal muscle myosin to determine the molecular dimensions given in Table III. The skeletal muscle myosin dimensions are almost 200% more precise than reported earlier. The equally precise dimensions of the aortic protein are the same, within experimental error, as those of skeletal muscle myosin. That these hydrated contractile proteins have similar asymmetries is further illustrated by calculating for each myosin the ratio of the observed frictional coefficient to the minimal coefficient determined from Stoke's law for a spherical molecule. For aortic myosin f/f_{min} is 3.58, and for rabbit myosin the value is 3.57.

Table III: Molecular Dimensions of Myosin[a]

aortic			skeletal muscle			
d (nm)	L (nm)	J	d (nm)	L (nm)	J	exptl
2.67	144	53.9	2.75	147	53.6	$S, \bar{\nu}$
2.47	169	68.7	2.52	175	69.1	$\eta, \bar{\nu}$
2.42	170	70.2	2.47	175	70.8	S, η
2.60	178	68.7	2.66	184	69.1	$\bar{\nu}S, \bar{\nu}\eta$
2.54 ± 0.04	165 ± 6	65.4 ± 3.3	2.60 ± 0.06	170 ± 7	65.6 ± 3.5	av ± SD

[a] The molecular dimensions of the proteins are calculated from the rigid pearl necklace model using the equations of Kirkwood (Riseman & Kirkwood, 1950; Kirkwood & Auer, 1951): $s_{20,w} = [(1 - \bar{\nu}\rho_0)d^2 \ln J]/18\bar{\nu}_0$; $[\eta] = \bar{\nu}J^2/3750 \ln J$; and $\bar{\nu} = N_0\pi d^3 J/6M$. J is the molecular axial ratio, d, the molecular diameter, and L, the molecular length.

The intrinsic viscosities, highly sensitive to the molecular axial ratio, are virtually the same for the two myosins. Differences in asymmetry then are likely to be very small. The difference in sedimentation coefficients for the two proteins, while significant, appears due to the lower molecular weight and greater apparent partial specific volume of aortic myosin rather than due to a difference in overall molecular geometry.

Circular dichroism spectra indicate that aortic myosin may have 200–400 more amino acid residues in the α-helical conformation than does skeletal muscle myosin. The α helicity observed here for skeletal muscle myosin is in keeping with that found by others for the protein (McCubbin et al., 1966; Oikawa et al., 1968; Frederiksen & Holtzer, 1968; Murphy, 1974) but is significantly lower than the recent report by Wu & Yang (1976) who used the unusual solvent $(NaPP_i)_{40}(7.5)$. Furthermore, the amino acid analysis indicates more helix-promoting arginine and leucine residues in the smooth muscle myosin. The secondary structures of the myosins may be the same, or a slightly more extensive helical tail could exist in aortic myosin than in skeletal muscle myosin, or it might be that the light chains of the smooth muscle protein are more helical than their skeletal muscle counterparts.

The structure of aortic smooth muscle myosin is quite similar to that of skeletal muscle myosin. Nevertheless, the two proteins are distinct. The difference in light chain composition may be responsible in part for the observed differences in the physical properties of the myosins. The characterization of purified aortic myosin light chains is underway and may resolve the contribution of these subunits to the properties of the intact myosin. A less flexible, less hydrated aortic smooth muscle myosin with a longer helical tail and a correspondingly smaller head region would fit the data presented here. Such a myosin might be expected to form thick filaments whose geometry is unlike that of skeletal muscle myosin. The structure of aortic myosin synthetic thick filaments is now under investigation. The properties of aortic myosin observed here and interpreted in terms of molecular structure may have a bearing on contractility in vascular smooth muscle.

Acknowledgments

The author is indebted to Dr. Jin-Jyi Chang, who performed

the amino acid analyses. Lindsay K. Bishop provided essential technical assistance for this work. Fresh porcine aortas were a generous gift of D. G. Odom, Jr., at Odom's Sausage Co., Madison, TN.

References

Barany, M., Barany, K., Gaetjens, E., & Bailin, G. (1966) *Arch. Biochem. Biophys. 113*, 205–221.
Chen, Y.-H., Yang, J. T., & Martinez, H. M. (1972) *Biochemistry 11*, 4120–4131.
Chen, Y.-H., Yang, J. T., & Chou, K. H. (1974) *Biochemistry 13*, 3350–3359.
Cohen, C., Lowey, S., & Kucera, J. (1961) *J. Biol. Chem. 236*, PC23–24.
Fiske, C. H., & SubbaRow, Y. (1925) *J. Biol. Chem. 66*, 375–400.
Frederiksen, D. W. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2706–2710.
Frederiksen, D. W., & Holtzer, A. (1968) *Biochemistry 7*, 3935–3950.
Greenfield, N., & Fasman, G. (1969) *Biochemistry 8*, 4108–4116.
Hamoir, G. (1973) *Philos. Trans. R. Soc. London, Ser. B 265*, 169–181.
Hamoir, G., & Laszt, L. (1962) *Nature (London) 193*, 682–684.
Hartshorne, D. J., & Askoy, M. (1977) in *Biochemistry of Smooth Muscle* (Stephens, N. L., Ed.) pp 363–378, University Park Press, Baltimore, MD.
Holtzer, A., & Lowey, S. (1959) *J. Am. Chem. Soc. 81*, 1370–1377.
Huriaux, F., Pechere, J. F., & Hamoir, G. (1965) *Angiologica 2*, 15–43.
Kirkwood, J. G., & Auer, P. L. (1951) *J. Chem. Phys. 19*, 281–283.
Kotera, A., Yokoyama, M., Yamaguchi, M., & Miyazawa, Y. (1969) *Biopolymers 7*, 99–106.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Lowey, S., & Cohen, C. (1962) *J. Mol. Biol. 4*, 293–308.
McCubbin, W. D., Kay, C. M., & Oikawa, K. (1966) *Biochim. Biophys. Acta 126*, 597–600.
Moore, S. (1972) in *Chemistry and Biology of Peptides* (Meienhofer, J., Ed.) pp 629–653, Ann Arbor Science Publishers, Ann Arbor, MI.
Moore, S., & Stein, W. H. (1963) *Methods Enzymol. 6*, 819–831.
Murphy, A. J. (1974) *Arch. Biochem. Biophys. 163*, 290–296.
Oikawa, K., Kay, C. M., & McCubbin, W. D. (1968) *Biochim. Biophys. Acta 168*, 164–167.
Pollard, T. D., & Weihing, R. R. (1974) *CRC Crit. Rev. Biochem. 2*, 1–65.
Pollard, T. D., Thomas, S. M., & Niederman, R. (1974) *Anal. Biochem. 60*, 258–266.
Potter, J. D. (1974) *Arch. Biochem. Biophys. 162*, 436–441.
Riseman, J., & Kirkwood, J. G. (1950) *J. Chem. Phys. 18*, 512–516.
Schuster, T. (1963) Thesis: *Solution Properties of Myosin*, pp 194–196, Washington University, St. Louis, MO.
Shih, J. W., & Hash, J. H. (1971) *J. Biol. Chem. 246*, 994–1006.
Sobieszek, A. (1977) in *Biochemistry of Smooth Muscle* (Stephens, N. L., Ed.) pp 413–443, University Park Press, Baltimore, MD.
Spackman, D. H., Stein, W. H., & Moore, S. (1958) *Anal. Chem. 30*, 1190–1206.
Szent-Gyorgyi, A. G. (1953) *Arch. Biochem. Biophys. 42*, 305–320.
Wachsberger, P., & Kaldor, G. (1971) *Arch. Biochem. Biophys. 143*, 127–137.
Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406–4412.
Wu, C.-S., & Yang, J.-T. (1976) *Biochemistry 15*, 3007–3014.

Conformational Transitions and Vibronic Couplings in Acid Ferricytochrome *c*: a Resonance Raman Study†

A. Lanir, Nai-Teng Yu,* and R. H. Felton

ABSTRACT: Resonance Raman spectral changes in ferricytochrome *c* as a function of pH between 6.7 and 1.0 are reported and the structural implication is discussed in terms of the "core-expansion" model advanced by L. D. Spaulding et al. [(1975) *J. Am. Chem. Soc. 97*, 2517]. The data are interpreted as indicating that the iron in high-spin acid ferricytochrome *c* (at pH 2.0) with two water molecules as axial ligands lies in the plane of the porphyrin ring. At pH 1.0 there is a different high-spin form of cytochrome *c* which has an estimated iron out-of-plane distance of ~0.46 Å. The effect of a monovalent anion at pH 2.0 is to produce a thermal spin mixture with predominant low-spin species. Excitation at ~620 nm in acid cytochrome *c* (pH 2.0) enhances only three depolarized ring vibrations at 1623, 1555, and 764 cm^{-1}. Marked enhancement of depolarized modes relative to polarized and anomalously polarized modes is attributed to the vibronic coupling between porphyrin $\pi \rightarrow \pi^*$ and porphyrin $\pi \rightarrow$iron(d_π) charge-transfer states.

Cytochrome *c* undergoes an interesting structural transition at pH 2.5 (Boeri et al., 1953; Gupta & Koenig, 1971; Fung & Vinogradov, 1968; Lanir & Aviram, 1975) which converts the native low-spin heme to a high-spin form with a concomitant displacement of the axial ligands by two water molecules (Lanir & Aviram, 1975). Addition of anions such as Cl^- or ClO_4^- causes some reversal in the spectral and magnetic changes which accompany the titration (Boeri et al., 1953; Aviram, 1973). The question of whether the iron in acid, high-spin ferricytochrome *c* is in-plane or out-of-plane is of

† From the School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia 30332. *Received December 12, 1978.* This work was supported in part by the National Institutes of Health (Grants GM 18894 and AM 14344).

* Recipient of National Institutes of Health Research Career Development Award EY 00073 (1976–1981).

0006-2960/79/0418-1656$01.00/0