[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY AND THE DEPARTMENT OF BIOLOGY HARVARD UNIVERSITY]

The Molecular Weight, Size and Shape of the Myosin Molecule

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Light scattering, viscosity and ultracentrifuge measurements on solutions of myosin indicate that the molecule is a rod 1620 Å. long and 26 Å. thick and has a molecular weight of 493,000. These molecular parameters are unaffected by variation of (1) ionic strength from 0.4 to 2.0 *M* at *p*H 7, (2) *p*H from 6–9 at ionic strength 0.6 *M*, (3) temperature from 1–30° at *p*H 6–7 and ionic strength 0.60 *M*, and (4) ATP concentration. Using the data, it is shown that the myosin molecule cannot be a single helical polypeptide chain. Other possible chain structures are discussed. In conjunction with recent data on the meromyosins these results indicate that one H-meromyosin and one L-meromyosin fragment are combined end-to-end in the intact myosin molecule. Combined with dimensions from recent electron microscope studies these data require that the myosin-containing filaments in the A-band of striated muscle be 10 myosin molecules long and about 4–5 molecules in diameter.

Introduction

Recent studies of resting, contracted and stretched striated muscle fibrils by light[2] and electron-microscopy[3,4] have led to a model of muscular contraction that focuses attention on the interaction of actin and myosin even more strongly than did the earlier work of the Szeged school.[5] At the same time, microscopic,[6] biochemical[7] and immunochemical[8] experiments have led to increased knowledge of the positions of the various protein components in the sarcomer. Further progress toward a molecular theory of muscle action must depend, in part, upon a more precise determination of the mass and dimensions of these protein components and of the physico-chemical nature of the interaction between them. The interaction of actin and myosin, whether in the muscle itself, in the gel state, or in solution, has long been known to be sensitive to such variables as ATP concentration, ionic strength, *p*H and temperature.[5] Consequently, a complete study of the formation and properties of the actomyosin complex must involve measurements of the effects of these variables. As a first step in such a study, it is essential to perform control experiments on the individual protein components themselves.

It is also true that techniques of macromolecular characterization have been much improved since the earlier work and, furthermore, it recently has become clear that a good part of earlier difficulties were caused by aggregation of the myosin.[9,10] For these reasons we have undertaken a study of the molecular state of rabbit myosin in solution. Following a preliminary report of results obtained under the most common experimental conditions (0.6 *M* KCl, 25°, *p*H 6–7),[11,12] we wish to present here a more complete summary of light scattering, viscosity and sedimentation measurements of myosin solutions under these, and a variety of other conditions.

Recent work has shown that the aforementioned aggregation of the myosin molecules occurs spontaneously in a side-to-side manner.[10] We have studied the effects of various reagents on this aggregation reaction. The results of this latter investigation are reported in the accompanying paper.

Experimental

Reagents.—All solutions were prepared with reagent grade chemicals and water that was either redistilled in an all Pyrex system, or treated with ion-exchange resin. The ATP (adenosine triphosphate) was obtained as the crystalline, disodium salt from the Pabst Brewing Company. It was neutralized with potassium hydroxide before use.

Preparation of Myosin.—Myosin was prepared from rabbit muscle by the method of Szent-Gyorgyi[5] and Mommaerts.[13]

All operations subsequent to the killing of the rabbit were carried out at a temperature of 5°, with pre-cooled solutions. Only the muscles of the back and hind legs were used. The extractant was one liter of a solution of the following composition: 0.3 *M* KCl, 0.1 *M* KH_2PO_4, 0.05 *M* K_2HPO_4, 0.015 *M* neutralized Versene. After precipitation and redissolution of the myosin, actomyosin was precipitated by lowering the ionic strength to about 0.3 and centrifuging the solution in the No. 21 rotor of the Spinco model L preparative ultracentrifuge at 10,000 r.p.m., in Lusteroid tubes with special caps made of bakelite impregnated Nylon.[12] The myosin was then re-precipitated and the precipitate dissolved in enough 2 *M* KCl to bring the ionic strength to about 0.6 *M*; the amount of 2 *M* KCl added depended on the bulk of the precipitate. The solution was stirred slowly overnight.

Suspended fat was removed by centrifuging in the No. 21 rotor at 15,000 r.p.m. for 3 hr. and then pipetting out the lower 3/4 of the solution with a capillary pipet inserted without removing the tube cap. This solution was then recentrifuged and the lower 3/4 collected in like manner. Additional centrifugation revealed no fatty layer and did not diminish the turbidity. The solution was dialyzed *vs.* 0.6 *M* KCl solution and then stored in the cold room after adding a crystal of thymol. At no time after the death of the rabbit and before the actual measurement of the physical property of interest was the protein exposed to temperatures above 5°; and at no time after cutting up the muscle was the protein exposed to metal ions or metal surfaces. Because of the tendency of the protein to aggregate, studies on the monomer were performed within 70 hr. after death.

Concentration Determination.—Protein concentrations were determined by Nesslerization.[14] One to three-ml. aliquots (containing about 0.6 mg. of protein each) were di-

(1) Most of this work was carried out while the authors were at the Sterling Chemistry Laboratory, Yale University, New Haven, Conn. Now at Department of Chemistry, Washington Univ., St. Louis, Mo.

(2) J. Hanson and H. E. Huxley, *Nature*, **172**, 530 (1953).

(3) H. E. Huxley, *Biochim. Biophys. Acta*, **12**, 387 (1953).

(4) H. E. Huxley, *J. Biophys. Biochem. Cytolcgy*, **3**, 631 (1957).

(5) A. Szent-Gyorgyi, "The Chemistry of Muscular Contraction," 2nd Ed., Academic Press, New York, N. Y., 1951.

(6) H. E. Huxley and J. Hanson, *Biochim. Biophys. Acta*, **23**, 229 (1957).

(7) J. Hanson and H. E. Huxley, *ibid.*, **23**, 250 (1957).

(8) H. Finck, H. Holtzer and J. M. Marshall, *J. Biophys. Biochem. Cytology*, **2**, Number 4, part 2, supplement, 175 (1956).

(9) K. Laki and W. R. Carroll, *Nature*, **175**, 389 (1955).

(10) A. Holtzer, *Arch. Biochem. Biophys.*, **64**, 507 (1956).

(11) A. Holtzer and S. Lowey, THIS JOURNAL, **78**, 5954 (1956).

(12) S. Lowey, Ph.D. Thesis, Yale University, 1958.

(13) W. F. H. M. Mommaerts and R. G. Parrish, *J. Biol. Chem.*, **188**, 545 (1951).

(14) F. C. Koch and T. L. McMeekin, THIS JOURNAL, **46**, 2066 (1924).

gested with concd. H_2SO_4, along with a blank and an $(NH_4)_2SO_4$ standard solution (0.100 mg. N/ml.). The colorless digest then was diluted to the 35-ml. mark with water and 15 ml. of Nessler reagent was added by pipet. One-half hour was allowed for complete development of color. Optical density at 425 mμ was compared to the blank in a Beckman Model B or DU spectrophotometer. Nitrogen concentration was calculated by comparison with the standard solution. The protein was assumed to be 16.1% nitrogen.[5] Reproducibility in determinations on duplicate samples was usually $\pm 3\%$.

Refraction Index Increment.—dn/dc for myosin in 0.6 *M* KCl was measured at 436 mμ in a Brice–Phoenix differential refractometer manufactured by the Phoenix Precision Instrument Company.[15] The cell constant was determined with NaCl and KCl solutions over the same range of Δn values as used for the protein solution. The absolute Δn values for the salt solutions were taken from the data of Kruis.[16] Six values of the cell constant, obtained in this way, agreed to $\pm 0.5\%$. Four myosin solutions in the concentration range 0.23—0.95% protein were dialyzed *vs.* 0.6 *M* KCl and the refractive index difference between solution and dialyzate was measured. The protein concentration of each solution was determined by the Nessler method. An average value of 0.208 ml./g. $\pm$ 2% was obtained for dn/dc. The poor precision is entirely ascribable to the error in determining protein concentrations. Volumetric dilution of a single stock solution would give misleadingly precise values of dn/dc because of the danger of an error in the absolute value of the stock concentration.

This value of refractive increment differs slightly from the one previously assumed (0.200),[11] but the older scattering data, based on the latter figure, are corrected easily. Our value differs appreciably from the one obtained by Rupp and Mommaerts (0.191) on a large number of samples.[17] The cause of the discrepancy is unknown but probably involves the concentration determination rather than the value of Δn. Pending further information, it is undoubtedly wiser to use one's own value since systematic concentration errors in dn/dc and in $c/R\theta$ have a chance to cancel out, at least partially.

Viscosity.—Viscosity measurements were made with Ostwald-Fenske viscometers at 25.1 $\pm$ 0.02°. Flow times for 0.6 *M* KCl in the various viscometers ranged from 244.0 to 331.0 seconds. Flow times for myosin solutions ranged from 50 to 100 seconds over solvent flow time. The long efflux times made kinetic energy corrections negligible.

Solvent was filtered through UF sintered glass filters before use. Solutions were prepared for viscometry by centrifugation for 1 hr. at 15,000 r.p.m. The upper $^3/_4$ of the solution was pipetted out with a capillary pipet, without removing the tubes or rotor from the centrifuge. Portions of this stock solution were diluted to desired concentrations by addition of solvent and very slow, magnetic stirring. Efflux times on the same solution were reproducible to ± 1 second but only for a limited number of trials. With a larger number of trials, fiber formation in the capillary and foaming at the meniscus led to erratic readings. The concentration of the centrifuged stock solution was determined and values of η_{SP}/c were plotted *vs.* c and extrapolated to zero concentration.

Sedimentation.—All sedimentation runs were made in the Spinco Model E ultracentrifuge, usually operated at 59,780 r.p.m. with a 12 mm. cell. For concentrations below 0.1% the B rotor and 30 mm. cell were used at the maximum allowable speed of 50,740 r.p.m. The Kel-F cell centerpiece was used in all cases to protect myosin from metal contact. The operating temperature was usually between 20 and 30°, depending on room temperature. The rotor temperature was measured before and after each run and 0.8° subtracted from the average to correct for adiabatic expansion and contraction.

When the sedimentation patterns were to be used as a criterion of homogeneity of monomer solutions, the runs were made in the cold. This precaution is essential; otherwise a small but detectable amount of dimer is formed during the run. With careful pre-cooling of the rotor the mean temperature can be kept to about 1°.

Sedimentation constants were calculated from plots of log (distance) *vs.* time. The usual corrections for solvent viscosity and buoyancy were made. The results were plotted as $1/S_{20,w}$ *vs.* concentration and extrapolated to zero concentration.

Light Scattering.—All water used to clean pipets and cells was freed of dust by distillation in an all-Pyrex still designed to prevent splashing in the receiving vessel. Acceptable water shows only a few dust motes when examined in the light beam at low angles. Salt solutions were filtered with UF filters and examined before use.

Protein solutions in all-plastic tubes were clarified by 5 hr. centrifugation at 15,000 r.p.m. in the Spinco No. 21 rotor. The temperature in the rotor was 4°. Contrary to the observations of others,[17] we have found that passage of myosin through grade F sintered glass filters results in extensive denaturation. Further, even for solvent, grade UF filters are necessary, if valid scattering data are to be obtained. The solutions were removed from the rotor by seating a rack and pinion pipet holder upon the rotor (without removing the rotor from the well), and lowering the pipet half-way into the tube, using this device.[18] Mild suction then was applied automatically to remove the upper half of the solution. The pipet was removed, using the rack and pinion and the contents allowed to run down the inside wall of a scattering cell that had been rinsed exhaustively with dust-free water and dried in a vacuum desiccator. In experiments on myosin monomer, the light scattering pipet was the first contact, since the death of the rabbit, that the protein had with temperatures greater than 4°. Ten to fifteen minutes then elapsed before the actual measurement of the scattering. During this time the solution came to about 20° and was examined visually in the light beam.

If the solution was acceptable, the scattering was measured at fifteen angles between 30 and 135° using the unpolarized blue light (436 mμ) in a Brice–Phoenix photometer.[19]

The scattering cells used were of the Erlenmeyer type, with negligible back reflection.[20] The cells were calibrated in the usual manner.[21]

Results were interpreted using the Zimm method based on the well known equation[20]:

$$Kc/R\theta = 1/M_wP(\theta) + 2Bc$$

The effect of concentration on the scattering was not measured by dilution of the stock solution, since it was found that stirring in the cell led to aggregate formation and the appearance of "dust" in the solution. However, this is not a very serious difficulty in this system since, as in most proteins at high ionic strength, the second virial coefficient B is very close to zero. In our earlier work,[11,12] whenever two concentrations were measured, the values of $Kc/R\theta$ agreed within the experimental errors due to the individual determinations of concentration.[22] Consequently, the value of $Kc/R\theta$ at a single concentration, or the average value for two concentrations, was used as the correct value at $c = 0$ and plotted *vs.* $\sin^2(\Theta/2)$. The results of a more nearly complete determination of the scattering for six concentrations of the same sample entirely justify this procedure (*cf.* below).

The average radius of gyration $(\overline{\rho^2})^{1/2}$ then was determined from the initial slope of the $Kc/R\theta$ *vs.* $\sin^2(\Theta/2)$ plot in the usual way.[20]

Experimental Results

Purity of the Preparation.—Myosin prepared in the manner described above and tested immediately in the ultracentrifuge at low temperatures, shows only one hypersharp peak (Fig. 1). Admittedly this is not a rigorous criterion of monodispersity, but in this case, where any polydispersity is likely to be caused by the presence of aggre-

(15) B. A. Brice and M. Halwer, *J. Opt. Soc. Am.*, **41**, 1033 (1951).

(16) A. Kruis, *Z. physik. Chem.*, **B34**, 13 (1936).

(17) J. C. Rupp and W. F. H. M. Mommaerts, *J. Biol. Chem.*, **224**, 277 (1957).

(18) A. Holtzer and E. P. Geiduschek, unpublished work.

(19) B. A. Brice, M. Halwer and R. Speiser, *J. Opt. Soc. Am.*, **40**, 768 (1950).

(20) B. Zimm, *J. Chem. Phys.*, **16**, 1099 (1948).

(21) A. Holtzer, H. Benoit and P. Doty, *J. Phys. Chem.*, **58**, 624 (1954).

(22) Because of varying loss of protein during the long centrifuge times required, the concentration of the solution in the cell must be determined directly.

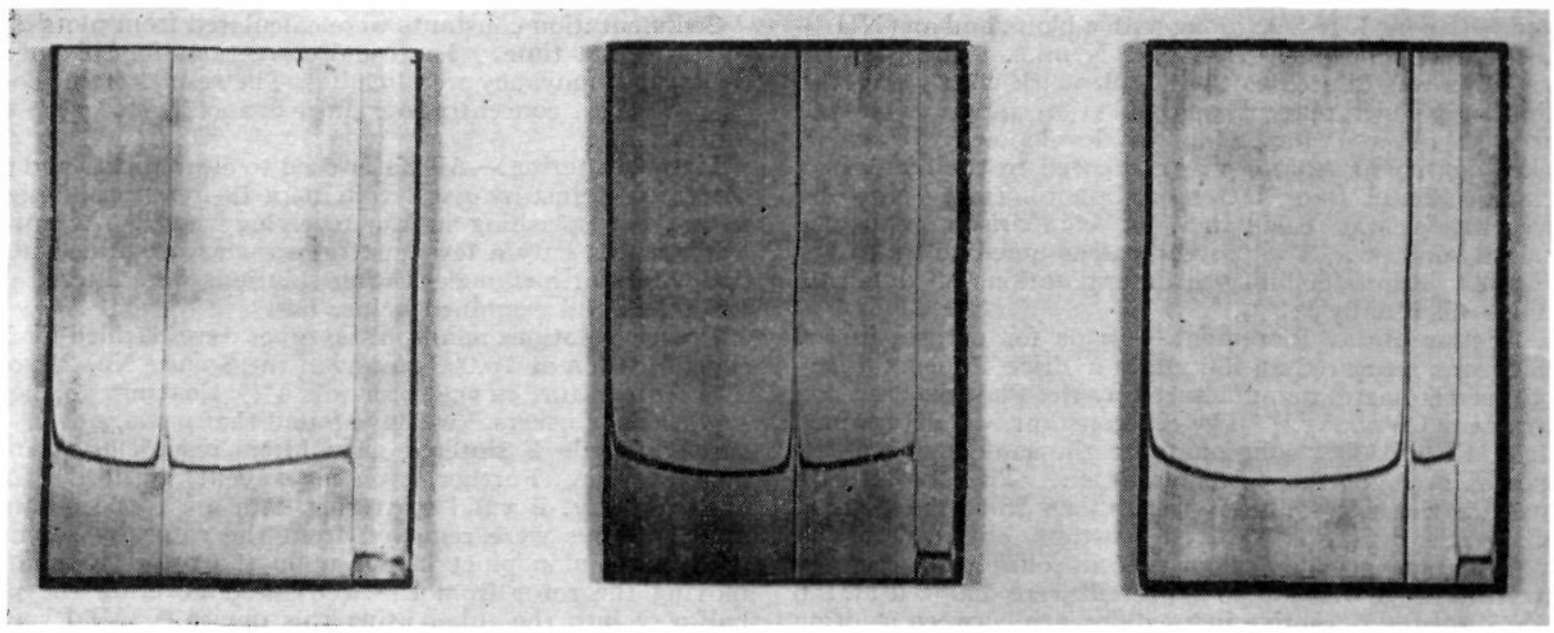

Fig. 1.—Sample MXII: 0.276% myosin in 0.6 *M* KCl (*p*H 6.3) at 1°. Speed, 59,780 r.p.m.; bar angle, 60°, time after reaching full speed; (a) 34 min., (b) 98 min., (c) 162 min.

gates of the myosin itself, homogeneity in the ultracentrifuge is a necessary, if not a sufficient, test. Further, it would be extremely difficult to measure homogeneity by any other method. Osmotic pressure measurements are notoriously imprecise in this region of molecular weight, and the pronounced tendency of myosin to aggregate under shear precludes the use of flow birefringence.[23] We accordingly assume throughout that measurements on solutions ultracentrifugally homogeneous refer to a monodisperse protein.

Viscosity in 0.6 *M* KCl, *p*H 6–7, 25°.—Results of viscosity measurements on four different monomeric preparations are shown in Fig. 2. As is

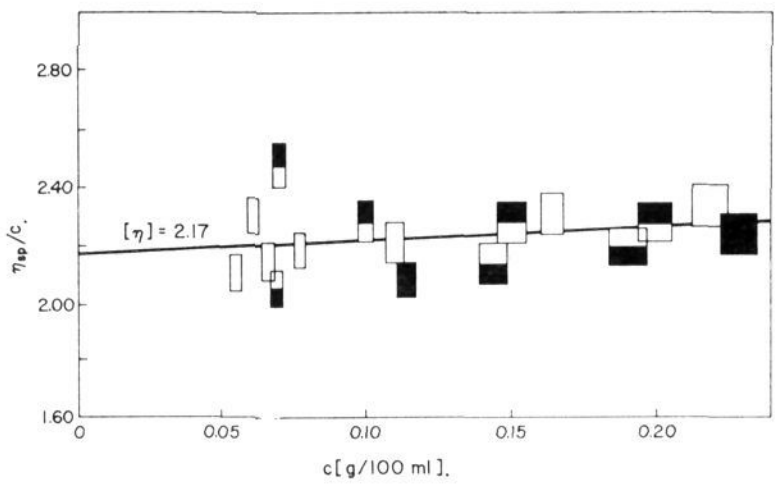

Fig. 2.—Reduced specific viscosity of myosin in 0.6 *M* KCl at 25° as a function of protein concentration: unfilled squares, MVII; filled squares, MVIII; upper half-filled, MX; lower half-filled, MXVI.

usual in protein solutions at high ionic strengths, there is little concentration dependence of η_{SP}/c. The least-squares straight line is given by $\eta_{SP}/c = 2.17 + 0.467c$. The intrinsic viscosity obtained, 2.17 ± 0.1 dl./g., is in good agreement with some previous work.[5,24] Sample to sample reproducibility is likewise seen to be good.

Sedimentation in 0.6 *M* KCl, *p*H 6–7, 25°.—Sedimentation data obtained for four different monomeric samples are plotted in Fig. 3. All points fall closely on the same line, given by least squares as: $10^{-13}/S_{20,w} = 0.156 + 0.114c$ and yield an intrinsic sedimentation constant of 6.40 ± 0.1 *S*. This compares with a value of 6.75 *S* obtained in the only study directly comparable.[25]

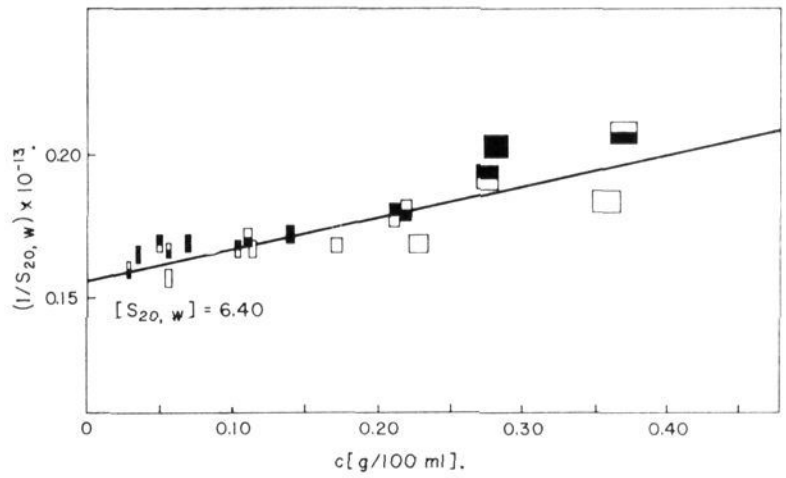

Fig. 3.—Reciprocal sedimentation constant of myosin in 0.6 *M* KCl at 25° as a function of protein concentration: unfilled squares, MVIII; filled squares, MXI; upper half-filled, MXII; lower half-filled, MVII.

However, the latter figure was obtained without making the correction for adiabatic expansion and contraction. The authors report that the use of this correction brings their value to 6.85 *S*. The difference is, we feel, barely outside experimental error. We have found that preparations may be very slightly aggregated (without actually showing a clear leading peak) and that these samples give a small apparent increase in the sedimentation rate of the main peak. It may be that this explains the small discrepancy.

Light Scattering in 0.6 *M* KCl, *p*H 6–7, 25°.—Some light scattering results already have been reported.[11,12] These were done as described above. In order to characterize the concentration dependence more exactly, six separate concentrations of the same preparation (Mxx) were simultaneously centrifuged and then pipetted into separate cells.

(23) Fractionation on modified cellulose columns and boundary spreading analysis at very low concentrations using interference optics remain as possibilities.

(24) H. Portzehl, *Z. Naturforsch.*, **5b**, 75 (1950).

(25) R. G. Parrish and W. F. H. M. Mommaerts, *J. Biol. Chem.*, **209**, 901 (1954).

All six were exceptionally clean (for unknown reasons) and were immediately measured in the photometer. Solutions waiting their turn were kept in the refrigerator and allowed to warm for 10 minutes before the measurement. The concentration of each was then determined by Nesslerization. The results are shown in the Zimm plot of Fig. 4.

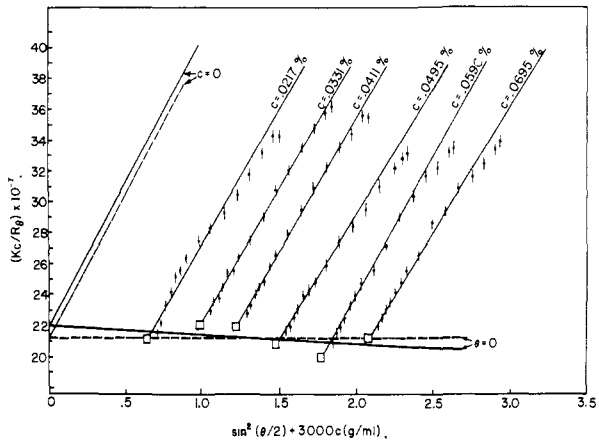

Fig. 4.—Zimm plot of MXX in 0.6 M KCl at 25°. Solid line at $\theta = 0$ drawn by least squares. Dashed line at $\theta = 0$ for B assumed to be zero. Details in text.

The considerable imprecision shown is explainable in terms of the rather large error in concentration measurements from sample to sample. In the usual Zimm method the stock is diluted in the cell and the amount of the solvent added is determined by weight (or, alternatively weighed amounts of stock are added to solvent). The individual values of the concentrations relative to stock are, therefore, extremely precise, and the plot is more impressive in appearance. Part of this exactness is illusory, however, because of possible inaccuracy in the value of the stock concentration.

In this case, since dilution in the cell is impossible because of the ensuing aggregation, the individual concentrations were each extrapolated to zero angle, and the least-squares straight line through the zero angle points was extrapolated to zero concentration. The resulting value of the second virial coefficient: -0.8×10^{-4} mole-cc./g.[2] is indeed small, and it is doubtful whether it is experimentally distinguishable from zero. It is seen from Fig. 4 that the $B = 0$ line fits the data virtually as well as the least squares line. Consequently, in the absence of more precise information, the value of B may be assumed to be zero.

In principle the zero concentration line should be drawn by extrapolating the measurements at each angle, by least squares, to zero concentration. The data are obviously not worth this computational effort, and since the initial slopes of all six measured concentrations are so similar, the $c = 0$ line was drawn to the proper intercept (obtained from the $\theta = 0$ line) with a slope that was taken as the average of the various individual slopes.

The results of these light scattering experiments on sample Mxx, some other new results (MXVI), and the previously obtained values, corrected for the experimental value of dn/dc, are summarized in Table I.

TABLE I

Sample	$M \times 10^{-3}$	$(\overline{p^2})^{1/2}$ (A.)
MVII	528	487
MVIII	468	473
MIX	487	470
MXVI	510	470
MXX	472	440
Av.	493 ± 50	468 ± 50

Reported molecular weight values for myosin have shown a consistent downward trend over the years.[9,11,17,26–28] In view of the precautions taken here to ensure the absence of aggregates, we feel that the error we have allotted to the value given above is to be taken seriously. In three other laboratories, recent studies, using light scattering[29] and the Archibald method,[30,31] have indicated a molecular weight of about 420,000. The agreement, we feel, is within experimental error. Our own measurements by the Archibald method give a value very close to 450,000.

The value of the radius of gyration is quite insensitive to the presence of aggregates[10] and is independent of systematic errors in dn/dc or concentration; it is our belief that the results for this parameter may be accepted with considerable confidence.

The Effect of Temperature.—At temperatures close to 1°, the concentration dependence of the sedimentation constant followed closely the least-square line: $10^{-13}/S_{20,w} = 0.148 + 0.176c$ (Fig. 5).

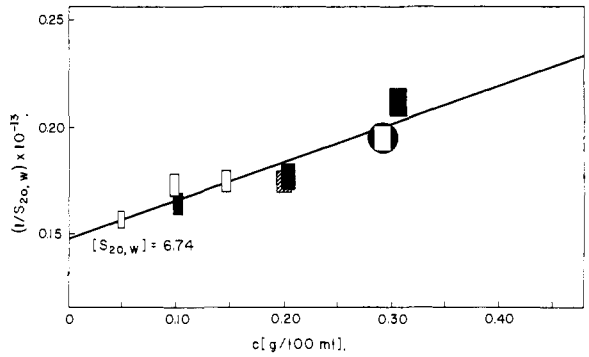

Fig. 5.—Reciprocal sedimentation constant of myosin in 0.6 M KCl at 1° as a function of protein concentration: unfilled squares, MXVI; filled squares, MXVII; barred square, MXIII. Filled circle is for MXVII at 25°.

Thus, the intrinsic sedimentation constant is 6.7 ± 0.1 S, slightly higher than at 25°, and the dependence on concentration is somewhat greater in the cold. However, the observed differences are barely outside the expected experimental precision, and since measured rotor temperatures are less reliable at the low temperature, it is doubtful that the difference is real. There is little question that

(26) O. Snellman and T. Erdos, *Biochem. Biophys. Acta*, **3**, 523 (1949).
(27) H. Portzehl and H. H. Weber, *Z. Naturforsch.*, **5b**, 2 (1950).
(28) W. F. H. M. Mommaerts, *Biochim. Biophys. Acta*, **4**, 50 (1950).
(29) J. Gergely, Biophys. Soc. Abstracts, p. 46, 1958.
(30) W. F. H. M. Mommaerts and B. B. Aldrich, *Biochim. Biophys. Acta*, **28**, 627 (1958).
(31) P. H. Von Hippel, H. K. Schachman, P. Appel and M. F. Morales, *ibid.*, **28**, 504 (1958).

not enough is known about the required correction factors to establish the reality of such small changes in sedimentation constant. For example, the partial specific volume $\bar{v}$ usually used for myosin, 0.728 ml./g., was measured at 26°.[25] In the only study of the temperature coefficient of $\bar{v}$ known to us it was found that the partial specific volume of hemoglobin decreased by 0.00038 ml./g.°C. in the relevant temperature range.[32] Applying the same coefficient to myosin would suggest a value $\bar{v}_{1°} = 0.719$ ml./g. If the low temperature sedimentation constant is corrected for this effect, it becomes 6.5 S in almost exact agreement with the value at higher temperature. As a more pragmatic test of the equivalence of $S_{20,w}$, measured at various temperatures, a single concentration of one of the samples was run at 25°, in the middle of the series of low temperature experiments. It is also plotted on Fig. 5 and is indistinguishable from the rest of the data.

These observations are in disagreement with those of Parrish and Mommaerts[25] and of Laki and Carroll,[9] who find that $S_{20,w}$ decreases with decreasing temperature in this range by about 0.5%/degree. The results of Laki and Carroll, however, were measured at only a single concentration of myosin and consequently can give no molecular information. The data given by Parrish and Mommaerts, although not reported in sufficient detail to permit exact analysis, suggest that the effect disappears at infinite dilution. This would argue very strongly against the inference that any change in molecular configuration occurs with temperature.

To examine this point more closely, viscosity measurements were performed at 25° and at 0.7° on the same sample (MXVI). The results are shown in Fig. 6. The two appear at first glance to be quite distinct, but a study of Fig. 2, (which also contains the data for MXVI at 25°) shows that if the low temperature points of Fig. 6 were to be plotted on Fig. 2, they would not stand out particularly. In other words, the differences shown in Fig. 6 may well be within probable error. Further, it is a deficiency of this experiment that measurements at both temperatures were performed in the same viscometers. Consequently, the flow times at 0.7° were much longer than those at the higher temperature. It may be that the longer exposure to shear caused some aggregates to form in spite of the low temperature. In agreement with this hypothesis, the results at 0.7° were somewhat less reproducible. Further work is planned on this point.

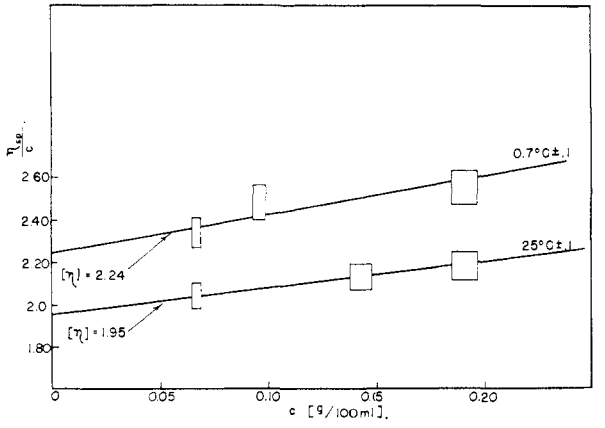

Fig. 6.—Reduced specific viscosity at MXVI in 0.6 M KCl as a function of concentration at 0.7° and 25°.

However, even if we accept the entire difference as real, the molecular changes involved are seen to be vanishingly small. The intrinsic viscosity is at most, 15% greater at the lower temperature. This would require an increase in axial ratio of only 7% (see equation 2, below). Molecularly, this would mean changes in length and/or diameter totaling 7%. The differences in $S_{20,w}$ observed by Parrish and Mommaerts amount to some 10% at a concentration of 0.25% and appear to be smaller at higher dilutions. Thus, from equation 1 below, we would expect the decrease in diameter with decreasing temperature to be smaller than 5% in the range 0–25°. If we arbitrarily, therefore, assume a 3% change in diameter, the viscosity results require that the length increase by about 4%. There is no macromolecular method currently known that is capable of detecting such small changes. For example, it would be entirely futile to measure the light scattering as a function of temperature in the hope of measuring a change in length of 4%!

An alternative explanation in terms of a reversible, endothermic dimerization cannot be brought into accord with the experimental observations since a side-to-side aggregation would increase $S_{20,w}$ (equation 1) by a factor of about two. Side-to-side aggregation may therefore be ruled out. An end-to-end aggregation would increase it by about 15% but would also increase the intrinsic viscosity by a factor of almost four. Furthermore, all the light scattering evidence indicates that, when myosin does aggregate, it does so almost strictly side-to-side and in an irreversible manner.[10] It should be emphasized that aggregation studies (see accompanying paper) do indicate that a temperature dependent configuration change may occur. However, the process is not instantaneous and is irreversibly and immediately followed by side-to-side aggregation. This process, therefore, has none of the features required for the phenomenon under current discussion.

In summation, our measurements of $S_{20,w}$ reveal a slight increase with decreasing temperature, in disagreement with earlier work. In our judgment all the observed discrepancies are not too large to be ascribed to a combination of experimental imprecision and inadequate knowledge of correction factors, and we would conclude that the sedimentation constant (corrected to water at 20°) at infinite dilution is independent of temperature between 1° and 26°. Our viscosity experiments indicate a small increase in intrinsic viscosity on lowering the temperature, but the change is also probably not significant. In any case, the magnitudes of all these changes would require alterations in molecular dimensions too small to be quantitatively assessed by any known method.

We conclude that the alleged temperature dependence of hydrodynamic properties of myosin

(32) Adair and Adair, *Proc. Roy. Soc.* (*London*), **A190**, 341 (1947).

solutions, which once appeared to be one of the outstanding features of this protein,[25] is, in all probability, devoid of molecular significance. It is likely that the effects were caused by a small amount of irreversible aggregation.

Perhaps it should be added that a dependence of $S_{20,w}$ on rotor speed also has been reported.[25] Unfortunately, a centrifuge run at 25° takes about an hour, if a precise value is to be obtained. At this temperature a considerable amount of aggregation occurs during this time. At lower rotor speeds, of course, runs take correspondingly longer. Frequently, in the early stages of aggregation, the aggregated material does not show up as a separate peak until quite far down in the cell. Estimates of sedimentation constants made in such solutions are consequently always too large. It is not surprising, therefore, that lower rotor speeds showed a greater increase of $S_{20,w}$ with temperature. In our experience the important variable is the total time the sample was kept at the given temperature, not the speed of centrifugation.

The Effect of Ionic Strength at *p*H 6–7.—In studying the effects of ionic strength (and of *p*H, see next section), we were interested in changes of molecular parameters. Consequently, these measurements were not always performed on solutions strictly monomeric. However a control solution of the same preparation in 0.6 *M* KCl, *p*H 6–7, was always run simultaneously. In all cases it was found (by measuring two concentrations) that the second virial coefficient was essentially zero. The refractive increment was corrected for differences in milieu, using an assumed value of 0.200 ml./g. in 0.6 *M* KCl, the densities of the other media, and the Gladstone–Dale relation.[33] Since absolute values were not required, we did not correct these molecular weights when the precise value of d*n*/d*c* became known.

The results and some of the controls for several samples are shown in Fig. 7. For the sake of clarity the control for MXVII is omitted. It coincided almost identically with the indicated control for MXII. It is clear that the molecule is unaffected by changes in ionic strength ranging from 0.40–2.0 *M* at *p*H 6.3. In 0.3 *M* KCl, however, aggregation occurs and precipitation soon follows on exposure to room temperature. Confirmation was obtained by heavy aggregation seen in the centrifuge patterns in 0.3 *M* KCl. At all other ionic strengths studied the sedimentation constants (corrected to water at 20°) are the same as in 0.6 *M* KCl. It is worth noting that the aggregation in 0.3 *M* KCl occurs with little change in molecular radius, indicating a similarity in mechanism to the aggregation in 0.6 *M* KCl, but proceeding at a more rapid rate.

These results explain the observation of Rupp and Mommaerts[17] that "high values of τ' (turbidity) occur at low KCl concentrations." The conventional interpretation of the decrease in solubility of myosin at low ionic strength in terms of the shift in isoelectric point caused by ion binding is probably correct.[5]

(33) P. Outer, C. Carr and B. H. Zimm, *J. Chem. Phys.*, **18**, 830 (1950).

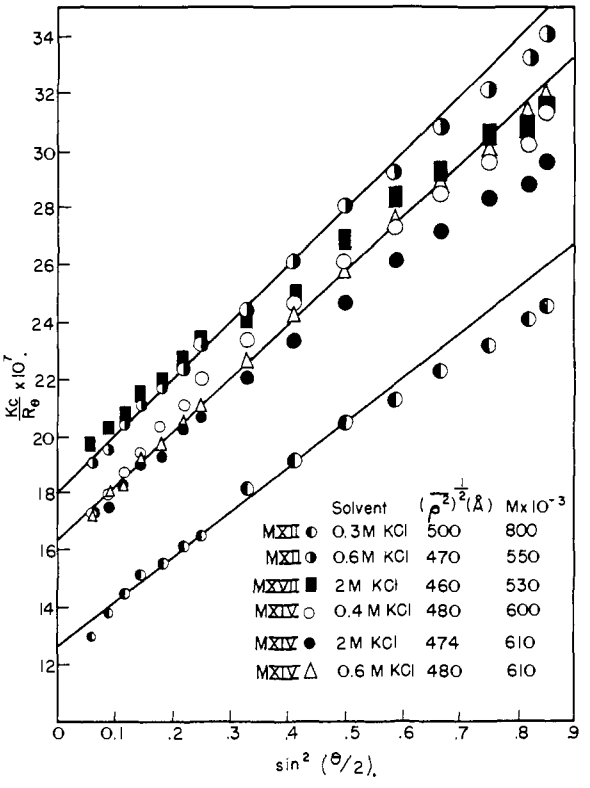

Fig. 7.—Reciprocal scattering envelope of myosin as a function of ionic strength at *p*H 6.3.

It is interesting that 2 *M* KCl, which has such a profound dissociating effect on the actomyosin complex,[5] does not alter the gross molecular properties of myosin at all.

The Effect of *p*H.—The *p*H of the myosin solutions was adjusted by dialyzing the samples against large volumes of buffered KCl solutions. The results for ionic strength 0.6 *M*, *p*H 6.3–9.0, are shown in Fig. 8. The control (0.6 *M* KCl, *p*H 6.3) for sample MXVI was not plotted because of overcrowding: it gave a molecular weight of 580,000 and a radius of 480 Å. The only apparent difference of any significance is thus the molecular weight of 630,000 (to be compared to 580,000) in *p*H 9 borate. This difference is barely outside experimental error and the value for the radius suggests strongly that the only process occurring here is a small amount of aggregation of the usual type.

The *p*H region 4–6 is inaccessible because of the precipitation of the protein, but, if the ionic strength is lowered from 0.6 *M*, the protein becomes soluble again at *p*H's lower than 4.[5] We found that, when dialyzed *vs.* salt-free, *p*H 2.9 buffer, myosin at first precipitated and then slowly dissolved; at $\mu = 0.15$ and the same *p*H, a small initial precipitate formed, which rapidly dissolved; and at this *p*H and higher ionic strengths the protein was insoluble. Consequently, we performed the light scattering at $\mu = 0.15$, *p*H 2.88, where we found molecular weights of several million and radii of about 800 Å. (Fig. 9). The ultracentrifuge showed a broad peak indicative of a highly polydisperse system. It is interesting to note that the ratio of

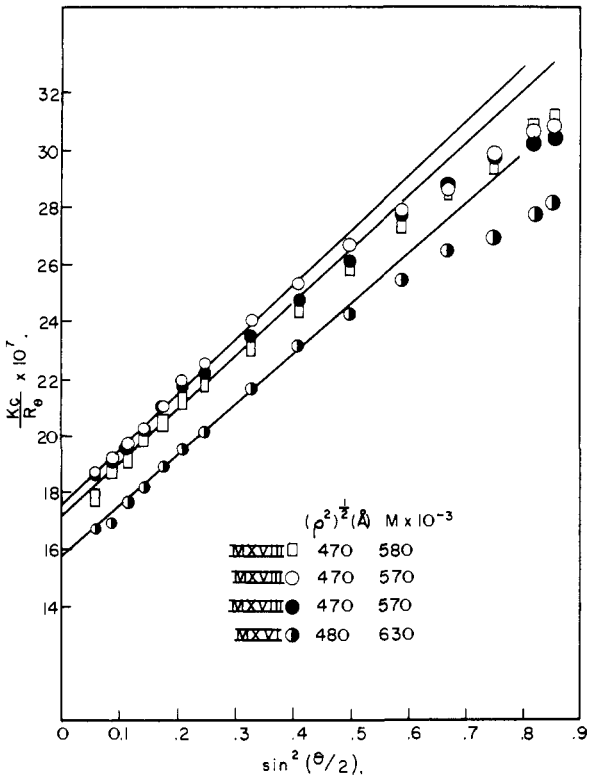

Fig. 8.—Reciprocal scattering envelope of myosin as a function of *p*H at ionic strength 0.6 *M*: unfilled squares, 0.1 *M* phosphate, *p*H 7.8; unfilled circles, 0.1 *M* borate, *p*H 8.5; filled circles, 0.6 *M* KCl, *p*H 6.3; half-filled circles, 0.1 *M* borate, *p*H 9.0.

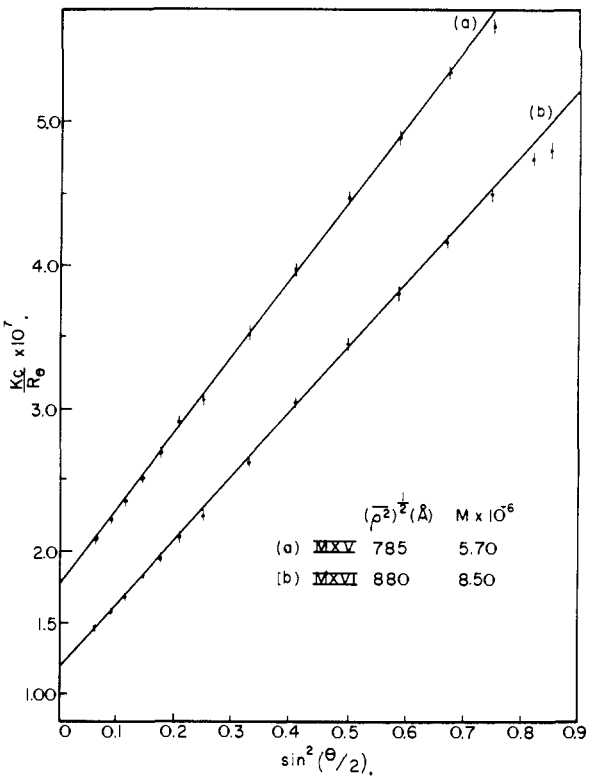

Fig. 9.—Reciprocal scattering envelope of two myosin samples at *p*H 2.9 in 0.1 *M* phosphate, 0.05 *M* KCl.

radius to mass indicates that, even in these highly aggregated samples, a substantial amount of the aggregation is side-to-side.

From these measurements we conclude that the gross molecular configuration of myosin is unaffected by varying (1) the ionic strength from 0.4 to 2.0 *M* KCl at *p*H 7, (2) the *p*H in the range 6–9, at $\mu = 0.6$, and (3) the temperature from 1 to 26° at $\mu = 0.6$, *p*H 6–7. Similar studies showed that the presence of 10^{-4} to 10^{-3} *M* ATP at $\mu = 0.6$, *p*H 7, also left the molecular parameters unchanged. At *p*H 2.9, $\mu = 0.15$, the myosin is inhomogeneous and highly aggregated, the average aggregate being over ten times as heavy as the monomer, and only twice as long.

It must not be inferred from this that the interaction between the myosin and these various media is unimportant. We simply imply that the profound effect these conditions have on the stability of the actomyosin complex must be a result of subtler, local interactions not accompanied by any gross changes in the molecular size or shape of the myosin. Indeed, the rate of aggregation of myosin *is* affected by these variables (*cf.* accompanying paper), and the extensive binding of various small ions by myosin is well known and incontrovertible.

Discussion

Gross Molecular Configuration.—The newer, more precise values for the molecular weight of myosin in no way alter our earlier conclusion that the molecule is rod-like in shape.[11,12] If the molecule were a uniform rod, the observed radius of gyration would require a length L of 1620 Å. To test the rod model we may use the present data, the equations of Kirkwood, *et al.*,[34,35] derived for a rigid string of spherical beads

$$[S] = \frac{(1 - \bar{v}\rho_0)d^2}{18\bar{v}\eta_0} \ln (6M\bar{v}/N\pi d^3) \quad (1)$$

$$[\eta] = \frac{24\bar{v}J^2}{9000 \ln J} \quad (2)$$

and the definition of partial specific volume for this model

$$\bar{v} = N\pi d^2 L/6M \quad (3)$$

where ρ_0 and η_0 are the density and viscosity of solvent, N is Avogadro's number, d is the molecular diameter and J is the axial ratio, L/d. Using also the value 0.728 cc./g. for the partial specific volume,[25] we find the values for the diameter summarized in Table II.

TABLE II

Source	d(Å.)
Sedimentation—light scattering—partial specific vol. (1)	27.8 ± 7%
Viscosity—light scattering—partial specific vol. (2)	23.5 ± 15%
Light scattering—partial specific vol. (3)	26.6 ± 15%
Av.	26.0 ± 3.0

The agreement is seen to be within the experimental error estimated from the errors for the individual parameters. The average value indicates a diameter of 26 Å.

(34) J. G. Kirkwood and P. Auer, *J. Chem. Phys.*, **19**, 281 (1951).
(35) J. G. Kirkwood and J. Riseman, *ibid.*, **18**, 512 (1950).

The random coil model readily is shown to be inadequate using the well known relations[36]

$$(\overline{\rho^2})^{1/2} = \frac{(0.193)}{\sqrt{6}} \frac{M}{N_0} \frac{(1 - \bar{v}\rho_0)}{\eta_0[S]} \quad (4)$$

$$(\overline{\rho^2})^{1/2} = \left[\frac{[\eta]M}{(6)^{3/2}(2.2 \times 10^{21})}\right]^{1/3} \quad (5)$$

The first expression gives a value of 279Å. ± 15%, and the second yields 322 A ± 8% for the radius of gyration. Both of these are far lower than the measured value of myosin.

Accepting the rod model, one may ask how these dimensions are to be related to the polypeptide chain structure of myosin. Since myosin is a member of the group of proteins giving an α-type X-ray diagram, we may assume, provisionally, the α-helix as the main structural arrangement. The amino acid analysis indicates that there are 4220 moles of amino acid residues per 500,000 grams of protein.[37] This would require 4160 residues per molecule. Using the known axial translation of the α-helix, we find that this many residues would form a rod 1.5 Å./residue × 4160 residues = 6250 Å. long, and according to molecular models[38] and polypeptide data,[39] about 10–15 Å. wide! It is evident, then, that the molecule is not a single helical chain, in agreement with the long standing observation that intact myosin contains no detectable end-groups. However, if four helices were to be close packed side-to-side, using the same number of residues, the length would be 1560 Å. and the diameter about 20–30 Å. in reasonable agreement with experimental values. There is other evidence, of course, that the structure of this molecule is more complex than this simple picture,[40] but it may serve as a rough guide.

Myosin Sub-structure.—We also may ask how the data for the intact protein are to be reconciled with the meromyosin sub-units obtained by enzymatic digestion of myosin. Contrary to earlier results,[41,42] recent studies[12,43,44] indicate that the molecule is broken, by tryptic digestion, into two parts of approximately equal length, the lighter fragment (L-meromyosin) having molecular weight 126,000 and the heavier (H-meromyosin) 324,000. About 6% of the total material is lost as dialyzable fragments.[45] These figures suggest a myosin molecular weight of 480,000 in rather close agreement with our observed value.

It also is shown readily that even the lighter fragment cannot be a single α-helix. The amino acid data for L-meromyosin show 794 moles of residues per 10^5 g. protein.[37] This means 954 residues per molecule. This would require a helical length of 1430 Å., about double the estimated length of L-meromyosin. The light fragment therefore has dimensions compatible with a close, side-to-side packing of two α-helices. The H-meromyosin fragment, on this model, would therefore have to have six helices in close packed array.

Perhaps it is not supererogatory to add that the newer data for the meromyosins yield a theoretical $P(\theta)^{-1}$ curve in almost exact agreement with the experimental curve for intact myosin, when the non-uniform distribution of mass along the myosin rods is accounted for.[46,47]

It also should be mentioned that this picture of myosin as a non-uniform rod alters somewhat the calculation of its length from the experimental radius of gyration. For a rod made up of meromyosin sub-units, with the proper masses and lengths, we calculate

$$\rho^2 = L^2/14.5$$

where 14.5 replaces the factor 12 for the uniform rod. The light scattering radius thus corresponds to a total length of 1780 Å., slightly longer than a uniform rod would be.

Myosin in the Sarcomer.—The most recent studies using the electron microscope suggest that the myosin-containing filaments in striated rabbit muscle stretch across the entire A band, including the H-zone, a distance of some 15,000 Å.[4] This would require about 10 myosin molecular units, arranged end-to-end. Except in the H-zone where they are somewhat thicker, and at the ends, where they taper, the myosin-containing filaments are about 115 Å. in diameter or about 4–5 myosin molecules thick.[4] This would mean that, if they are close packed, not all of the myosin molecules in the filaments have some free surface available for the formation of the presumed complex with the secondary filaments.

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