Molecular and Enzymatic Properties of Cardiac Myosin A as Compared with Those of Skeletal Myosin A*

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Previous studies have suggested that the molecular and enzymatic parameters of cardiac myosin are quite similar to those of its skeletal counterpart (1). Thus, the weight average molecular weight, $M_w$, weight intrinsic viscosity, $[\eta]$, and intrinsic sedimentation constant, $s_{20,w}$, are comparable with the corresponding data on the skeletal protein (2). Also, the enzymatic adenosine triphosphatase activity of cardiac myosin was found to possess some features similar to those of the skeletal enzyme. However, a distinct difference was observed in the value of the maximal velocity ($V_{max}$) of cardiac ATPase as compared with the skeletal enzyme. In order to elucidate the cause of this difference, more detailed studies of molecular and enzymatic properties of cardiac myosin were undertaken.

In this paper, further similarities in molecular and enzymatic properties of cardiac myosin were established. In its secondary structure, native cardiac myosin was shown to be not significantly different from its skeletal counterpart, as revealed by optical rotatory dispersion measurements. Cardiac myosin was found to be dissociable by aqueous guanidine hydrochloride into subunits similar in size to those obtained with skeletal myosin under comparable dissociation conditions. The kinetic data on cardiac myosin ATPase point to the existence of similarities between the two enzymes. However, the comparatively low value of $V_{max}$ and the slightly lower value of $K_m$ (reciprocal of $K_w$) with the cardiac enzyme probably reflect some differences in the structure of the 2 molecules, and this is demonstrated further by the different behavior of the cardiac molecule toward attack by the proteolytic enzyme, trypsin. Since the secondary structural characteristics of the two proteins are similar, it is concluded that the observed differences in enzymatic properties may reside in slight alteration of the tertiary structure of the cardiac molecule, probably in the "hydrophobic regions."

**EXPERIMENTAL PROCEDURE**

**Protein Preparation**—Myosin A was prepared from healthy normal dog hearts excised while still beating. The Nembutal-anesthetized dogs were kept under positive intermittent oxygen pressure during the excision of the hearts. The procedure used in our study is an adaptation of the method of Szent-Györgyi (3) as modified by Botta (1). The method involves extraction of myosin with a solution containing 0.1 m KCl, 0.05 m KH$_2$PO$_4$, 0.01 m MgSO$_4$, and 0.002 m ATP at pH 6.7 during 7 to 10 minutes; removal of myosin B at an ionic strength of 0.15 in the presence of ATP and Mg$^{++}$ at pH 7.5, followed by precipitation of myosin A at an ionic strength of 0.04; and repetition of the latter operation some three times. In contrast to skeletal muscle, it was found that cardiac muscle thus extracted yielded a comparatively large amount of myosin B, and therefore the separation of the latter was twice repeated. Myosin dissolved in 0.5 m KCl was dialyzed overnight against 0.2 m KCl-0.01 m Tris-0.001 m ATP at pH 7.4 and freed of any remaining myosin B contaminant by 1 to 2 hours' centrifugation at 50,000 $\times g$. In some cases, e.g. preparation of myosin for optical rotatory dispersion studies, the final dialysis was effected in the absence of ATP, and myosin was precipitated a fourth time, also in the absence of ATP, in order to avoid any ATP-protein binding. The material at this stage sediments as a single component in the ultracentrifuge (1). Preliminary chromatographic results on DEAE-cellulose (conditions described by Brahm (4), in 0.2 m KCl-0.01 m Tris in the presence of ATP) satisfied the criterion of protein homogeneity as well. Only homogeneous preparations were used for further studies.

Skeletal myosin was prepared according to the method of Szent-Györgyi (3).

Protein concentrations were determined by the method of Lowry et al. (5) and standardized by Kjeldahl protein determination.

**Preparation of Myosin in 5 M Guanidine Hydrochloride**—Cardiac myosin A solution was pretreated with a 10-fold excess of N-ethylmaleimide (with regard to -SH groups of myosin) for several hours at 4°C. The dialysis of myosin against 5 m guanidine hydrochloride was carried out at room temperature during a period of 1 week. The solvent was then dialyzed for 4 to 5 times; the first two changes of guanidine·HCl contained N-ethylmaleimide in order to avoid any possible formation of disulfide bridges. After dialysis, the volume of myosin solution in guanidine·HCl was adjusted to a given volume with 5 m guanidine·HCl solution. Protein concentrations in 5 m guanidine·HCl were alternately checked by optical density measurements at 276 m$\mu$ based on an $E_{276}^{	ext{max}}$ of 5.07, after Kielley and Harrington (2).

**ATPase Determinations**—ATPase activity was determined by following phosphate liberation according to the chemical method of Fiske and SubbaRow (6) or by electrometric titration by following proton liberation in the pH-Stat (Radiometer TTT1 equipped with a titrator and a titrigraph) during ATP hydrolysis in accordance with the equation,

$$\text{ATP}^- + \text{H}_2\text{O} \rightarrow \text{HPO}_4^{2-} + \text{ADP}^- + \text{H}^+$$

The pH-Stat reaction vessel was stirred by passage of water...
from a large constant temperature bath through the jacket surrounding the vessel. Plots of phosphate (or proton) liberation versus time gave straight lines, and the rates of dephosphorylation were inferred from the slopes of these zero order curves. The standard conditions of an ATPase determination were: 0.5 M KCl, 10^{-3} M ATP, 5 × 10^{-3} M CaCl2, and 0.1 M Tris at pH 8 and 25°C. The 0.1 M Tris buffer was not used in the case of the experiments in the pH-Stat.

**Sedimentation Velocity**—These measurements were made at 59,780 r.p.m. and at 20°C with the Spinco model E analytical ultracentrifuge equipped with an RTIC unit for temperature regulation. Sedimentation constants of cardiac myosin in 5 M guanidine hydrochloride were calculated from plots of log (distance) versus time. In order to correct the sedimentation constants in guanidine to the values pertaining in water, the experimentally determined values of 1.2651 centipoises and 1.1205 g per ml for the viscosity and density, respectively, of a 5 M guanidine hydrochloride solution were used. The quantity (b) in guanidine was assumed to be approximately 1% less than b in aqueous salt solution, as found by Kielley and Harrington (2) for skeletal myosin in the same solvent. This procedure may result in erroneous values of s_{20,w} if there is appreciable preferential binding of guanidine-HCl by the protein (7); this is probably not a serious factor in this study, however, since the binding of guanidine-HCl by skeletal myosin is only of the order of 5% of b (2).

**Viscosity**—A 1-ml Ostwald-type viscometer was used for all viscosity measurements. The capillary tubing of the viscometer was approximately 13 cm long and 0.8 mm in internal diameter. Outflow time for water at 20°C was 100 seconds.

**Molecular Weight**—Molecular weight determinations by the Archibald "approach to equilibrium" method were conducted as outlined by Schachman (7), and were performed at a speed of 9945 r.p.m. with the schlieren phase plate ranging from 70° to 80°. Fluorocarbon, FC-43, was introduced into a 12-mm Kel-f cell before the protein in order to facilitate measurements at the cell bottom; however, extrapolation was so extremely difficult here as to vitiate the reliability of any such measurements. Consequently, molecular weights were evaluated from the meniscus position exclusively, by the equation (8),

\[
M = \frac{RT}{(1 - \beta \rho) w^2} \frac{\langle dc \rangle_m}{\langle dx \rangle_m}
\]

and the equation of Klainer and Kegel for correction of concentration at the meniscus (9),

\[
c_m = c_0 - \frac{1}{x_m^2} \int_{x_m}^X x^2 \langle \frac{dc}{dx} \rangle_m \, dx
\]

where M, R, T, \beta, and \rho have their usual designations, \omega is the angular velocity, \omega_m and \langle \frac{dc}{dx} \rangle_m are the concentration and concentration gradient, respectively, at the position x_m, and X is an arbitrary x-coordinate in the plateau region (\langle \frac{dc}{dx} \rangle = 0); c_0 is the initial concentration of protein in the cell, determined by a companion run at 59,780 r.p.m. in a 12-mm, 4° sector synthetic boundary cell. A value of 0.720 ml per g (1% lower than \delta in H2O) was used for the partial specific volume of cardiac myosin in 5 M guanidine-HCl, after Kielley and Harrington (2). The technique of measuring the photographic plates is described in a previous publication (10). No appreciable salt distribution was detected at the speed employed.

**Optical Rotation**—Rotatory dispersion measurements were obtained with a Rudolph model 260 recording spectropolarimeter at the "medium" sensitivity range, where the reproducibility of the observed rotations is approximately ±0.01°. The wave length range employed was 320 to 700 μm, and the measurements were made on 1% solutions of native cardiac myosin in 0.5 M KCl and cardiac myosin in 5 M guanidine-HCl, contained in 5-cm path length cells. The data for the native protein were plotted according to the Moffitt equation (11),

\[
|\alpha|_\lambda = \left( \frac{100}{M} \right) \left( \frac{\alpha^2 + 2}{3} \right) \left( \frac{a_0 \lambda^2}{(\lambda^2 - \lambda_0^2)} + \frac{b_0 \lambda^4}{(\lambda^2 - \lambda_0^2)^2} \right)
\]

where \lambda_0 is 2100 Å and M is the average residue weight of 115 (12). The constant b_0 was considered proportional to the helix content. The data for cardiac myosin in 5 M guanidine-HCl were evaluated from a modified one-term Drude plot (13).

**Tryptic Digestion of Myosin**—The tryptic proteolysis of myosin was followed in the pH-Stat (Radiometer model TTT1 pH meter equipped with automatic recorder). Water from a constant temperature bath was circulated through the jacket surrounding the reaction vessel, and the temperature was maintained at 25°C. Some 4.2 ml of myosin in 0.5 M KCl (concentration, 4.2 mg per ml) were introduced into the pH-Stat cell, and the pH was adjusted to 7 with 0.01 M NaOH. Worthington trypsin in 0.005 M HCl was added to the solution in the ratio of 1:86 parts by weight, and the release of hydrogen ions was recorded continuously in the pH-Stat. The pH-Stat data were converted into an analytical graphical form similar to that used by Mihalyi and Harrington (14).

Another sample of cardiac myosin incubated with trypsin was run concurrently with the pH-Stat experiment. The reaction conditions were identical, except that the solvent medium, in addition to containing 0.5 M KCl, was buffered at pH 7 with 0.01 M phosphate. At desired time intervals, 1-ml samples of the digest were withdrawn, and the reaction was stopped by the addition of soybean trypsin inhibitor, in the weight ratio of 2.5 parts of inhibitor to 1 part of enzyme. The sample was then subjected to ultracentrifugal analysis. In the case of those sedimentation patterns in which two protein components were present, a correction taking into account the familiar radial dilution effect, but not the Johnston-Ogston effect, was introduced in order to determine relative concentration of the two components. For this purpose, the equation of Trautman and Schumaker (15) was used, viz.

\[
c_{12}^s = \left( \frac{c_{12}^m}{c_{12}^o} \right)^2 \cdot c_{12}^{obs}
\]

where \(c_{12}^s\) is the actual concentration of the slower component, \(c_{12}^{obs}\) is the uncorrected concentration of the slower component actually obtained from the area measurement (determined from a 20-fold enlargement of the plate), \(c_{12}^m\) is the position of the maximal ordinate at time t, and \(x_{20}\) is the meniscus position. A similar correction was made for the fast component.

**RESULTS AND DISCUSSION**

**Optical Rotatory Dispersion Studies on Native Cardiac Myosin**

A recent physicochemical study from our laboratory has established the molecular parameters of dog cardiac myosin (1).
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FIG. 1. Plot of \( [\alpha]_\lambda (\text{M} / 100) / (\text{X}^2 - \lambda^2)^{-1} \) versus (\( \text{X}^2 - \lambda_0^2 \)) for cardiac myosin in 0.5 M KCl. M is the average residue weight, \( [\alpha]_\lambda \) is the refractive index of the solvent, \([\alpha]_\lambda \) corresponds to the observed rotation at the wave length \( \lambda \) in question, and \( \lambda_0 \) is the dispersion constant (2120 Å).

The molecular weight is 758,000 and the weight intrinsic viscosity, \([\eta]_w\), is 2.09 dl per g, and the \( s_{20, w}^0 \) is 5.8 S. In order to compare the secondary structural characteristics of cardiac myosin with its skeletal counterpart, an optical rotatory dispersion study was carried out on the cardiac protein. At pH 7 in 0.5 M KCl, \([\eta]_w^0\) was found to be \(-31^\circ\), as opposed to a value of \(-27^\circ\) for the skeletal molecule (2). A Drude plot of the dispersion data deviated from the straight line typical of normal dispersion behavior; however, as seen in Fig. 1, the measurements did fit a Moffitt plot. The percentage of helix content was calculated from the \( b_0 \) value, evaluated from the slope of the plot on the assumption that figures of 0° and 64° characterize a random coil and a fully coiled right-handed \( \alpha \)-helix, respectively, of synthetic polypeptides (11). On this basis, the estimated \( b_0 \) value of \(-374^\circ\) corresponds to a helix content of 58%, which is essentially in agreement with the value of 56% for skeletal myosin (19). Evidently, then, there are no major secondary structural differences between cardiac and skeletal myosin.

Enzymatic Properties of Cardiac Myosin

The average value of ATPase activity, \( V_{\text{max}} \), determined with several cardiac myosin preparations, was 4 \( \mu \)moles of PO\(_4\) per g of enzyme per second (for conditions of the test, see “Experimental Procedure”). This is approximately one-third the value for skeletal myosin A.

\(^2\) The molecular weight of skeletal myosin is still an unresolved problem, in view of two recently reported widely divergent values. Lowey and Cohen (16), with the Archibald technique, have reported an \( M \) value of 470,000 with no concentration dependency, whereas Kielley and Harrington (2), using the same technique, have observed a marked concentration dependency for their molecular weight data and have deduced a value of 619,000 at infinite dilution. Our data on cardiac myosin (1) also show a marked concentration dependency and yield an \( M \) of 758,000 at infinite dilution, which is in decided disagreement with the value of 225,000 previously reported for this same protein (17). The higher \( M \) reported for dog cardiac myosin (758,000), as compared with skeletal, may be a reflection of a species difference. Recent hydrodynamic studies in our laboratory suggest that the monomeric molecular weight for dog cardiac tropomyosin is 90,000, as opposed to 33,000 for rabbit skeletal tropomyosin (18).

Effect of Ca\(^{2+}\) and Mg\(^{2+}\)—It is well known that Ca\(^{2+}\) is an activator of skeletal myosin and Mg\(^{2+}\) an inhibitor (in the presence of 0.5 M KCl). Experiments were carried out, therefore, to test the influence of these ions on cardiac myosin. Fig. 2 reveals that, under the conditions of the experiment (0.5 M KCl, 2 \( \times 10^{-3} \) M ATP, 0.1 M Tris buffer, pH 8.0, at 25°C), Mg\(^{2+}\) is an inhibitor and Ca\(^{2+}\) is an activator of cardiac myosin; these results are again reminiscent of the corresponding findings with skeletal myosin.

Kinetic Studies—Fig. 3 demonstrates the applicability of the Michaelis-Menten kinetic law with the cardiac enzyme; at saturating concentration of CaCl\(_2\) (5 \( \times 10^{-3} \) M) \( K \) is about 10\(^4\), slightly lower than the \( K \) values of 5 to 8 \( \times 10^4 \) reported by Morales and Hotta (20) and by Ouellet, Laidler, and Morales (21) for skeletal myosin. Furthermore, as shown in Fig. 4, the variation of the velocity of ATP hydrolysis by myosin with temperature (at high substrate concentration, \( 10^{-3} \) M ATP) yields a straight line Arrhenius plot. The heat of activation, \( \Delta H^\ddagger \), evaluated from the slope of this plot according to the equation,

\[
\Delta H^\ddagger = -\frac{Rd(\ln V_{\text{max}})}{ d(1/T) } - Rt
\]

is 12.9 kcal mole\(^{-1}\), in good agreement with the figure of 12.4 kcal

![Fig. 2. Effect of Ca\(^{2+}\) and Mg\(^{2+}\) on cardiac myosin ATPase (conditions, 0.5 M KCl, 0.1 M Tris, 2 \( \times 10^{-3} \) M ATP, pH 8, at 25°C). X, Mg\(^{2+}\); O, Ca\(^{2+}\).](http://www.jbc.org/)

![Fig. 3. Lineweaver-Burk plot of the reciprocal of the rate of hydrolysis as a function of the reciprocal of ATP concentration.](http://www.jbc.org/)
mole$^{-1}$ recorded by Ouellet, Laidler, and Morales (21) for skeletal myosin.

The inactivation of myosin was studied by incubating the enzyme solution in 0.5 M KCl at pH 7.4 in a constant temperature bath. At measured time intervals, the enzyme samples were withdrawn and pipetted into the reaction vessels, where ATPase activity was measured under standard conditions. A pH of 8 was chosen for the test, since the rate of inactivation is pH-independent from 7.5 to 8.5 (22, 23). In our experiments, the rate of inactivation was found to be a first order process with respect to time. At three different temperatures the following values of the first order rate constant of deactivation, $k_d$, were observed: $35^\circ$, $k_d = 5.71 \times 10^{-4}$ sec$^{-1}$; $30^\circ$, $k_d = 2.73 \times 10^{-4}$ sec$^{-1}$; $25^\circ$, $k_d = 0.27 \times 10^{-4}$ sec$^{-1}$. The variation of the rate of inactivation as a function of temperature followed the Arrhenius law. The plot of $\log k_d$ against $1/T$ indicated an energy of activation in the range between 50 and 60 kcal mole$^{-1}$ (of the order of 54), which is in agreement with the figures of 56 and 57 kcal mole$^{-1}$ recorded by Pelletier and Ouellet (22) and by Yasui et al. (23) at pH 8.0 and 7.5, respectively, for skeletal myosin.

**Physicochemical Properties of Cardiac Myosin in 5 M Guanidine-HCl**

Kielley and Harrington (2) have demonstrated that rabbit skeletal myosin is dissociated in aqueous 5 M guanidine-HCl into polypeptide chains of similar, if not identical, mass (219,000). It was of interest to observe whether the cardiac myosin molecule exhibited similar behavior in this solvent system.

Initially, the degree of unfolding of cardiac myosin in 5 M guanidine-HCl was established by optical rotatory dispersion measurements in this solvent. The specific levorotation of myosin increases from an $[\alpha]_{589}^2$ of $-31^\circ$ to $-127^\circ$ when the protein is transferred from 0.5 M KCl to 5 M guanidine-HCl. Moreover, the value of the Drude parameter, obtained from a plot of $\lambda_0[\alpha]$ versus $\alpha$ (see Fig. 5), was evaluated as 215 mg. By the criteria of $\lambda_c = 215$ mg and $[\alpha]_c = -127^\circ$, complete transition to the random polypeptide form seems to have occurred when the protein was transferred to 5 M guanidine-HCl. Cardiac myosin in this solvent could have one of three possible conformations on the basis of only $\lambda_c = 215$ mg (24): a random coil, a $\beta$ structure, or a left-handed helix. However, the $[\alpha]_c$ value is in better agreement with the values expected for a random coil than for the other two conformations (24).

The protein, in 5 M guanidine-HCl, was then subjected to ultracentrifugal analysis in a synthetic boundary cell (Fig. 6). A single sedimenting peak, uncontaminated by low or high molecular weight material, was obtained at all of the concentrations employed (1 to 4.5 mg per ml), which suggests that cardiac myosin in 5 M guanidine-HCl sediments as a single weight class. A plot of $s_{20,w}$ versus concentration (grams per 100 ml) was found to be linear over a concentration range of 1 to 4.5 mg per ml and yielded, by least squares analysis, the equation,

$$s_{20,w} = 3.58 - 1.76 \times c$$
The low intrinsic sedimentation constant of 3.58 S for cardiac myosin in 5 M guanidine-HCl suggested that the molecule was dissociated by this solvent into smaller molecular weight units. As ancillary information confirming the decrease in size of cardiac myosin in 5 M guanidine-HCl, the reduced viscosity of the molecule was determined in this solvent as a function of protein concentration. A plot of the data is presented in Fig. 7.

The points in the figure represent average values obtained by analysis at several time intervals. The molecular weight shows a marked concentration dependency and yields a value for \( M \) at infinite dilution of 207,000, which is essentially in agreement with the value obtained with skeletal myosin (2). Since the cardiac myosin subunits approach random chains in 5 M guanidine-HCl, it is possible to determine their approximate molecular weight by employing the Scheraga-Mandelkern equation (25). If a \( \beta \) value of \( 2.5 \times 10^6 \), the value commonly accepted for a random chain (25), is assumed, the molecular weight deduced from this calculation is 180,000, which is in reasonable agreement with that determined by direct measurement.

Table 1 summarizes the physicochemical properties of both cardiac and skeletal myosin in 5 M guanidine-HCl. It is to be noted that the values for the cardiac molecule are essentially in agreement with the data on skeletal myosin under comparable dissociation conditions. This may be taken as further evidence against the hypothesis that native cardiac myosin is a naturally occurring subunit of skeletal myosin (17) since both molecules are approximately of the same size and behave similarly in dissociating media.

**Tryptic Digestion of Cardiac Myosin**

It is known that the primary, secondary, and tertiary structures of a protein all have great influence on the susceptibility of the molecule to attack by proteolytic enzymes; i.e. either factor alone or a combination of all three may favor or hinder the cleavage of the protein by the enzyme. In order to compare the structural features of cardiac and skeletal myosin with regard to proteolytic enzyme susceptibility, cardiac myosin was submitted to tryptic digestion, and the proteolysis was followed in both the pH-Stat and the ultracentrifuge.

The pH-Stat data were converted into an analytical graphical form similar to that used by Mihalyi and Harrington in following the tryptic digestion of skeletal myosin (14). The first order rate law, in its integrated and logarithmic form, yields

\[
2.3 \log (A_o - A) = 2.3 \log A_o - k t
\]

where \( A \) is the number of bonds cleaved in time \( t \) (deduced from alkaline uptake) and \( A_0 \) is the number of susceptible bonds in the molecule. Thus, by plotting \( \log (A_0 - A) \) against time, one obtains a straight line for a first order reaction, with slope equal to \(-k\) and ordinate intercept equal to \( \log A_0 \). Such a representation is shown in Fig. 9 for the tryptic proteolysis of cardiac myosin at an enzyme-substrate ratio of 1:86 at pH 7 and 25°C. There is an initial lag period of some 10 minutes, but beyond this time a single straight line relationship is obtained, indicating adherence of this phase of the reaction to first order kinetics; the first order velocity constant was evaluated from the slope of the plot as \( 0.8 \times 10^{-3}\) min^{-1}. This reaction differs in several respects...
Fig. 9. First order plot of the trypsin digestion of cardiac myosin as determined in the pH-Stat at pH 7 and 25°C. \( A_0 \) refers to the initial concentration of susceptible bonds, and \( A_t \), to the concentration of digested bonds. Enzyme to protein ratio = 1:86.

from the trypsin digestion of skeletal myosin: first, only with the cardiac protein is there a latent period of some 10 minutes, during which no proton liberation occurs; second, unlike the skeletal protein, for which the kinetics are described in terms of two simultaneous first order reactions, a fast and a slow one, the proteolysis of cardiac myosin occurs essentially at a single, uniform rate; and third, the cardiac myosin molecule is much more resistant to trypsin attack than its skeletal counterpart: the calculated \( k \) for digestion of the cardiac enzyme is 30 times smaller than the \( k \) value for the slow reaction and some 200 times smaller than the \( k \) value for the fast reaction described with skeletal myosin (14).

Sedimentation patterns obtained from samples removed at various time intervals from the digestion mixture are shown in Fig. 10. All of the photographs were taken under comparable conditions of bar angle (50°) and time (64 minutes). The first (A) represents the initial cardiac myosin peak at zero digestion time; B, taken after 12 minutes of digestion, illustrates some diminution of the native myosin peak and the appearance of a slower, broad peak, no doubt made up of peptides cleaved off the parent molecule; photographs C to E inclusive show the progressive decrease in the amount of the fast peak and the simultaneous buildup of the slow, broad peak; finally, F, representing 155 minutes of digestion time, shows only the slow broad peak. As further aid in interpreting these ultracentrifuge patterns, the sedimentation coefficients of the components were determined and their relative concentrations were calculated from the areas and corrected for radial dilution. A summary of the data is presented in Table II, from which the following points emerge. First, there is a progressive decrease in the amount of the fast component, paralleled by a steady increase in the amount of the slow component. This would indicate that the trypsin proteolysis of cardiac myosin is characterized by a continual transformation of the native molecule to lower molecular weight peptides. Second, the high \( s_{20, w} \) values relative to native myosin for the fast component at 30, 50, and 70 minutes of digestion suggest that aggregation phenomena are definitely occurring in this process. The rapidly spreading fast boundary observed at each of these times is probably made up of some undigested myosin and aggregates of partially digested myosin. Third, the rapidly spreading slow peak of relatively low \( s_{20, w} \) is no doubt made up of a large spectrum of digestion products, principally of relatively low molecular weight.

It is to be noted that this process with cardiac myosin is appreciably different from the trypsin digestion of skeletal myosin (14). Photograph G in Fig. 10 illustrates the well known appearance of the two meromyosins formed from a trypsin digest of skeletal myosin. With cardiac myosin, no meromyosin sub-

![Figure 9: First order plot of the trypsin digestion of cardiac myosin.](image-url)

![Figure 10: Ultracentrifuge patterns of myosin samples digested for various time intervals.](image-url)
weight peptide, which was made to estimate quantitatively the amount of low molecular from area measurements of visible patterns only, and no effort was made to estimate quantitatively the amount of low molecular weight peptide, which may not have moved away from the meniscus during the experiments.

The data presented here and in our previous study (1) indicate the existence of similarities in molecular parameters of cardiac myosin relative to skeletal as evidenced by a similar $M_o$, $[\eta]$, and $\bar{s}_{20, w}$. In addition, cardiac myosin undergoes dissociation in aqueous 5 m guanidine hydrochloride into smaller subunits, and these possess hydrodynamic parameters comparable with those obtained from skeletal myosin under similar dissociation conditions. Moreover, this similarity in molecular parameters and behavior in guanidine-HCl may be taken as evidence against the supposition that cardiac myosin is a naturally occurring subunit of skeletal myosin (17).

Table II

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<th>Digestion time (min)</th>
<th>Slow component</th>
<th>Fast component (undigested myosin and secondary products)</th>
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<tr>
<td></td>
<td>Relative concentration*</td>
<td>$s_{20, w}$</td>
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<tr>
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<td>100%</td>
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The observed differences in susceptibility to trypsin, and the kinetic parameters of enzymatic ATPase activity for cardiac and skeletal myosin, may reflect some alterations in the tertiary and secondary structures of the two molecules. Despite the fact that the total percentage of helix, as inferred from the $[\eta]$ value, is very similar for the two proteins, the ways in which the helical regions are distributed within the structure might be quite different. The important role of the hydrophobic regions for the enzymatic activity of cardiac myosin (27) supports the contention that slight differences in the hydrophobic regions of the two proteins might exist. It may well be that the two phenomena are interrelated; i.e. the tertiary structure in the hydrophobic regions has an important bearing on the helical structure in the case of myosin (27).

CONCLUSIONS

The molecular and enzymatic studies on cardiac myosin have permitted an extensive characterization of this protein, and a comparison to be made with its skeletal counterpart. From this comparison, similarities and distinct differences between the two proteins have emerged.

The observed differences in susceptibility to trypsin, and the kinetic parameters of enzymatic ATPase activity for cardiac and skeletal myosin, may reflect some alterations in the tertiary and secondary structures of the two molecules. Despite the fact that the total percentage of helix, as inferred from the $[\eta]$ value, is very similar for the two proteins, the ways in which the helical regions are distributed within the structure might be quite different. The important role of the hydrophobic regions for the enzymatic activity of cardiac myosin (27) supports the contention that slight differences in the hydrophobic regions of the two proteins might exist. It may well be that the two phenomena are interrelated; i.e. the tertiary structure in the hydrophobic regions has an important bearing on the helical structure in the case of myosin (27).

SUMMARY

This study reports additional molecular and enzymatic properties of cardiac myosin A and their comparison with those of skeletal myosin A.

1. Cardiac myosin A undergoes dissociation in aqueous 5 m guanidine-HCl into subunits of hydrodynamic properties that are comparable to those obtained with the skeletal protein. The $M_o$ is 207,000, the $[\eta]$, 1.00 dl per g, and the $s_{20, w}$, 3.58 S. By the criteria of $\lambda_c = 215$ m and $[\alpha]_c = -127^o$, complete transition to the random poly peptide form seems to have occurred in this solvent.

2. As with the skeletal enzyme, cardiac myosin is activated by Ca$^{2+}$ and inhibited by Mg$^{2+}$ (at high KCl concentration); it follows Michaelis-Menten kinetics, and its temperature dependence follows an Arrhenius plot. The thermal inactivation of cardiac myosin is characterized by first order kinetics with respect to time and possesses an energy of activation of the order of 54 kcal mol$^{-1}$.

3. The secondary structural characteristics of cardiac and skeletal myosin are essentially the same: the helical content of cardiac myosin, as inferred from optical rotatory dispersion, is 58%. However, some structural differences exist between the 2 molecules, as evidenced by their different behavior toward attack by trypsin. The cardiac protein does not yield products analogous to the meromyosins, and the digestion process is
characterized by a slow progressive degradation of the native molecule to relatively lower molecular weight peptides.

This is interpreted as indicating slight differences in the tertiary structure, in the distribution of the secondary structure of the 2 molecules, or both, probably caused by somewhat dissimilar hydrophobic regions.

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Molecular and Enzymatic Properties of Cardiac Myosin A as Compared with Those of Skeletal Myosin A
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