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High-Resolution Differential Scanning Calorimetric Study of Myosin, Functional Domains, and Supramolecular Structures†

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ABSTRACT: High-resolution differential scanning calorimetry (DSC) has been employed to study the thermal stability of myosin, its major constitutive fragments (S-1, light chains, and rod), and also reconstituted thick filaments. The thermal denaturation of soluble myosin was complex and was characterized by a multistep endothermic process for the temperature range from 41 to 60 °C. The shape of the endotherm was highly dependent on the pH and the ionic strength of the solution, although the ΔH_{cal} (calorimetric enthalpy) of denaturation (1715 ± 75 kcal/mol) was insensitive to these changes for the solvent conditions used in this study. This value also agrees, within experimental error, with the sum of the denaturation enthalpies obtained for isolated fragments (1724 ± 79 kcal/mol). In identical conditions of ionic strength, pH, and heating rate, the computer-calculated differential endotherms of domains belonging to S-1 and light chains were superimposable with those of the isolated fragments. Their responses to changes in the solvent condition were also similar. We suggest that the observed functional independence of the major domains in myosin reflects also the independence of their structural stability. The thermal unfolding of the isolated rod was multiphasic and readily reversible (95%). It occurred between 41 and 60 °C, with an ΔH_{cal} of 1058 ± 59 kcal/mol. The melting of S-1 showed a single peak at 46.3 ± 0.1 °C with an ΔH_{cal} of 255 ± 12 kcal/mol. Light chains melted at 51.0 ± 0.2 °C with an ΔH_{cal} of 85 ± 15 kcal/mol. Despite the reversibility of the rod, the thermal denaturation of myosin was irreversible. When the ionic strength of the solution was gradually reduced to obtain synthetic filaments, the transition temperatures of domains converged, and the shape of the endotherm appeared sharp, with little effect on the overall enthalpy of denaturation (ΔH_{cal} of 1679 ± 98 kcal/mol). The peak of the overall transition of myosin filaments was 45 °C in 0.2 M KCl and increased to 49 °C in 0.1 M KCl. Electron micrographs showed different morphologies for filaments grown in an imidazole buffer and that grown in a phosphate buffer although the endotherms of these samples appeared similar. pH had a strong effect on the endotherms of these filaments. Lowering the pH from 7.0 to 6.7 in 0.1 M KCl increased the T_m of the main peak by 1.4 °C, resulting in the separation of the endotherm into two distinct transitions. These two transitions were broad, and their ΔH_{vH} values were much smaller than the ΔH_{cal}. Analysis suggests that the structural domains that constitute a myosin molecule also exist in these filaments and that the stability of these structural domains is equally sensitive to modulation by the ionic strength and the pH of the solution, consistent with the possibility of local structure events in the molecular mechanisms of muscle contraction.

In the attempt to elucidate the molecular mechanisms of muscle contraction, great emphasis has been given to the study of the thermal stability of myosin. Myosin is a chimeric protein. The globular heads (S-1) retain both its ATPase activity and the ability to move actin cable in vitro (Harada et al., 1987) even when they are separated from the fibrous tails in which the aggregation properties of myosin are located (Young et al., 1963). The tendency for myosin to coagulate during heat denaturation (Lowey & Holtzer, 1959) has limited the study with optical spectroscopies, such as absorption,

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fluorescence, circular dichroism, and light-scattering spectroscopies, and viscometry to conditions in which myosin is soluble in solution. Little is known about the thermal behavior of the protein in organized structures. Attempting to overcome this difficulty, Goodno and co-workers (Goodno & Swenson, 1975a,b; Goodno et al., 1976) have monitored solution pH during the thermal denaturation of myosin and its fragments. Proton was absorbed upon the thermal unfolding of myosin and S-1. However, no systematic studies have been reported for synthetic filaments of myosin. If a localized conformational change is responsible for contraction, whether it is in the helical rod (Harrington, 1971, 1979) or in the globular head (Botts et al., 1989), it must be energetically feasible, and the local change must also be independent of the overall structural transition. The presence of independent melting domains has been demonstrated previously for the rod (Potekhin & Privalov, 1978; Potekhin et al., 1979). However, these results do not ensure that myosin and its more complex assemblies would also behave as rod does in solution and that the domains which exist in rod would exist in these molecular assemblies. Coagulation of myosin after thermal denaturation seems to refute such an assumption.

To answer these questions and to investigate the possible role of local structural changes in muscle contraction, we have undertaken a systematic study of the energetics of the thermal unfolding of myosin, its proteolytic fragments, reconstituted thick filaments, and myofibrils using high-sensitivity differential scanning microcalorimetry. DSC is a unique technique for investigating thermal stability of macromolecules and their insoluble assemblies. The turbidity of a sample does not interfere with the DSC measurement, and most importantly, a DSC measurement can provide energetic information which is essential for the thermodynamic analysis of the contraction process. This paper is one part of such systematic studies.

Materials and Methods

Materials. Chemicals were purchased from Baker (Phillisburg, NJ), and α-chymotrypsin type VII, TLCK treated, was purchased from Sigma (St. Louis, MO). Unless specified, proteins and chemicals used in these experiments were of the purest grade available.

Protein Purification. Myosin was purified from paravertebral muscles (latissimus dorsi) of white New Zealand male rabbits according to Segall and Harrington (1967). After the ammonium sulfate precipitation step, the myosin solution was applied to a DEAE-Sephadex A-50 ion-exchange column for further purification, following the procedure of Geoffrey and Harrington (1970). Pure myosin was used fresh or stored at 4 °C for not more than 1 week.

Rod was obtained by α-chymotryptic digestion of purified myosin. Myosin was dialyzed overnight against imidazole buffer (10 mM, pH 7.00), containing 0.1 M KCl and 1 mM EDTA. The protein concentration was adjusted to 1–3 mg/mL, and α-chymotrypsin (final concentration of 80–100 μg/mL) was added. The reaction was run at 4 °C for 10 min and was stopped by adding PMSF to a final concentration of 0.1 mM. The digested solution was adjusted to pH 5.2 and centrifuged for 5 min at 3000*g* (5000 rpm using an SS-34 rotor) in a Sorvall RC2-B centrifuge. The pellet which contained the rod was resuspended in a buffer containing 50 mM KP_i, 0.5 M KCl, and 10 mM EDTA at pH 7.0 to a final protein concentration of 1–3 mg/mL. Three volumes of 96% ethanol were added, and the mixture was stirred at room temperature for 1 h. Precipitated proteins were recovered by centrifugation (3000*g* for 5 min), resuspended in the same buffer, and degassed under vacuum for 15 min to remove ethanol. The protein solution was dialyzed overnight against the same buffer. The undigested and denatured myosin precipitated and was removed by centrifuging for 1 h at 38000*g* (20000 rpm in a Spinco 50.2 Ti rotor, average radial distance 81.2 mm, using a Beckman L8-M class H centrifuge). Rod was recovered from the supernatant by lowering the pH to 5.2, resuspending in the same buffer at a protein concentration of 0.1–0.5 mg/mL, and centrifuging for 2 h at 150000*g* (40000 rpm in a 50.2 Ti rotor). Purified rod was finally recovered by using pH precipitation. The purity of the rod preparations used for these experiments was 95–98%, assessed by SDS gel electrophoresis, and preparations with greater than 5% contamination were discarded. The only contaminants present were two bands with lower molecular weights (supposedly light meromyosin).

S-1 was purified following the procedure of Weeds and Taylor (1975). Further ammonium sulfate fractionation was done. The fraction from 45% to 50% was collected, pooled, and dialyzed overnight against the buffer used for DSC experiments. Fresh samples or samples prepared within a few days were used in DSC experiments.

Myosin light chains were obtained from purified myosin by using the urea denaturation method described by Wagner (1982). The proteins obtained from the ion-exchange column were further purified with a Sephadex G-100 column (40 × 2.5 cm) preequilibrated with a buffer containing 20 mM KP_i, 0.5 M KCl, and 1 mM EDTA at pH 7.0. The protein in the peak fractions was precipitated by ammonium sulfate (the fraction retained between 40% and 60%). The purity and molar ratio were checked by SDS gel electrophoresis. Three bands were observed with apparent molecular weights of 25K, 20K, and 16K. The relative amounts were determined by densitometric analysis and compared with the relative amounts of the corresponding bands in the SDS gel of myosin. The stoichiometry of these bands was 1:2:0.65.

The standard synthetic thick filament preparation was prepared according to Koretz (1982). Filaments of different forms were grown both by varying the concentration of KCl and by using different buffers: 10 mM imidazole buffer, pH 7.00, containing 1 mM EDTA and KCl varying from 0 to 0.3 M (steps of 0.1 M) and 10 or 20 mM potassium phosphate buffer, pH 7.00, containing 1 mM EDTA and KCl varying from 0 to 0.5 M. Formation of supramolecular structures was monitored by using a Zeiss A-20 electron microscope, at a magnification of 20000× unless otherwise specified. Grids were coated with Formvar and made hydrophilic by glow discharge in a vacuum evaporator. The adsorbed proteins were negatively stained with 1% uranyl acetate as described by Pollard (1982).

Proteins were characterized by SDS–PAGE using the standard technique described by Laemmli (1970). Acrylamide concentrations were 4% and 10% for stacking and separating gels, respectively. Molecular size was determined by using a 16 × 16 cm gel. Protein purity was checked by using a Bio-Rad Mini-Protean II apparatus, and gels were scanned with a Zenith soft laser scanning densitometer, Model SL-504-XL. Protein concentration was determined either by the absorption at 280 nm or by the method of Lowry et al. (1951). The extinction coefficients used ($\epsilon^{1\%}_{280nm}$) were 5.5 for myosin (Geoffrey & Harrington, 1970), 2.1 for rod (Hvidt et al., 1982) and 7.0 for S-1 (Weeds & Pope 1977).

Microcalorimetric Measurements. Measurements were done with an MC-2 differential scanning microcalorimeter (Microcal Inc., Amherst, MA), with a computerized data acquisition system, linked to an AT&T PC-6300 computer and

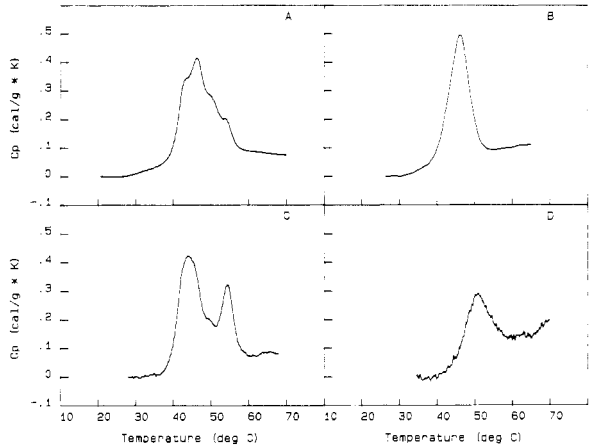

FIGURE 1: DSC endotherms of myosin in solution (panel A), rod (panel C), chymotryptic S-1 (panel B), and isolated light chains (panel D). The composition of the buffer was 20 mM potassium phosphate, pH 7.00, 0.5 M KCl, and 1 mM EDTA. DSC scan rate was 1.5 K/min. Protein concentration was 8 mg/mL for myosin, 4 mg/mL for rod, 7.8 mg/mL for S-1, and 1.2 mg/mL for light chains. Light chains were prepared as described, and a mixture with approximate stoichiometry 1:2:0.65 was used. See text for details.

a Hewlett Packard Colorpro plotter. Data analysis was performed by using a package provided by the manufacture (EMS Software) which is based on the deconvolution procedure of Freire and Biltonen (1978a,b). In some measurements, an earlier model, MC-1, by the same manufacturer was used. MC-1 was not equipped with a digital data acquisition system. These results were analyzed by the deconvolution program CPFIT kindly provided by Dr. E. Freire. The principle behind this program is the same as that mentioned above. The formalisms of equilibrium thermodynamics were used to analyze the DSC results of S-1 and synthetic thick filaments with the sole purpose of calculating the van't Hoff enthalpy and the cooperative ratio of the domains in order to obtain some additional information on the cooperativity of the system. This approach was suggested and has been used by other authors (Privalov & Potekhin, 1986; Tatunashvili & Privalov, 1984; Edge et al., 1985). Protein concentrations from 1 to 16 mg/mL and DSC scan rates from 0.2 to 1.5 K/min were used. The calorimeter cell capacity was 1.24 mL.

RESULTS

Thermal Unfolding of Myosin, Rod, S-1, and Light Chains. The excess heat capacity curves for myosin, rod, S-1, and light chains are reported in Figure 1, and thermodynamic parameters for the thermal unfolding of these molecules are summarized in Table I. The endotherm of myosin (Figure 1A) was characterized by a peak at 46 °C and three shoulders at 43, 49, and 54 °C. The total enthalpy change of these transitions was 1715 ± 75 kcal/mol. Given a value of 2425 amino acid residues per strand (Strehler et al., 1986), ΔH/residue was calculated to be 353 cal. The unfolding was not reversible (this point will be elaborated later). The reproducibility of ΔH_{cal} was greater than 95% for identical runs of the same myosin preparation and 90% or greater for different preparations of myosin.

In contrast, the unfolding of the rod (Figure 1C) was readily reversible (95%). There were two peaks at 43 and 54 °C. The ΔH_{cal} for a double-stranded rod of 252K was 1058 ± 59 kcal/mol, which gave a ΔH per residue of 481 cal (using a value of 1099 amino acid residues per strand; Strehler et al., 1986). The shape of the endotherm of the rod was extremely sensitive to changes in pH between 6 and 7.5 (Bertazzon & Tsong, 1987). However, for all pH values tested, the endotherms were well approximated by six quasi-two-state transitions, in agreement with Potekhin and Privalov (1978).

Table I: Enthalpies of Unfolding of Myosin, Subfragments, and Synthetic Thick Filaments[a]

	ΔH_{cal} (kcal/mol)	Δh_g^{-1} (cal/g)	ΔH/residue (cal)
rod[b]	1058 ± 59	4.2 ± 0.23	481
subfragment 1	255 ± 15	2.9 ± 0.17	304
light chains[c]	85 ± 15	2.25 ± 0.4	258
myosin[b]	1715 ± 75	3.43 ± 0.15	353
thick filament[b]	1679 ± 98	3.39 ± 0.2	346

[a] All the measurements were done in solution containing 20 mM KP_i, pH 7.0, 0.5 M KCl, and 1 mM EDTA, except for the thick filaments which were measured in a buffer containing 10 mM KP_i, pH 7.0, 0.1 M KCl, and 1 mM EDTA. Heating rate, 0.5 K/min. The uncertainties are expressed as standard deviation (SD) from the mean and are calculated on at least 10 determinations. [b] Refers to a dimer as basic unit. [c] Calculated as described under Materials and Methods. Refers to two light chains using a stoichiometry 1:2:0.65 and a final molecular weight of 37 700.

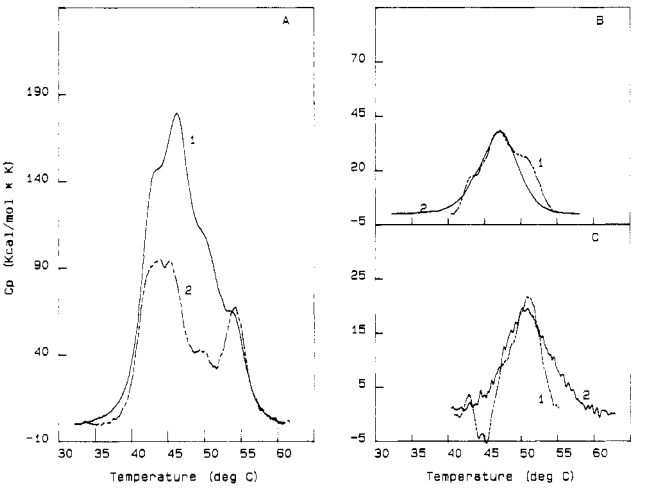

FIGURE 2: Comparison of the DSC endotherms of myosin and subfragments. (Panel A) Curve 1 represents the endotherm of myosin after subtraction of the base line; curve 2 represents the endotherm of the rod. Panel B represents a computer-calculated differential endotherm (normalized per mole of S-1) of the two previous endotherms (1) and is compared to the experimental endotherm obtained with S-1 (2). Experimental conditions were identical with those described in Figure 1. Panel C represents the differential endotherm of curve 1 and curve 2 of panel B (1) and is compared to the experimental endotherm of light chains (2).

Figure 1B shows the endotherm of purified S-1. The mass of S-1 heavy chains was estimated to be 87K by SDS–PAGE, in agreement with Strehler et al. (1986). The melting temperature was 46.3 °C, and a ΔH_{cal} of 255 ± 12 kcal/mol was obtained. The ΔH_{vH} (van't Hoff enthalpy) was 104 kcal/mol, and the ΔH per residue was 304 cal. The difference in ΔH_{cal} and ΔH_{vH} suggests the existence of subdomains. Deconvolution of the endotherm indicates that it could be reasonably fitted into three independent two-state transitions. However, the calorimetric endotherm is too symmetric to obtain a unique fit.

Purified light chains showed a single endotherm (Figure 1D), with the T_m at 51.5 °C and an ΔH_{cal} of 85 ± 115 kcal/mol. The average ΔH per residue was 258 cal.

Independent Melting of the Major Domains of Myosin and Reversibility of the Native Structure. Comparison of the endotherms obtained at a scan rate of 1.5 K/min of myosin and rod shows that the onset and the end point temperatures of the two curves coincide (Figure 2A). This coincidence is less obvious at lower heating rates. Because the endotherm of rod was independent of the heating rate whereas that of S-1 was not, one can use this property to identify the contribution

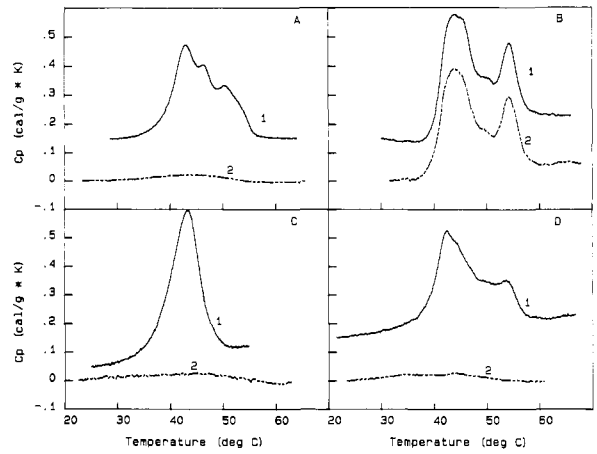

FIGURE 3: Reversible and irreversible transitions of myosin and its subfragments. Panel A represents the first (curve 1) and the second (curve 2) DSC scans of myosin. The transition was irreversible. Panel B represents the first (curve 1) and the second (curve 2) DSC runs of the rod. The reversibility was typically 95% or better. Panel C represents the first (curve 1) and the second (curve 2) DSC runs of S-1. Panel D represents the first (curves 1) and the second (curve 2) DSC runs of 1.3×10^{-5} M rod double chain and 2.6×10^{-5} M S-1. These endotherms were measured at pH 7.0 in 20 mM KP_i, 0.5 M KCl and 1 mM EDTA. The heating rate was 0.5 K/min.

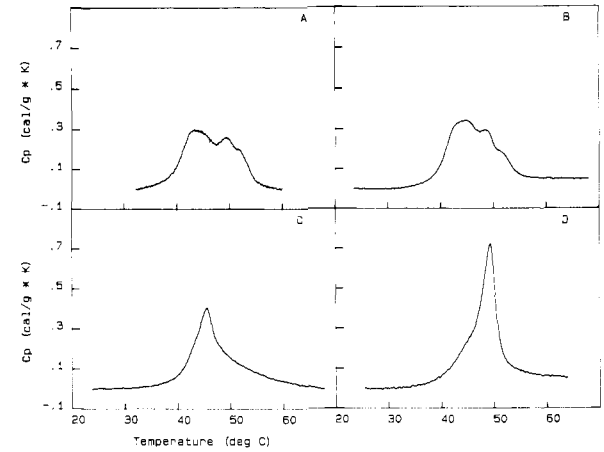

FIGURE 4: Dependence of ionic strength on the thermal stability of myosin. All samples were in a 20 mM potassium phosphate buffer containing 1 mM EDTA, at pH 7.0. These samples were dialyzed against the same buffer containing different concentrations of KCl. Panel A, 0.4 M KCl; panel B, 0.3 M KCl; panel C, 0.2 M KCl; panel D, 0.1 M KCl. The DSC scan rate was 0.5 K/min. The protein concentration was 5.9 mg/mL.

of S-1 in the endotherm of myosin. In Figure 3A,C, the main peak of the endotherm of myosin was identified to be due to the transition of S-1 when the heating rate was set at 0.5 K/min. The differential curve of the endotherms of myosin and rod (curve 1) was compared with the experimental curve of S-1 (curve 2) in Figure 2B. The difference between curve 1 and curve 2 was further obtained to compare with the experimental curve of light chains in Figure 2C (curve 1 being the difference spectrum and curve 2 the experimental endotherm).

The thermal unfolding of myosin (Figure 3A), S-1 (Figure 3C), and the light chains was irreversible, whereas the rod (Figure 3B) showed a high degree of reversibility (95%). The irreversibility of myosin would seem inconsistent with the suggestion that these fragments are nearly independent of each other with respect to unfolding. One would expect that at least that part of the endotherm originating from the rod segment in myosin would be reversible. It is highly probable that nonspecific interactions such as chain tangling or adhesion of exposed hydrophobic residues contributed to the irreversibility of myosin. To check this assumption, an appropriate ratio of rod and isolated S-1 were mixed and the thermal unfolding followed with DSC (Figure 3D, curve 1). In a rerun of the same sample, the endotherm of the rod disappeared (Figure 3D, curve 2), indicating that the presence of S-1 interfered with the rod which would be consistent with nonspecific interaction between these fragments.

Thermal Stability of Filamentous Myosin. In solutions of low ionic strength, myosin aggregates to form different types of filamentous structures (Pinset-Harstrom & Truffy, 1979; Kaminer & Bell, 1966). We have investigated the melting behaviors of these filaments by comparing the DSC results with the result of electron microscopy. Figure 4 shows the endotherms of myosin in four different concentrations of KCl. Little change in the shape of endotherms was detectable in 0.4 and 0.3 M KCl where the molecule was still soluble (Figure 4A,B). When aggregates began to form, however, the shape of the melting curve changed dramatically. For example, in 0.2 M KCl, the endotherm showed a sharp peak, or T_m, at approximately 45 °C (Figure 4C). When the KCl concentration was reduced to 0.1 M, a shift of the main endotherm to ~49 °C was observed (Figure 4D). Below 0.1 M KCl, no further changes were detected (data not shown). The ΔH_{cal} of the unfolding remained relatively constant with a mean value of 1679 ± 98 kcal/mol.

The changes in the endotherms of aggregates found in 0.2 and 0.1 M KCl did not appear to correlate with formation of particular types of filament (Figure 5A–D). Synthetic thick filaments formed by dialysis in a 10 mM imidazole buffer, pH 7.00, containing 0.1 M KCl and 1 mM EDTA were similar morphologically to filaments obtained in the same buffer containing 0.2 M KCl (Figure 5A,C). However, their DSC spectra (Figure 6A,B) resembled those obtained in the phosphate buffer (Figure 7C,D), in which myosin formed different types of filaments. The structures observed when phosphate was used instead of imidazole were primarily noncompact filaments (Figure 7A,C).

Imidazole was not a suitable buffer for DSC because of its large ΔH of protonation. Its pK_a varied 0.8–1 pH unit when the temperature of the solution was raised from 10 to 50 °C. The differences observed in the DSC spectra of thick filaments in imidazole and in phosphate were related to the pH variation of the buffer (Figure 6A). When the pH of the phosphate buffer was reduced to 6.7, the spectra obtained with the two buffers were superimposable, suggesting that the strong pH sensitivity observed for rod in solution (Bertazzon & Tsong, 1987) was also present in the thick filaments.

Each of the two endotherms (curves 1 and 2 of Figure 6A) could be resolved into two major transitions. At pH 7.0, the first transition had a T_m at 46.5 °C, a ΔH_{cal} of 985 kcal/mol, and a ΔH_{vH} of 96 kcal/mol, and the second transition had a T_m at 49.3 °C, a ΔH_{cal} of 715 kcal/mol, and a ΔH_{vH} of 294 kcal/mol (Figure 6B). When the pH was decreased to 6.7, the first transition stayed at a T_m of 46.2 °C, with a ΔH_{cal} of 856 kcal/mol and a ΔH_{vH} of 88 kcal/mol, while the second transition moved to a higher temperature, with a T_m of 51.7 °C, a ΔH_{cal} of 850 kcal/mol, and a ΔH_{vH} of 300 kcal/mol (Figure 6C). In all these cases, the van't Hoff enthalpies were much smaller than the calorimetric enthalpies. Thus, these transitions can be regarded as the sum of several quasi-two-state transitions. Because the transition temperatures of these domains are close, an unambiguous deconvolution was difficult to achieve. An alternative approach is to subtract the endotherms of S-1 which were obtained under identical conditions of pH, ionic strength, and heating rate. When 2 mol of

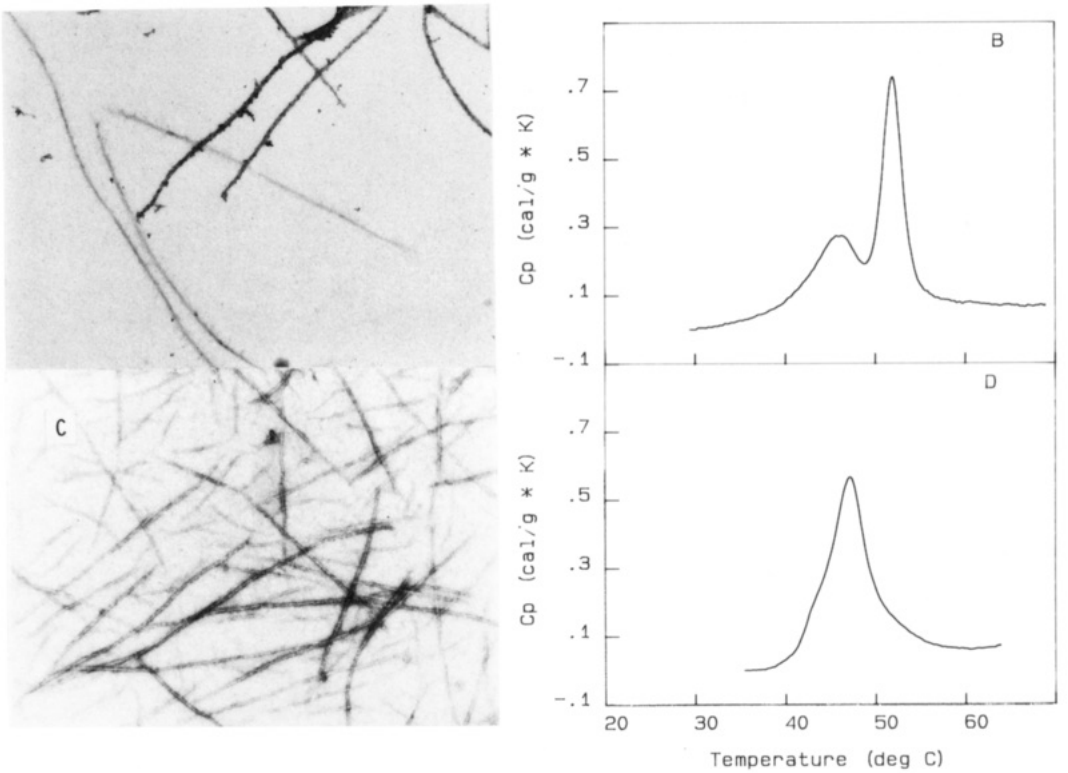

FIGURE 5: Comparison of the morphology of myosin filaments and DSC endotherms. Panel A is an electron micrograph of myosin dialyzed (overnight) against a 10 mM imidazole buffer containing 0.1 M KCl and 1 mM EDTA at pH 7.0, a condition which favors the formation of thick filament. The magnification is 12000×. Panel B shows a DSC endotherm of the sample of panel A. Panel C is an electron micrograph of the sample when the KCl concentration was 0.2 M, and panel D is the endotherm. There is a change in the shape of the endotherms when compared to data of Figure 4 due to pH drift of the imidazole buffer as indicated in Figure 6.

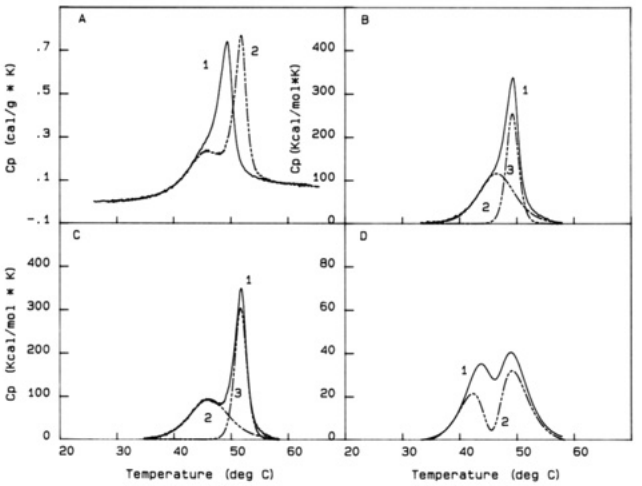

FIGURE 6: pH dependence of the myosin endotherm in a 10 mM potassium phosphate buffer containing 0.1 M KCl and 1 mM EDTA. (Panel A) Curve 1 was obtained at pH 7.0, and curve 2 was obtained at pH 6.7. The DSC scan rate was 0.5 K/min in both cases. Panels B and C show the fitting of the endotherm (curve 1) into two major transitions (curves 2 and 3). Curve 1 of panel D was obtained by subtracting the endotherm of S-1 obtained under identical conditions from curve 2 of panel B, at pH 7.0. The same procedure was applied to the condition at pH 6.7 (curve 2 of panel D).

S-1 was subtracted, at pH 6.7, a residual endotherm can be observed at a T_m of 41.5 °C, with an apparent enthalpy of 144 kcal/mol, and a second transition with a T_m at 49.5 °C, with a ΔH_{cal} of 309 kcal/mol (Figure 6D).

Discussion

Information on the thermal stability of native myosin has been limited because of its tendency to coagulate upon thermal denaturation, thus making many physicochemical techniques unsuitable for studying. This limitation has been overcome by using DSC which directly measures the enthalpy changes associated with a conformational transition without any a priori assumption. Goodno and Swenson (1975a,b) previously demonstrated that the melting temperature of myosin, following proton absorption upon thermal unfolding, was similar to that observed for its fragments. Where applicable, our results are in general agreement with their observations. However, the method of measuring solution pH to monitor thermal unfolding does not directly provide energetic information. Neither is it suitable for studying thermal denaturation of more complex molecular assemblies of myosin. It has been demonstrated that a complex endotherm of rod can be resolved into several transitions arising from independent domains (Potekhin & Privalov, 1978; Potekhin et al., 1979; Privalov, 1982). This also appears to be the case for myosin. Our study shows not only that the melting temperatures of S-1, rod, and light chains appear similar when isolated but also that the molar enthalpy of the myosin is the sum of the enthalpies of these fragments, both in the soluble form and in the filament forms. Our data on the specific enthalpy for rod, 4.2 ± 0.23 cal/g, are slightly lower than those reported by Potekhin and Privalov (1978), which is 4.66 cal/g.

The irreversibility of myosin denaturation is due to a nonspecific process and did not substantially change the enthalpy values and our data analysis. First, the enthalpy values for myosin and its functional domains were unafected by heating rate in the range 0.2–1.5 K. Second, the enthalpy contribution of rod in the myosin agrees with the enthalpy of the melting of the isolated rod.

The molar enthalpy of denaturation remains the same for myosin when it forms filaments, suggesting that filament formation is mainly an entropic process. This result is in agreement with Joseph and Harrington (1968), who have

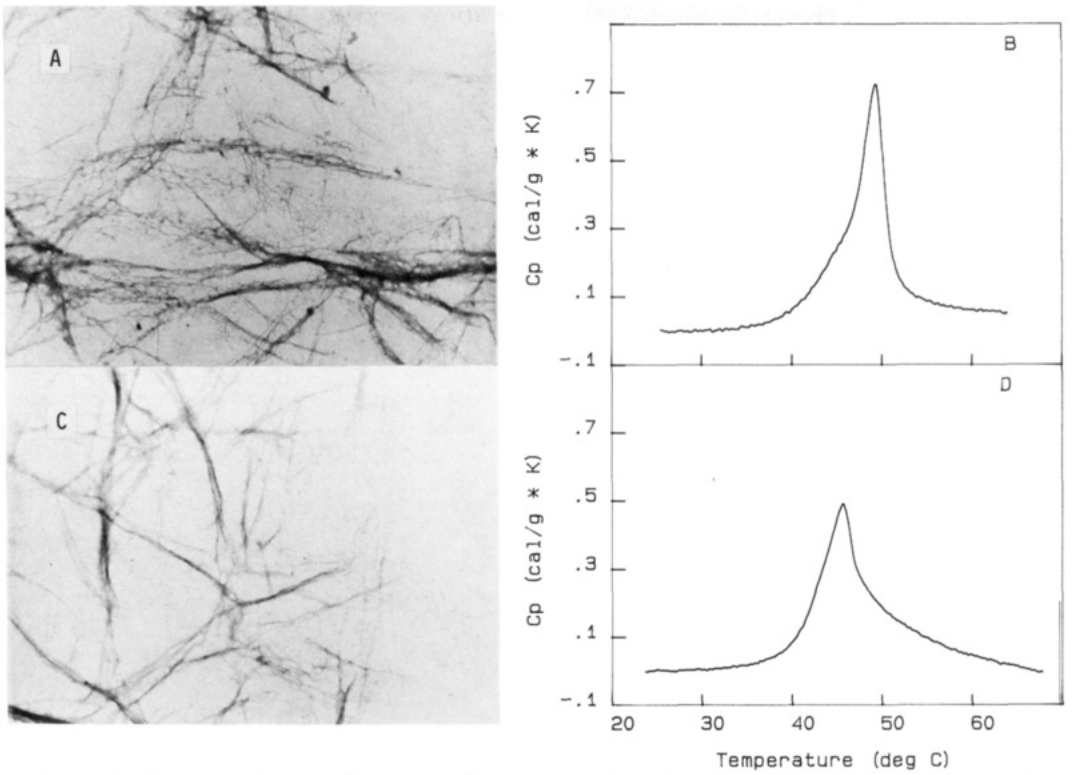

FIGURE 7: Effect of inorganic phosphate on the morphology and DSC endotherm of myosin filaments. Panels A and B show the electron micrograph and the DSC spectrum, respectively, of myosin filaments in 20 mM potassium phosphate buffer at pH 7.0, containing 1 mM EDTA and 0.1 M KCl. Panels C and D show the electron micrograph and DSC spectrum, respectively, of myosin filaments in the same buffer but containing 0.2 M KCl. Although the morphology of these aggregates is similar, their DSC spectra are quite different.

shown that the equilibrium constant of filament formation is independent of temperature. Comparison of the electron microscopic and the DSC results indicates that the specific arrangement of myosin in a filament does not appear to be critical for the shape of the endotherm nor does the enthalpy of the thermal denaturation depend on the morphology of these filaments. Phosphate seems to make the filament less rigid, as is also suggested from scanning tunneling microscopic (STM) images (Bertazzon, Lee, Evans, and Tsong, unpublished observation), thus impeding regular close packing of the filaments (Figure 7). However, phosphate does not significantly change the endotherm of myosin.

The major factors affecting the shape of the endotherm for myosin and its filaments are the concentration of KCl and the solution pH, as is the case for isolated rod and S-1. Joseph and Harrington (1968) measured a release of 11 mol of KCl and an absorption of 0.68 mol of hydrogen ion per mole of myosin during the filament formation. The proton absorption could be due to the protonation of a group in a specific domain. A slight decrease in the solution pH has been shown to change the shape of the endotherm of rod (Bertazzon & Tsong, 1987), LMM (Bertazzon & Tsong, 1989), and Long S-2 (Swenson & Ritchie, 1980). Thermally the least stable domain of LMM and rod, situated at the LMM/S-2 junction (Privalov, 1982), is probably split into two parts during the tryptic digestion (Swenson & Richie, 1980). The T_m of this domain decreases when the pH of solution decreases whereas all other domains show an increase in T_m. This seems to be the case also for the filaments. If the domains are independent in the filaments, the endotherm of S-1 contributes to the enthalpy, and when subtracted, a residual enthalpy of approximately 140 kcal/mol is observed at 42 °C and another contribution at 49 °C. The T_m of the higher temperature transition increases with a reduction in pH (Figure 6D). We may therefore conclude that the formation of filaments does not significantly change the independent behavior of domains. The change in shape of the endotherm is due to the change in the concentration of KCl. The analysis shown in Figure 6 supports this conclusion and rules out any significant enthalpy effect due to intermolecular interaction in the process of filament formation. These results support that a local structure change during the muscle contraction is a viable mechanism.

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Zinc Release from *Xenopus* Transcription Factor IIIA Induced by Chemical Modifications†

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ABSTRACT: *Xenopus* transcription factor IIIA (TFIIIA) contains two tightly bound intrinsic Zn^{2+} ions that are released through treatment with either *p*-(hydroxymercuri)benzenesulfonate (PMPS) or diethyl pyrocarbonate (DEP) as monitored by the metallochromic indicator 4-(2-pyridylazo)resorcinol (PAR). The inactivation of TFIIIA by DEP as detected by an in vitro 5S RNA gene transcription assay was correlated with the extent of modification of histidine residues and Zn^{2+} release. Following reaction with PMPS, the 7S particle was dissociated into free TFIIIA and 5S RNA. This dissociation could be correlated with the extent of modification of cysteine residues as well as the Zn^{2+} release. The dissociation of the 7S particle was reversed by the addition of excess thiol reagent. However, the reversibility could be inhibited by EDTA, suggesting that Zn^{2+} was required for the binding of TFIIIA to 5S RNA. In the presence of PMPS- or DEP-modified TFIIIA or Zn^{2+}-depleted TFIIIA, the fluorescence emission maximum of the hydrophobic probe, 8-anilinonaphthalenesulfonate, was blue-shifted by 30 nm, while only less than a 10-nm blue shift was observed in the presence of either the 7S particle or TFIIIA. These results indicate that the two Zn^{2+} ions in TFIIIA are coordinated with the cysteine and histidine residues and are required for maintenance of the proper conformation of TFIIIA.

Xenopus transcription factor IIIA (TFIIIA)[1] is one of several protein factors required for the transcription of 5S RNA genes. It binds to the intragenic control region (ICR) of the 5S RNA genes with a high affinity to promote the initiation of transcription (Engelke et al., 1980; Sakonju et al., 1981) and associates with the 5S RNA transcript in immature oocytes in the form of a 7S nucleoprotein complex (Picard & Wegnez, 1979; Pelham & Brown, 1980). TFIIIA is also a metalloprotein that contains two tightly bound intrinsic Zn^{2+} essential for the binding of TFIIIA to the ICR of the 5S RNA genes (Hanas et al., 1983b). In order to account for the TFIIIA interactions with DNA, a model has been

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[1] Abbreviations: TFIIIA, transcription factor IIIA; ICR, intragenic control region; PAR, 4-(2-pyridylazo)resorcinol; PMPS, *p*-(hydroxymercuri)benzenesulfonate; DEP, diethyl pyrocarbonate; ANS, 8-anilino-1-naphthalenesulfonate; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; Gdn-HCl, guanidine hydrochloride; Tris-HCl, tris(hydroxymethyl)-aminomethane hydrochloride; TNG buffer, 10 mM Tris-HCl, pH 7.5, 0.2 M NaCl, and 5% glycerol.

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