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Effects of Ions and pH on the Thermal Stability of Thin and Thick Filaments of Skeletal Muscle: High-Sensitivity Differential Scanning Calorimetric Study†

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ABSTRACT: Differential scanning calorimetry (DSC) is unique for studying conformational changes in supramolecular structures because it is immune to interference by the turbidity and other optical artifacts of a sample solution. We have employed DSC to study thermal stability of myosin and actin in their filamentous forms (i.e., thick and thin filaments). The thermal stability of the myosin monomer, as well as polymers, showed remarkable sensitivities to pH and to the ionic strength of the solution. At pH 7.5, the endotherm of myosin filaments was broad and resembled that of the monomer in solution. Reducing the pH to 6.3 split the endotherm of the filament into two major transitions. The first one, with a T_m of 47 °C, a ΔH_{cal} of 805 kcal/mol, and a cooperative ratio (CR) of 0.1, was relatively insensitive to the pH changes whereas the second one which represented approximately 80% of the helical structure was pH sensitive. The second transition released 2.17 H^+ per mole at 0.17 M KCl and was defined by a T_m of 53.9 °C, a ΔH_{cal} of 917 kcal/mol, and a CR of 0.35. The major fragment contributing to the splitting of the endotherm was interpreted to be S-2 because the T_m of purified S-2 in a similar medium also shifted from 39.5 °C at pH 7.3 to 49.6 °C at pH 6.0. KCl had similar effects on the shape of the endotherm of the thick filament. A decrease of KCl from 0.2 to 0.1 M enhanced the effect of pH on the second transition. A calculated release of 8.9 K^+ per mole was associated with the melting of the major part of the helix. The T_m vs pH curve had an inflection point at pH 6.8 in 0.17 M KCl. These conditions mimicked the physiological conditions. The two major transitions of myosin filaments did not show a strong cooperativity (CR 0.1 and 0.4, respectively), implying that the domains observed in the monomer [Bertazzon, A., & Tsong, T. Y. (1989) *Biochemistry 28*, 9784–9790] were also present in the filament. In actin, a partial polymerization was observed when the concentration of Ca^{2+} in the medium was increased (1–10 mM), as assessed by analysis of the DSC endotherms (the CR increased from 0.7 to 1.25). This effect of calcium was not observed in the fully polymerized protein; however, a reduction of the pH from 7.9 to 5.9 increased the T_m from 68.2 to 74.3 °C, with apparently no effect on the cooperative ratio. These results suggest that, in contrast to the myosin filament, the thin filament behaved as a fully cooperative structure. The effect of pH on the thermal stability of F-actin was consistent with the release of 1.4 H^+ per mole upon melting.

The thermal stability of most components of the sarcomere has been studied in detail in solution. However, the aggregation of myosin to form filaments precludes any physical study based on spectroscopy or viscometry. By monitoring the change in solution pH as a function of temperature, Goodno and Swenson (1975a,b) and Goodno et al. (1976) were able to circumvent the problem to a certain degree. The melting of the whole myosin and of the light meromyosin (LMM) was shown to be characterized by the absorption of protons at 41 °C. Melting of S-2, on the contrary, was accompanied by a

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release of protons. However, by the pH measurements, these authors were unable to detect melting of the myosin filament at KCl concentrations below 0.15 M.

The model of Harrington (1971, 1979) suggests that a helix-to-coil transition of a fragment in myosin rod (LMM/S-2 hinge) is responsible for the force generation in the molecular mechanism of muscle contraction. The melting of myosin and its fragments in solution has been extensively studied (Sutoh et al., 1978; Burke et al., 1973; Highsmith et al., 1977; Hvidt et al., 1982; Stafford, 1985; King & Lehrer, 1989), and the kinetics of the melting of S-2 were also investigated (Tsong et al., 1979, 1983). Although these data are compatible with the helix-to-coil transition model of Harrington, there are some discrepancies between the melting temperature obtained for myosin fragments in solution and the temperature of the generation of maximal tension in the myofibril when the myofibril was subjected to a laser temperature jump (Davis & Harrington, 1986).

In an attempt to resolve these discrepancies and to overcome the lack of information about the aggregated states of myosin and actin, we have used a systematic approach which involved both monitoring the thermal stability with high-sensitivity differential scanning calorimetry (DSC) and spectroscopic techniques (Bertazzon & Tsong, 1989a; Bertazzon et al., 1989). In this paper, we report a study of the effect of pH and KCl on the thermal stability of domains in filamentous myosin, in the physiological range of ionic strength, and compare the cooperative behavior of the thick and the thin filaments.

Materials and Methods

Protein Purification. Proteins were purified from New Zealand White male rabbits, and only paravertebral, fast-twitching muscles were used. Myosin was purified as previously described (Bertazzon & Tsong, 1989b). Actin was purified following the procedure described by Pardee and Spudich (1982). S-2 was prepared as described in the following paper (Bertazzon & Tsong, 1990). Protein concentration was determined from UV absorption, using coefficients $E^{1\%}_{280}$ of 5.5 for myosin (Geoffrey & Harrington, 1970) and $E^{1\%}_{290}$ of 6.3 for G-actin. The method described by Lowry et al. (1951) was used to determine the concentration of F-actin. The purity of the sample was checked by SDS–polyacrylamide gel electrophoresis, as described by Laemmli (1970). Gels were scanned with a Zenith Soft Laser Model SL-504-XL scanning densitometer.

Differential Scanning Microcalorimetry (DSC). DSC was performed with a Microcal MC2 (Amherst, MA) microcalorimeter, interfaced with an AT&T PC 6300 personal computer through an A/D converter (DT 2801). Data acquisition and data analysis software (EMF) were provided by the manufacturer; the analysis is based on the approach of Freire and Biltonen (1978a,b). The capacity of the cell was 1.241 mL. The samples were dialyzed overnight against the reference buffer before each run. The calorimetric run was obtained under excess N_2 pressure (2 atm). A base line obtained by running buffer vs buffer was subtracted from the data files before analysis. The enthalpy observed was not influenced by protein concentration between 2 and 15 mg/mL. The curves were fitted into a minimal number of independent transitions, assuming that the domains were independent as in the case of the protein in solution. The transitions were defined by a calorimetric enthalpy, which reflects the amount of structure involved in the melting process, a van't Hoff enthalpy, which reflects the width of the transition, a ΔC_p, which measures the heat capacity change from the native state to the denatured state, and a melting temperature, which indicates the stability.

The ΔC_p of an endotherm was obtained by the difference in the C_p values between the linearly extraporated pretransition base line and the posttransition base line. ΔC_p for G-actin was 0.05 ± 0.01 cal/(g·K) and 0.11 ± 0.03 cal/(g·K) for myosin. In our deconvolution analyses, the effect of ΔC_p was removed by drawing a line from the onset to the end point of an endotherm. The area between the DSC curve and this line measures the enthalpy of the total transition.

The ratio between the van't Hoff enthalpy and the calorimetric enthalpy ($\Delta H_{vH}/\Delta H_{cal}$ = cooperative ratio, or CR) reflects the cooperativity of the system. A value below unity suggests the presence of domains and/or intermediate steps in the melting process. A value higher than 1 indicates an increased cooperativity of the structure and/or aggregation.

The thermal melting of rod and S-2 were fully reversible although those of myosin, thick filaments, G-actin, and thin filaments were irreversible. We have used equilibrium thermodynamics in the analysis with the only purpose of defining the width of the transition, i.e., to obtain ΔH_{vH}, and calculate the cooperative ratio.

The total number of protons (or ions) involved in the process was calculated by using DSC results as previously described (Bertazzon et al., 1989) by assuming that the binding is a single-step process described

$$P + \Delta nH^+ = P{\cdot}\Delta nH^+$$

where P is the protein and Δn is the net number of protons absorbed per molecule upon unfolding. Equation 1 can then be derived:

$$\log [\alpha/(1 - \alpha)] = \log K_{app} + \Delta n \log [H^+] \qquad (1)$$

where α is the extent of change at a pH value, usually a change in T_m. From the slope of the T_m vs pH plot, Δn may be obtained.

Results

Modulation of the Thermal Stability of Thick Filament. The endotherms of myosin in the aggregated states are presented in Figure 1A,B. In Figure 1A, the concentration of KCl was 0.17 M. Small variations in pH changed the shape of the endotherm although the enthalpy of the denaturation (3.43 ± 0.15 cal/g) was constant throughout the range investigated. These myosin aggregates were initially prepared at pH 7.0 in a 20 mM potassium phosphate buffer with 0.1 M KCl and 1 mM EDTA. The ionic strength and pH were then gradually modified by an overnight dialysis into a desired medium. As the pH was reduced in the medium, there was a gradual change of the thermal stability of individual domains within the myosin. As a result, the shape of the endotherm changed. The main effect was the separation of the broad endotherm into two distinct transitions, one sharp peak at a higher temperature and a shoulder at a lower temperature. In Figure 1B, the pH was held constant at 6.7, and the concentration of KCl was varied. The effect was similar to that of lowering pH (i.e., the endotherm was split into two major components).

Deconvolution into Two Major Transitions. The experimental endotherm was fitted into a minimal number of transitions in Figure 2. At a KCl concentration of 0.13 M (panels A–C), the curve could be fitted into two major components. The numerical values for the two components are given in Table II. This procedure allowed an estimate of the amount of structure involved if we subtracted the contribution of S-1 (Bertazzon & Tsong, 1989b) and assumed that the calorimetric enthalpy for each transition was proportional to

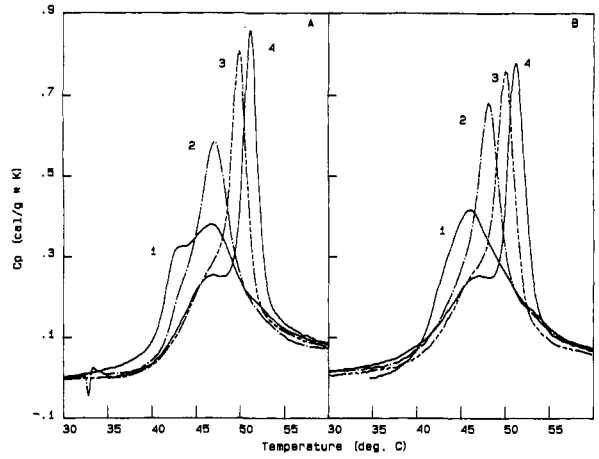

FIGURE 1: DSC melting curves of myosin in the filament state. (Panel A) In 0.17 M KCl: the pH of a potassium phosphate buffer, 20 mM, containing 1 mM EDTA, was adjusted to 7.5 (curve 1), 7.0 (curve 2), 6.5 (curve 3), or 6.3 (curve 4) through overnight dialysis. (Panel B) At pH 6.7: the KCl concentration was adjusted by overnight dialysis at 0.25 M (curve 1), 0.20 M (curve 2), 0.15 M (curve 3), and 0.10 M (curve 4). The buffer composition was potassium phosphate (20 mM)/EDTA (1 mM). The heating rate was 1.5 K/min.

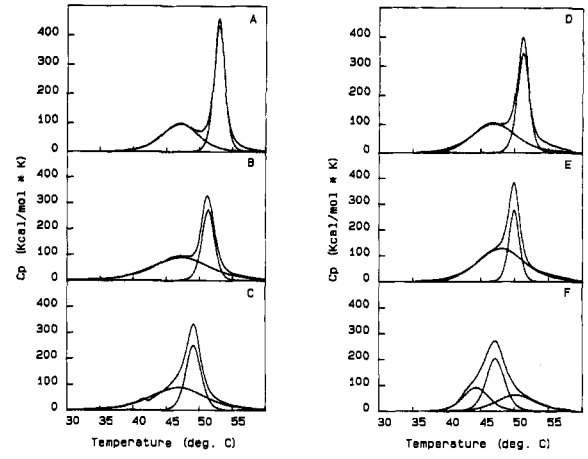

FIGURE 2: Resolution of the endotherm of myosin into major transitions. Panels A–C: Myosin filaments in 20 mM potassium phosphate, 0.13 M KCl, and 1 mM EDTA at pH 6.3, 6.7, and 7.0, respectively. Panels D–F: Myosin filaments in 20 mM potassium phosphate, 0.17 M KCl, and 1 mM EDTA at pH 6.3, 6.7, and 7.0, respectively. Deconvolution was done by using the procedure of Freire and Biltonen (1978). See text for details. Numerical values are given in Table II.

Table I: Effect of pH and K^+ on the T_m (°C) of Myosin Filaments[a]

	pH				
[KCl] (M)	7.5	7.0	6.7	6.5	6.3
0.10		49.7	51.4	52.3	54.9
0.13	46.9	49.2	51.2	51.8	54.0
0.15	46.3	48.7	50.1	50.8	52.9
0.17	46.6	47.6	49.1	49.8	51.1
0.20		46.9	48.0	49.0	49.9
0.25		46.2	47.3	48.2	49.8

[a] T_m is defined as the highest value of C_p in each endotherm. The DSC curves were obtained at 1.5 K/min heating rate, in potassium phosphate buffer (20 mM) and 1 mM EDTA at different values of pH and ionic strength.

the amount of structure involved. By comparison of the calorimetric enthalpy with the van't Hoff enthalpy, the cooperativity of the system may also be evaluated. At the lower pH value (pH 6.3 in panel A), the first transition was characterized by a ΔH_{cal} of 805 kcal/mol, a T_m of 47.3 °C, and a CR of 0.12. By increasing the pH, the enthalpy of the transition increased, but the transition broadened with a further reduction of the cooperative ratio (0.07 at pH 7.0, panels B and C). It has been shown (Bertazzon & Tsong, 1989) that this transition contained the contribution of the head groups (S-1) but the total enthalpy was about 150 kcal/mol higher than that expected for the two heads alone. The second transition appeared much sharper; however, the cooperative ratio of 0.35 indicates that it was not fully cooperative. At pH 6.3, the total enthalpy (917 kcal/mol) was less than that expected for the whole rod (1058 ± 50 kcal/mol) and greater than that of LMM (590 ± 35 kcal/mol). The same behavior was observed at higher KCl concentration (0.17 M, panels D–F), with the exception that at pH 7.0 the endotherm could no longer be resolved into only two transitions. At least three were needed (Figure 2, panel F). It appeared that at low ionic strength and low pHs, about 80% of the helical structure melted in the second transition.

Number of Protons and Ions Involved in the Unfolding. The T_m of the second transition was determined as a function of pH and ionic strength (Figure 3 and Table I). From the curves in Figure 3A,B, the number of protons and ions involved in the thermal unfolding can be determined. In the inserts, the log of the equilibrium constant was plotted vs pH and KCl concentration, respectively. From the slope of the curves, 2.17

Table II: Deconvolution of the Endotherms of Myosin Filaments[a]

	transition I			transition II		
pH	T_m (°C)	ΔH_{cal} (kcal/mol)	ΔH_{vH} (kcal/mol)	T_m (°C)	ΔH_{cal} (kcal/mol)	ΔH_{vH} (kcal/mol)
			[KCl] ≈ 0.13 M			
6.3	47.3	805	99	53.9	917	397
6.5	47.4	812	108	51.9	877	378
6.7	47.3	980	70	51.3	760	334
7.0	46.9	970	74	49.2	767	269
			[KCl] ≈ 0.15 M			
6.3	47.5	852	90	53.0	850	407
6.5	47.4	998	90	50.8	700	323
6.7	47.6	1110	88	50.1	644	343
			[KCl] ≈ 0.17 M			
6.3	47.0	899	93	51.2	801	358
6.5	47.6	1177	89	49.9	561	408
6.7	47.1	1069	94	49.1	633	333
7.0	44.3 (I)	466	158	47.2 (II)	738	225
				50.2 (III)	488	108

[a] The endotherms were resolved into a minimal number of domains. Each domain is defined by a calorimetric enthalpy, a melting temperature, and a van't Hoff enthalpy. A broad transition can be resolved into subdomains. The uncertainty for ΔH_{cal} and ΔH_{vH} was 5% (3–5 determinations).

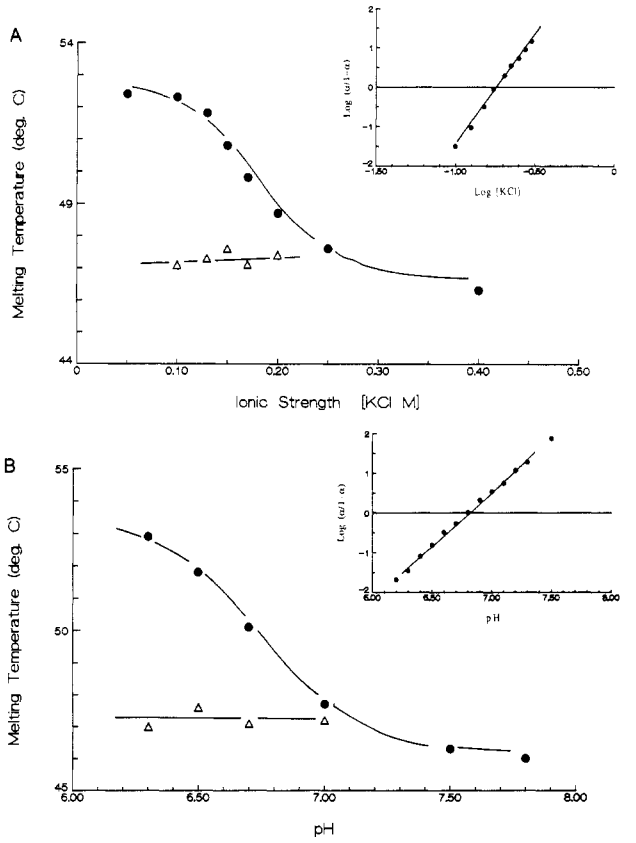

FIGURE 3: Dependence of the endotherm of myosin on [KCl] (panel A) and pH (panel B). Numerical values are reported in Table II. The melting temperature considered is the highest value of heat capacity (C_p) of the endotherm. Filled circles correspond to the second transition and triangles to the first transition (Table II). Equation 1 was used to calculate Δn(pH), the number of H^+ released upon unfolding (insert in panel B), and Δn(KCl), the number of K^+ released upon unfolding (insert in panel A). Δn(pH) was 2.7/mol, and $\Delta n(K^+)$ was 8.9/mol.

Table III: pH Dependence of the Melting of S-2 at High and Low Ionic Strength[a]

pH	T_m(0.5 M KCl) (°C)	T_m(no KCl added) (°C)
5.90	51.3	49.6
6.23	50.1	
6.45	48.6	
6.80	45.6	42.1
7.02	44.5	
7.30		39.5

[a] The melting temperature considered was the highest value of C_p in the endotherm. The conditions were 20 mM potassium phosphate buffer, 1 mM EDTA, and 0.5 M KCl, or no added KCl. The endotherms were reversible and independent of heating rate. The concentration of protein was varied from 1.8 to 3.5 mg/mL.

H^+ and 8.9 K^+ per mole were obtained. This indicates that the binding of 2.17 protons or 8.9 K^+ stabilized about 85% of the helix or that this part of the helix melted with a release of 2.17 protons.

Contribution of S-2 to pH Sensitivity. From our solution studies [see Bertazzon and Tsong (1990)], the fragment that showed the greatest sensitivity to pH reduction was S-2. A reduction of pH from 7.0 to 5.9 shifted the T_m from 39.5 to 49.6 °C in 20 mM KP_i and 1 mM EDTA. The result is shown in Figure 4, and Table III summarizes the effect of pH on the T_m of S-2 in the presence or absence of 0.5 M KCl.

Effect of Millimolar Calcium on the Polymerization of G-Actin. When G-actin polymerized to F-actin, the T_m of the thermal transition shifted from 57.2 to 67 °C. The calorimetric enthalpy also increased from 142 ± 10 to 168 ± 12

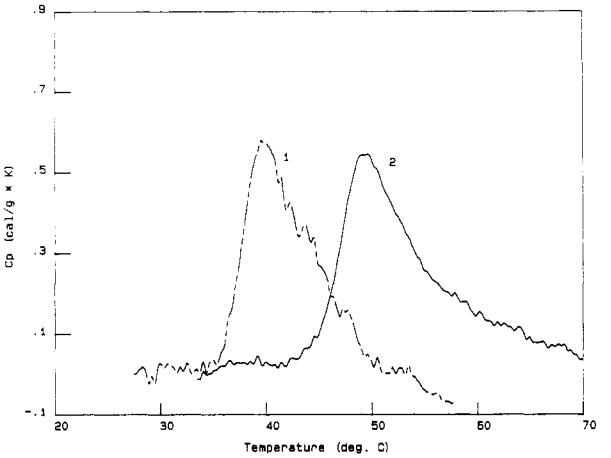

FIGURE 4: Endotherms of S-2 in low ionic strength medium. Curve 1 refers to pH 7.0 (protein concentration 1.2 mg/mL), and curve 2 was obtained at pH 5.9 (protein concentration 3.4 mg/mL). The composition of the medium was 20 mM potassium phosphate, 0.1 M KCl, and 1 mM EDTA. The heating rate was 1 K/min.

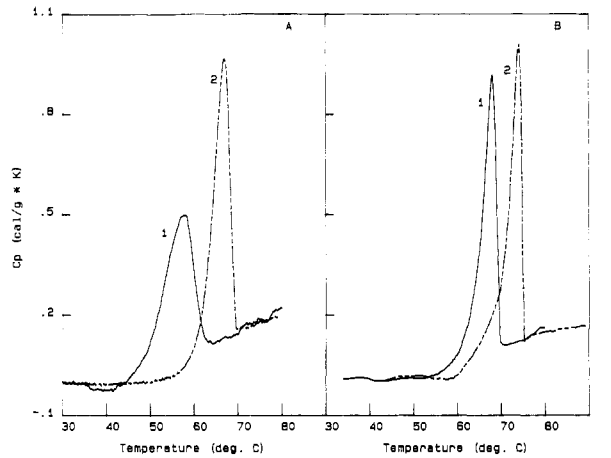

FIGURE 5: Effect of polymerization and pH on the melting of actin. Panel A: Curve 1 represents the endotherm of G-actin in 2 mM HEPES containing 0.2 mM $CaCl_2$, 0.2 mM Na-ATP, and 0.5 mM β-mercaptoethanol at pH 8.0. Curve 2 is the endotherm obtained after polymerization to F-actin, in 2 mM HEPES, 0.2 mM $CaCl_2$, 50 mM KCl, 1 mM Na-ATP, 2 mM $MgCl_2$, and 0.5 mM β-mercaptoethanol at pH 8.0. Panel B: F-Actin's endotherms were obtained at pH 7.9 (curve 1) and 5.9 (curve 2), in the same buffer as above. Data for intermediate pH values are given in Table V. Protein concentration was 3.4 mg/mL for G-actin and 5.2 mg/mL for F-actin. The heating rate was 0.5 K/min.

kcal/mol, although the ΔC_p remained at 2.3 ± 0.7 kcal/(mol·K). The most remarkable effect was on the width of the transition curve (Figure 5A). The cooperative ratio, 0.65, which suggested the existence of domains in G-actin, was reversed to 1.4, indicating an increased cooperativity for F-actin. G-Actin's thermal stability was very sensitive to changes in buffer composition. Ionic strength, pH, and specific ions (Mg^{2+}) promoted polymerization. Calcium (10^{-11}–10^{-10} M) has been shown to play a fundamental role in the structural integrity of the monomer (Bertazzon et al., 1989). When this high-affinity calcium was removed by EGTA, the enthalpy associated with the thermal transition of G-actin disappeared. Calcium in the millimolar range appeared to induce a partial polymerization as assessed from the DSC result reported in Table IV. When calcium concentration was increased from 0.2 to 8 mM, the stability of G-actin was increased along with a sharpening of the endotherm (CR from 0.82 to 1.25). The same experiment with a fully polymerized protein (F-actin) showed that the stability of F-actin was not affected by this

Table IV: Effect of Calcium on the Thermal Unfolding of G-Actin[a]

$[Ca^{2+}]$ (mM)	T_m (°C)	ΔH_{cal} (kcal/mol)	ΔH_{vH} (kcal/mol)	CR
0.2	62.7 ± 0.3	146 ± 6	120	0.82
0.4	63.9 ± 0.2	154 ± 7	130	0.84
1.0	65.9 ± 0.3	164 ± 7	145	0.88
2.0	66.9 ± 0.3	163 ± 10	170	1.04
4.0	67.4 ± 0.4	164 ± 6	184	1.12
8.0	67.0 ± 0.4	159 ± 8	187	1.25

[a] The difference from the mean is expressed as the standard error (SE) for three determinations. The cooperative ratio is given by the ratio $\Delta H_{vH}/\Delta H_{cal}$. G-Actin was dialyzed overnight vs. a buffer containing 2 mM HEPES, pH 7.0, 0.2 mM Na-ATP, and 0.5 mM β-mercaptoethanol. The heating rate was 0.5 K/min. The number of DSC runs at each calcium concentration was 3–5.

Table V: Effect of pH on the Thermal Stability of F-Actin[a]

pH	T_m (°C)	ΔH_{cal} (kcal/mol)	ΔH_{vH} (kcal/mol)	CR
5.9	74.3 ± 0.2	196 ± 12	283	1.44
6.4	74.0 ± 0.3	189 ± 10	288	1.51
6.9	71.8 ± 0.3	190 ± 13	269	1.41
7.3	70.4 ± 0.2	180 ± 9	267	1.48
7.6	69.1 ± 0.3	179 ± 12	258	1.44
7.9	68.2 ± 0.3	168 ± 12	253	1.50

[a] Data are expressed as mean ± SE of three determinations. F-Actin was dialyzed overnight against 2 mM HEPES buffer, 0.2 mM $CaCl_2$, 1 mM Na-ATP, 2 mM $MgCl_2$, 50 mM KCl, and 0.5 mM β-mercaptoethanol. The heating rate was 0.5 K/min. The number of DSC runs at each pH value was 3–5.

range of concentration of calcium.

Effect of pH and ATP on the Stability of F-Actin. F-Actin was further stabilized by H^+ as demonstrated by an increase in the melting temperature from 68.2 °C at pH 7.9 to 74.3 °C at pH 5.9 (Figure 5B and Table V). The quantification of the protons involved was obtained as described for myosin. The thermal unfolding involved a release of 1.4 protons per mole (Figure 6).

To see whether the ATPase activity of F-actin contributed to the enthalpy of denaturation, varying concentrations of ATP were added. No significant differences in the melting temperature and the total enthalpy of denaturation were observed for ATP concentrations as high as 10 mM.

Discussion

The stability of myosin in filamentous form was highly sensitive to H^+ and K^+. The effects of the two ions were similar. There were two major conformational transitions in the endotherm: The first one arises from the melting of S-1, a domain which was destabilized by proton absorption (Bertazzon & Tsong, 1989b), and a thermally highly stable domain that was stabilized by proton absorption as did short S-2 in solution. The second one was comprised of the melting of LMM and a part of the short S-2. DSC analysis allowed distinction of the opposite effects of H^+, or K^+, on the thermal stability of different segments of the myosin molecule close to physiological conditions.

Goodno and Swenson (1975a,b) have found that a thermal transition of myosin, with a T_m of 41 °C, is accompanied by absorption of protons. The structure is located in the LMM fragment (Goodno et al., 1976). These authors have also reported that the melting of S-2 is accompanied by a release of protons. The transition which occurs at 41 °C is not observed for KCl concentrations below 0.15 M. Our data indicate that the first of the two major transitions of the myosin endotherm contained part of the helical structure (150–200 kcal/mol). The whole transition was relatively insensitive to

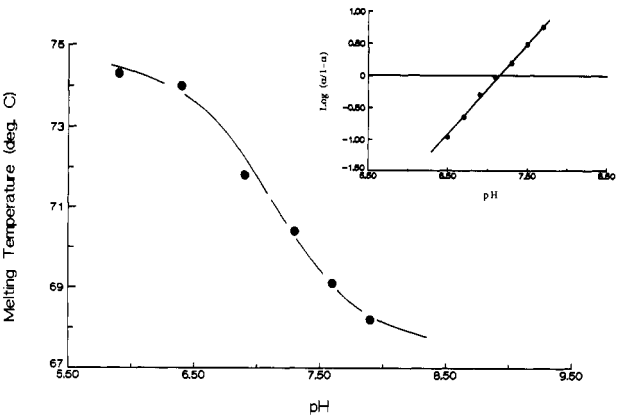

FIGURE 6: Effect of pH on the melting temperature of the thin filament. In the insert, the slope of the plot of log K versus pH gives the number of protons released in the process to be 1.4 per mole.

pH at low KCl concentrations (with a T_m around 47 °C). If we consider an average specific enthalpy of the helix (Δh) to be 4.24 cal/g and an average MRW (mean residue weight) of amino acids to be 115, this part of the helix would contain 150–190 residues which is approximately the size suggested by Harrington (1979) for the melting of the "hinge". The process we followed with the second major domain was related to about 80% of the helix and was characterized by a release of protons upon melting. The sigmoidal behavior of the T_m vs pH curve, at low ionic strength, allowed the calculation of the total number of protons involved in the process to be 2.17. It is known that short S-2 melts with a release of protons (Swenson & Ritchie, 1980; Tsong et al., 1983) and that part of the LMM demonstrates a similar behavior (Bertazzon & Tsong, 1989a). In solution at high ionic strength, the melting temperature of the transitions belonging to S-2 was most sensitive to pH variations compared to T_m's of transitions of LMM and S-1 [see Bertazzon and Tsong (1990)]. The pH dependence of the T_m of isolated S-2 reflected the pH-induced shift of the second peak in the myosin filaments, and the amount of helical structure (264–284 kcal/mol) which shifted from transition II at pH 6.3 and 0.13 M KCl to transition I at pH 6.7 and 0.17 M KCl was comparable with a part of S-2, about 65 kDa in size if we assume that the specific heat of unfolding was also 4.2 cal/g for S-2.

The endotherms for S-2 (Figure 4), G-actin (curve 1 of Figure 5A), and F-actin (Figure 5B and curve 1 of Figure 5A) were highly asymmetrical. Detailed deconvolutions of endotherms of S-2 are reported in Bertazzon and Tsong (1990). Deconvolutions of endotherms of G-actin have been reported (Bertazzon et al., 1990). The high asymmetry for the endotherm of F-actin could indicate a dissociation to actin monomer before, or precipitation after, unfolding.

Another conclusion derived from this study is that the thermal denaturation of the myosin filament did not demonstrate increased cooperativity when compared with the denaturation of the monomer in solution. The second transition of the filament was characterized by a cooperative ratio of about 0.4, indicating that although T_m's of several domains in a myosin were close these domains were still far from being fully cooperative. This phenomenon should be compared with the thermal behavior of F-actin. Analysis of the endotherm of G-actin (Bertazzon et al., 1989; Tatunashvili & Privalov, 1984) suggests the presence of two domains in the molecule. The existence of two domains in G-actin was also suggested by limited proteolytic digestion (Jacobson & Rosenbush, 1976) and the low-resolution X-ray structure (Kabsh et al., 1985). When actin polymerized to form F-actin, the endotherm be-

came much sharper than that of the monomer, and the cooperativity (CR of 1.4) exceeded the value expected for a pure two-state process, i.e., unity. Various ions were able to induce polymerization of G-actin, e.g., Mg^{2+}, K^+, and H^+.

Millimolar concentrations of calcium facilitated the formation of polymers and increased the T_m and the CR of the endotherm, though not reaching the values of the fully polymerized protein. The lack of effect of calcium on F-actin suggests that these low-affinity binding sites were not exposed in the polymerized form and that the overall stability of the thin filament was not calcium dependent. In contrast, the endotherm of F-actin was sensitive to pH changes between 6 and 8.

A model describing the cooperativity of F-actin (Erickson, 1989) suggests that the longitudinal and diagonal bonds in the inner domain are responsible for the rigidity of the helical backbone, where the flexibility of the outer domain permits a certain degree of movement and the possibility for functional interactions with myosin during the formation of cross bridges. The observed effect of calcium, as well as other ions, is probably exerted on the inner domain, which is relatively inaccessible to ions in F-actin.

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