Differences in the Thermal Stability of Acclimation Temperature-Associated Types of Carp Myosin and Its Rod on Differential Scanning Calorimetry†

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ABSTRACT: Differential scanning calorimetry (DSC) was employed for studying the thermal unfolding of myosin and its rod part prepared from carp acclimated to 10 and 30 °C. Differences in the thermal stability reflecting structural properties were clearly demonstrated by the DSC data obtained at pH 8.0 in 0.6 M KCl for the two types of carp myosin and rod. The transition temperatures on myosin and rod given by the major peaks for the 10 °C-acclimated carp were 33.9 and 47.4 °C and 33.0 and 44.0 °C, respectively, assuming two endotherms for this type. Since the shape of the first peaks at 33.9 and 33.0 °C was not symmetrical, two peaks having similar transition temperatures overlapped in this temperature range. When the data were analyzed using three endotherms, the three transition temperatures obtained for myosin and rod were 32.8, 34.9, and 47.4 °C and 32.9, 33.4, and 44.1 °C, respectively. Thus, the position of the first peak for the 10 °C-acclimated carp myosin did not change even after removal of the large subfragment-1 part, but the transition of the second peak shifted to a lower temperature by about 3 °C. The myosin and rod from carp acclimated to 30 °C showed three distinct peaks at 35.9, 39.7, and 49.1 °C and 34.5, 39.7, and 46.7 °C, respectively. The position of the largest peak for myosin remained unchanged, and the shift of the peak position of the highest temperature was about 3 °C, as obtained for the 10 °C-acclimated carp. The calorimetric enthalpies were 86, 146, and 69 kcal/mol for the 10 °C-acclimated rod and 95, 115, and 159 kcal/mol for the 30 °C-acclimated rod, respectively, in the order of the above endotherms. The DSC runs at pH 6.5 also gave similar results. Thermal unfolding responsible for these endotherms was mostly explained by melting of α-helices which could be determined by far-ultraviolet CD spectroscopy.

Sarcomeric myosins are complexed multidomain proteins consisting of an N-terminal two-globular head, subfragment-1 (S1), and a C-terminal rodlike tail which forms a coiled coil of α-helices (Harrington & Rodgers, 1984). The myosin rod is further functionally separable into light meromyosin (LMM) and subfragment-2 (S2). These three major domains are easily obtained by limited proteolysis with proteases commercially available such as α-chymotrypsin and trypsin. The myosin molecule itself is composed of noncovalently bound hexameric peptides: it contains two heavy chains with an approximate molecular weight of 200 000 and four light chains with a molecular weight of about 20 000, resulting in a total molecular mass of about 500 kDa. S1 has two noncovalently bound light chains and contains a site for ATP hydrolysis and a site for the interaction of actin. The three-dimensional structure of S1 has been intensively studied (Rayment et al., 1993).

The nature and interactions of the myosin rod molecule have also been well characterized (Harrington, 1979; Hvidt et al., 1982; McLachlan & Karn, 1982; Nyitray et al., 1983; Rodgers & Harrington, 1987; Rodgers et al., 1987; Rim et al., 1989; Offer, 1990). The rod portion is responsible for the assembly of myosin to form the functional thick filaments, and S1 protrudes from the filament surface to form cross-bridges with actin-containing thin filaments. The data obtained by techniques of molecular biology and electron microscopy suggest that the C-terminal tail part of the rod or LMM is extremely important for the formation of myosin filaments (Maeda et al., 1991; Atkinson & Stewart, 1992; Hodge et al., 1992). Myosin rods may also play an important role in the regulation of mechanochemical energy transduction. Investigations along these lines have been intensively carried out, especially focusing on the S2 region (Sutoh et al., 1978; Ueno & Harrington, 1984, 1986a,b; Lu & Wong, 1985; Lovell et al., 1988; Harrington et al., 1990; Sugi et al., 1992). Even given such extensive studies, the exact role of the S2 or hinge region in the rod which is essential to myosin functions and how these regions are regulated are not satisfactorily understood.

Differential scanning calorimetry (DSC) has remarkable advantages in demonstrating cooperative domain structures and domain interactions in proteins (Privalov, 1979; Privalov & Gill, 1988). Measurements obtained via DSC provide information on the thermodynamic behavior of proteins such as regarding the progress of the unfolding reaction (a van't Hoff enthalpy) and the calorimetric heat associated with the reaction. Since the development of the DSC techniques, studies on heat capacity related to the thermal transition of proteins with multiple domains have been precisely performed; the thermal unfolding of rabbit skeletal heavy

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meromyosin (HMM), S1, and S2 has been studied by DSC (Swenson & Ritchie, 1980; Shriver & Kamath, 1990). The unfolding of the S2 domain is reversible not only in the isolated form but also in HMM where S2 exists as a single domain (Shriver & Kamath, 1990). DSC has also been employed for examining thermal stability of myosin and actin in their filamentous forms (Bertazzon & Tsong, 1990a,b): the thermal stability of the myosin monomer, as well as of polymers, showed remarkable sensitivities to pH and to the ionic strength of the solution. In the myosin rod part, the lowest stability domain is located at the LMM/S2 junction and split into two parts, one associated with S2 and the other with LMM.

Fish skeletal myosins also have complexed multidomain structures containing two units of globular heads, S1, and a rodlike tail probably forming a coiled coil of α-helices similarly to mammalian myosins (Connell, 1958; Huriaux & Focant, 1978; Kimura et al., 1979; Watabe & Hashimoto, 1980; Matsuura et al., 1988; Hamai & Konno, 1990; Ochiai et al., 1990). The S1/rod junction is cleaved by limited proteolysis with α-chymotrypsin at a low ionic strength (Hamai & Konno, 1990; Ochiai et al., 1990; Kato & Konno, 1993). We have recently demonstrated that carp express different types of myosin heavy chains of fast skeletal muscle as a result of temperature acclimation, which differ from each other in the primary structure (Hwang et al., 1990; Watabe et al., 1992). In accordance with structural changes of the myosin molecule induced by low-temperature acclimation, acto-S1 Mg^{2+}-ATPase activity clearly increases (Hwang et al., 1991; Watabe et al., 1992). Furthermore, temperature acclimation caused changes in the rod region of the myosin molecule, since the apparent molecular sizes of LMM were different from each other for cold- and warm-acclimated types of myosin (Watabe et al., 1992).

The objective of this DSC study was to examine differences in the thermal stability of domains of different types of myosin and its rod from carp acclimated to cold and warm water temperatures. The participation of the α-helix in thermal stability was simultaneously demonstrated by CD spectrometry.

MATERIALS AND METHODS

Materials. Carp *Cyprinus carpio* (0.6−0.8 kg in body weight) were acclimated to either 10 or 30 °C for a minimum of 5 weeks, and their dorsal fast muscles were used for protein preparations.

Protein Preparations. Myosin was prepared as reported previously (Hwang et al., 1990). Myosin rod was prepared from myosin by the method of Weeds and Taylor (1975) modified by Hwang et al. (1991). Briefly, myosin at a concentration of 10−15 mg/mL was digested with *N*-tosyl-L-lysine chloromethyl ketone (TLCK)-treated α-chymotrypsin at an enzyme-to-myosin weight ratio of 1:130 at 10 °C for 30 min in 20 mM phosphate buffer (pH 7.0) containing 0.12 M NaCl, 1 mM EDTA, and 0.1 mM dithiothreitol (DTT). Digestion was stopped by the addition of phenylmethanesulfonyl fluoride (PMSF) to a final concentration of 0.5 mM. The digest thus obtained was subjected to centrifugation at 100 000*g* for 1 h, and the resulting pellet was dissolved in and dialyzed against 20 mM TRIS (pH 8.0) containing 0.6 M KCl, 5 mM $MgCl_2$, and 0.1 mM DTT. For some analyses, the rod fraction was further purified by the ethanol precipitation method (Hvidt et al., 1982) or by using a DEAE-Toyopearl 650M column equilibrated with 40 mM sodium pyrophosphate (pH 7.4) containing 0.1 mM DTT according essentially to the method of Rodgers et al. (1987).

Protein concentrations were determined by the biuret method of Gornall et al. (1949) using bovine serum albumin as the standard. The accuracy of this method for carp myosin and rod was confirmed by weight measurements after dehydration of protein solution.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). SDS−PAGE was carried out by the method of Laemmli (1970) using 7.5−20% polyacrylamide gradient slab gels containing 0.1% SDS. After electrophoresis, proteins were stained with 0.1% Coomassie brilliant blue R-250.

Differential Scanning Calorimetry (DSC). DSC was performed with a differential microcalorimeter MC2 (MicroCal Inc., Northampton) equipped with a personal computer system. The solvent used was either 20 mM TRIS (pH 8.0) containing 0.6 M KCl, 5 mM $MgCl_2$, and 0.1 mM DTT or 20 mM potassium phosphate (pH 6.5) containing 0.5 M KCl, 1 mM EDTA, and 0.1 mM DTT. The pH was adjusted at room temperature. Protein solutions were exhaustively dialyzed against the indicated buffers. The sample cell was loaded with 1.2 mL of a protein solution at a protein concentration range of 1.0−2.6 mg/mL, whereas the reference cell was filled with the buffer. DSC scans were performed at a rate of 45 °C/h in the temperature range from 10 to 70 °C under an excess N_2 pressure at about 220 kPa.

DSC data were analyzed using a software package, Origin, developed by the MicroCal. After subtracting the reference buffer data, the raw data obtained in the form of heat capacity as a function of measuring temperature were converted to excess molar heat capacity using the scan rate and protein concentration. Subunit molecular weights of myosin heavy chain and its rod were assumed to be 200 000 and 132 000, respectively (Harrington & Rodgers, 1984; Rodgers et al., 1987; Watabe et al., 1992). The heat capacity data were fit by using nonlinear least-squares, initially assuming that $\Delta H_{cal}/\Delta H_{vh} = 1$ where ΔH_{cal} and ΔH_{vh} are calorimetric and van't Hoff enthalpy, respectively. When the data were not fit satisfactorily, the heat capacity curves were subsequently fit by allowing ΔH_{cal} and ΔH_{vh} to float. After minimization by appropriate computer programs, the values for the thermal transition, i.e., the transition temperature (T_m), ΔH_{cal}, and ΔH_{vh}, and ΔC_p could be obtained.

CD Spectroscopy. CD spectra of myosin rod were measured at various temperatures ranging from 25 to 60 °C in the same buffer as in DSC measurements with a JASCO J-600 spectropolarimeter. A jacketted cell of 0.2 mm optical path length was used, and the temperature was controlled by circulating thermoregulated water. The measurements were carried out in the range from 250 to 200 nm at protein concentrations of 0.6−1.0 mg/mL.

RESULTS

Protein Characterization. Myosin isolated and purified to homogeneity from carp acclimated to 10 and 30 °C did not show apparent differences in the SDS−PAGE pattern from each other (Figure 1). Both types of myosin contained heavy chain having a molecular weight of about 200 000

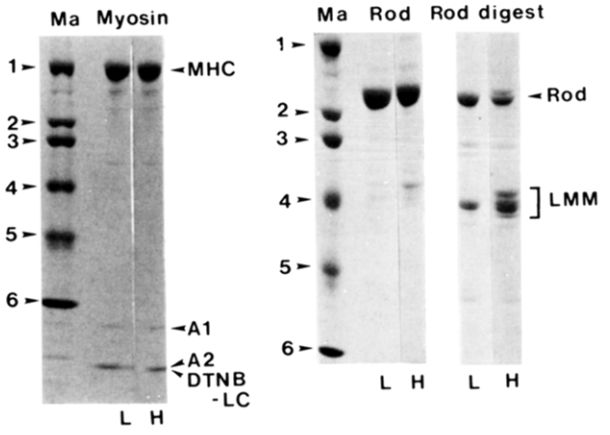

FIGURE 1: SDS−PAGE patterns of myosin, rod, and rod chymotryptic digest from the 10 (L) and 30 (H) °C-acclimated carp. The rod was purified by ion-exchange column chromatography. Proteins of 10 μg/lane were applied to the 7.5−20% gradient polyacrylamide slab gel and stained with Coomassie brilliant blue R-250. Abbreviations: MHC, myosin heavy chain; A1 and A2, myosin alkali light chains 1 and 2; DTNB-LC, myosin 5,5′-dithiobis(2-nitrobenzoic acid) light chain; LMM, light meromyosin. Molecular weight markers (Ma) used are 1, myosin heavy chain (205 kDa); 2, β-galactosidase (116 kDa); 3, phosphorylase b (97.4 kDa); 4, bovine serum albumin (66 kDa); 5, ovalbumin (45 kDa); 6, carbonic anhydrase (29 kDa).

and three kinds of light chain of about 20 000 in molecular weights. Limited proteolysis of filamentous myosins resulted in the production of the rod part which also did not show any difference between the two acclimated carp groups with an apparent molecular mass of 132 kDa (Figure 1). On the other hand, chymotryptic digestion of myosin rod in a buffer of a high ionic strength gave rise to different SDS−PAGE patterns between the two acclimation groups. The 30 °C-acclimated rod produced apparent three LMM fragments, while the 10 °C-acclimated one showed a major single LMM band with a component of lesser density (Figure 1). One of the assigned LMM bands might correspond to that of S2, since LMM and long S2 have a similar molecular weight (Sutoh et al., 1978). Faint bands in the range between molecular weight markers 5 and 6 may be composed of short S2 and degraded products of LMM (Sutoh et al., 1978; Kato & Konno, 1993). We showed previously that peptide maps of the rod in the SDS gels were clearly different between the 10 and 30 °C-acclimated carp, suggesting changes in the primary structures of rods resulting from temperature acclimation (Watabe et al., 1992). The appearance of a minor band in the remaining rod region after limited proteolysis of the 30 °C-acclimated rod may be due to the presence of rod isoforms in this preparation.

DSC Scans. Results of DSC at pH 8.0 in 0.6 M KCl for the two types of myosin from carp acclimated to 10 and 30 °C are shown in Figure 2. Two prominent endotherms having transition temperatures (T_m) of 33.9 and 47.4 °C were found for the 10 °C-acclimated myosin. However, the shape of the first peak was not symmetrical but somewhat skew, implying overlap of two peaks. The deconvolution analysis gave three endotherms having three T_m's of 32.8, 34.9, and 47.4 °C as shown in Figure 2A. Myosin from the 30 °C-acclimated carp gave two main peaks, a large peak with a shoulder and a smaller one, and three endotherm peaks at 35.9, 39.7, and 49.1 °C were found by a curve resolution (Figure 2B). The lowest T_m (35.9 °C) in the 30 °C-acclimated myosin was higher than that of the peak (32.8

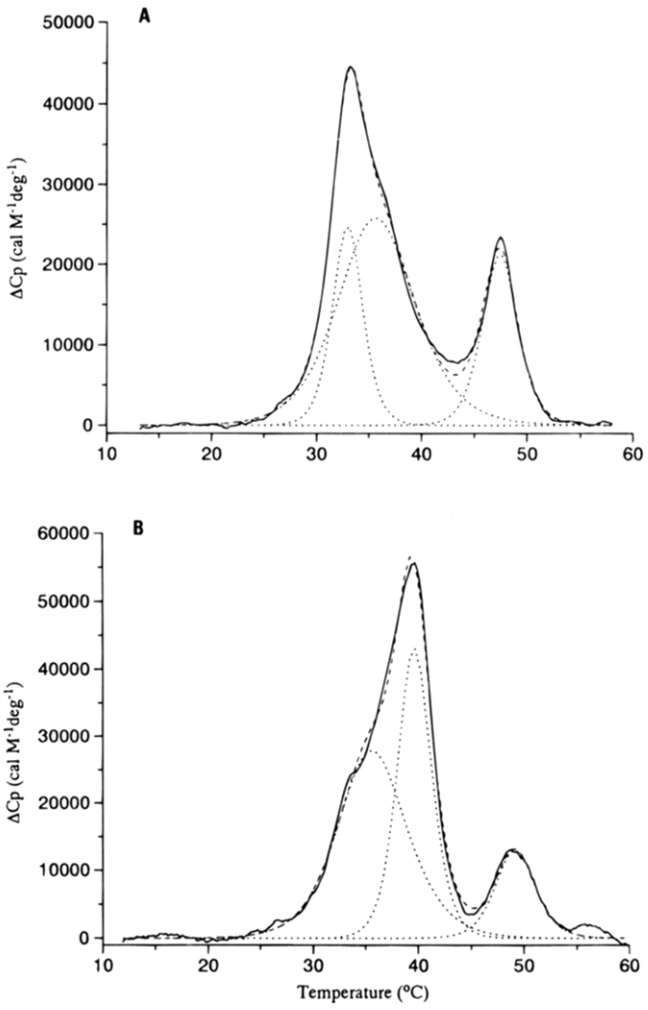

FIGURE 2: DSC scans of myosins from carp acclimated to 10 (A) and 30 (B) °C, together with their computer-calculated differential endotherms by the convolution analysis (dotted lines). DSC scans were performed in 20 mM TRIS (pH 8.0) containing 0.6 M KCl, 5 mM $MgCl_2$, and 0.1 mM DTT at protein concentrations of 2.63 (A) and 1.67 (B) mg/mL. The scan rate was 45 °C/h, and data were collected every 15 s. ΔC_p represents molar excess heat capacity.

°C) located at a lower temperature in the 10 °C-acclimated myosin. Since the reversibility of the unfolding reaction was not satisfactory with either type of myosin (data not shown), any further calorimetric data analysis was not performed.

DSC scanning patterns of the 10 and 30 °C-rod were very similar to those observed for intact myosin (Figure 3). For example, the 10 °C-acclimated rod exhibited two midpoints of the unfolding, whereas the 30 °C-acclimated counterpart clearly showed three endotherms. These patterns hardly changed irrespective of preparation methods for the rod, including ethanol precipitation and ion-exchange column chromatography (data not shown). The T_m values were also comparable for myosin and rod within the same acclimation group. The thermal denaturation of the rod from the 10 and 30 °C-acclimated carp was partially reversible (insets in Figure 3), in contrast to a good reversibility of 80−90% reported for rabbit myosin rod (Bertazzon & Tsong, 1990b), suggesting that carp myosin is more labile than rabbit myosin.

When the curve resolution of the endotherm into independent domains was performed, the 10 °C-acclimated rod showed the better fitting for three thermal unfolding com-

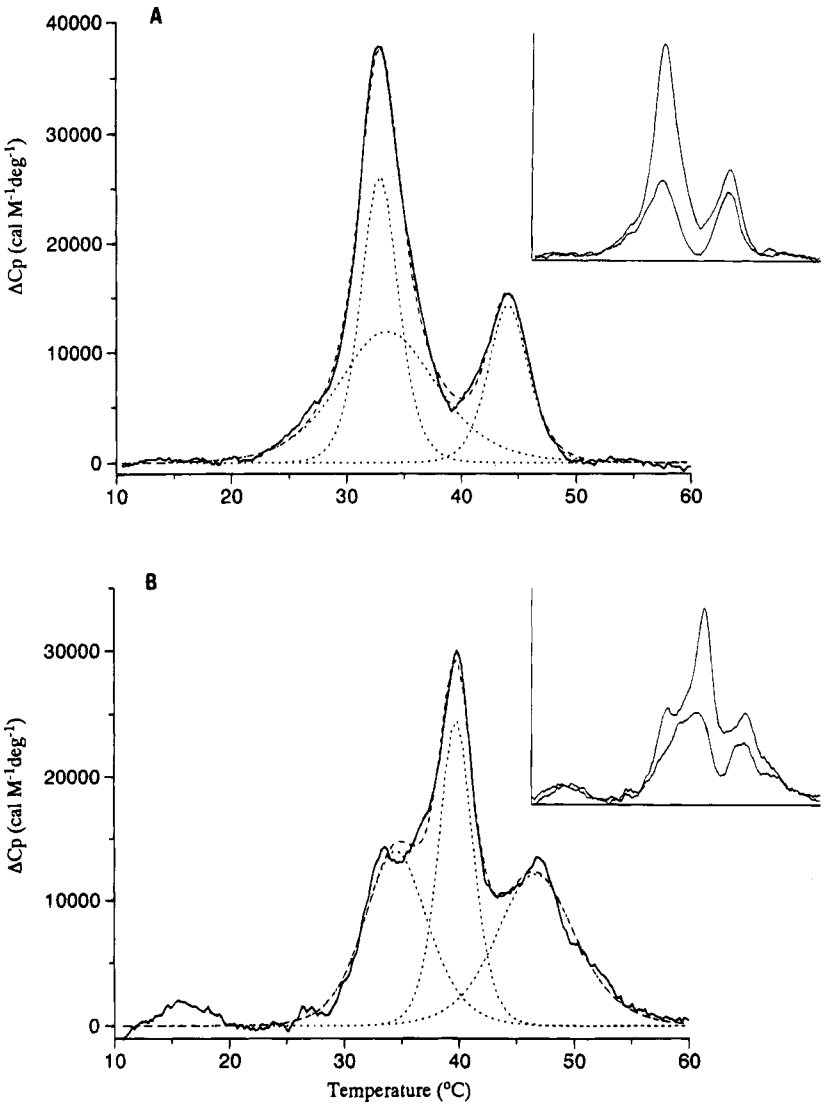

FIGURE 3: DSC scans of myosin rods from carp acclimated to 10 (A) and 30 (B) °C, together with their computer-calculated differential endotherms by the convolution analysis using three peaks (dotted lines). The results of repetition of DSC scans for myosin rods are shown in insets, where the upper curves indicate the first run and the lower curves indicate the second run. Protein concentrations were 1.32 (A) and 0.99 (B) mg/mL. Refer to the legend of Figure 1 for other experimental conditions.

ponents, as found for the 30 °C-acclimated rod (Figure 3). T_m values of the thermal unfolding of the 10 °C-acclimated rod, however, were markedly different from those of the 30 °C-acclimated rod. Thermodynamic parameters derived from deconvolution of the DSC data on the carp myosin rods are listed in Table 1. Bertazzon and Tsong (1990b) reported that rabbit myosin rod exhibited three main differential endotherms: the two peaks having the lowest and highest T_m values correspond to LMM endotherms, while the intermediate peak was identified as that of S2.

The three T_m values of the 10 °C-acclimated rod were lower than those of the corresponding three peaks in the 30 °C-acclimated carp. The most striking difference was observed for the T_m of the second endotherm. The difference of T_m between the 10- and 30 °C-acclimated rod was as large as 6.3 °C and much larger than those of the first and third T_m values (1.6−2.6 °C). The calorimetric enthalpy change (ΔH_{cal}) for the endotherm was in the range from 70 to 160 kcal/mol and was highest in the third endotherm from the 30 °C-acclimated rod. The DSC scanning patterns at pH 6.5 in 0.5 M KCl for carp myosin and rod were also similar to those obtained at pH 8.0 in 0.6 M KCl (data not shown), although the T_m values for corresponding peaks were somewhat shifted to higher temperatures at pH 6.5 compared with those at pH 8.0.

Table 1: Thermodynamic Parameters on the Thermal Unfolding of Myosin and Rod from Carp Acclimated to 10 and 30 °C[a]

	2 peaks (pH 8.0)		3 peaks (pH 8.0)		3 peaks (pH 6.5)	
	T_m (°C)	ΔH_{cal} (kcal/mol)	T_m (°C)	ΔH_{cal} (kcal/mol)	T_m (°C)	ΔH_{cal} (kcal/mol)
myosin						
10 °C-acclimated	33.9	294(0.4)	32.8	73 (3.3)	34.7	122 (1.8)
	47.4	85	34.9	228 (0.4)	37.5	249 (0.3)
			47.4	80 (2.7)	48.3	116 (1.7)
30 °C-acclimated			35.9	248(0.4)	36.3	274 (0.3)
			39.7	211 (0.8)	40.4	105 (1.8)
			49.1	91 (1.5)	50.9	52 (2.9)
rod						
10 °C-acclimated	33.0	249(0.5)	32.9	86 (2.2)	34.5	99 (1.6)
	44.0	89(1.7)	33.4	146 (0.6)	37.8	203 (0.5)
			44.1	69 (2.5)	51.1	78 (1.5)
30 °C-acclimated			34.5	95 (1.2)	36.2	127 (0.8)
			39.7	115 (1.6)	40.7	107 (1.9)
			46.7	159 (0.5)	51.9	78 (1.5)

[a] The values in parentheses are ratios of ΔH_{vh} to ΔH_{cal}.

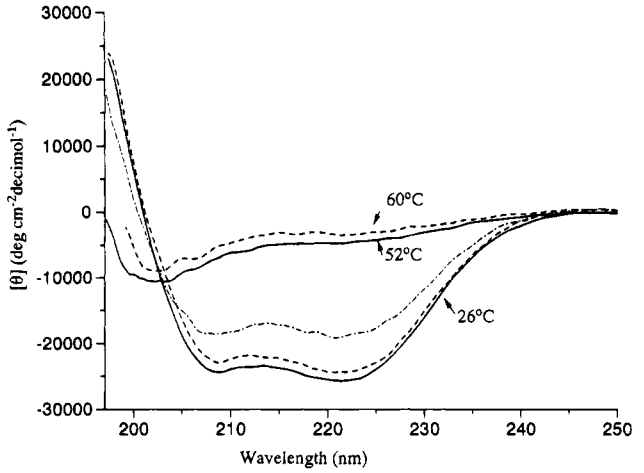

FIGURE 4: CD spectra of myosin rods from carp acclimated to 10 (solid lines) and 30 (dashed lines) °C at various temperatures. CD spectrometry was performed in 20 mM TRIS (pH 8.0) containing 0.6 M KCl, 5 mM $MgCl_2$, and 0.1 mM DTT. Reversibility was examined for the sample once heated at 60°C and subsequently measured at 26 °C (chain line with a dot). [θ] represents the mean residue ellipticity. Protein concentrations were 1.0 (solid lines) and 0.6 (dashed lines) mg/mL.

CD Spectra. Since the rod has α-helical content of over 90% (Lowey et al., 1969), the heat capacity changes observed in DSC may be related to the thermal unfolding of α-helix. CD spectra at pH 8.0 of myosin rod from the 10 and 30 °C-acclimated carp are shown in Figure 4 at representative low and high temperatures. The CD spectra at room temperature for both rods are the typical pattern of α-helix having two minima at 222 and 208 nm. Assuming that 100% of helical content corresponds to a value of −36 000 deg cm^{-2} dmol of [θ] (Yang et al., 1986), both types of myosin rod are deduced to have a helical content of 70%, following measurements at 25 °C. This value seems to be a bit smaller than those from rabbit skeletal muscle. However, the helical content at pH 6.5 and 26 °C was 80−90% for the two types of myosin rod.

The minimum at 222 nm increased with temperature at pH 8.0 in 0.6 M KCl, indicating the decrease in the helical content. Finally the curve at 50−60 °C did not change with further heating, showing that the rod molecules are unfolded. When the temperature was lowered to room temperature, the CD curve recovered to show a typical spectrum of α-helix but the recovery was not complete (about 75% for the experiments shown in Figure 4) as observed for the DSC experiments.

Plots of α-helical contents at pH 8.0 in 0.6 M KCl against temperature for myosin rod of the 10 and 30 °C-acclimated carp are shown in Figure 5. In addition, the derivatives of the curves together with the data for DSC analysis are superimposed in the same figure. DSC endotherms agree fairly well with peaks of the decreasing rate of α-helical content, especially for the rod from the 10 °C-acclimated carp. These results imply that thermal denaturation of carp myosin rod occurs due mainly to the unfolding of the α-helix.

DISCUSSION

Different types of myosin rod are expressed by temperature acclimation of carp (Watabe et al., 1992; see also Figure 1). Differences between the cold- and warm-acclimated rods prevailed in the primary structure, suggesting a functional significance of the changed region of the rod molecule in the regulation of energy transduction from S1 to thick filaments. We prepared myosin and rod from the 10 and 30 °C-acclimated carp and subjected them to the DSC runs. It is quite reasonable to consider that these preparations contain multiple isoforms of myosin and rod. Nevertheless, differences in the thermal stability reflecting structural properties were clearly demonstrated by the DSC data for the two types of carp myosin and rod (see Figures 2 and 3), suggesting that these preparations consisted of an acclimation temperature-specific dominant isoform together with minor components. Thermodynamic parameters (T_m, ΔH_{cal}) and the cooperative ratio ($\Delta H_{vh}/\Delta H_{cal}$) for myosin and rod from the 10 and 30 °C-acclimated carp are listed in Table 1. The transition temperatures on myosin and rod at pH 8.0 in 0.6 M KCl given by the major peaks for the 10 °C-acclimated carp are 33.9 and 47.4 °C and 33.0 and 44.0 °C, respectively, assuming two endotherms for this type. Since the shape of the first peaks at 33.9 and 33.0 °C is not symmetrical, two peaks having similar transition temperatures overlap in this temperature range. When the data were analyzed using three endotherms, the three transition temperatures obtained for myosin and rod are 32.8, 34.9, and 47.4 °C and 32.9, 33.4, and 44.1 °C, respectively. Thus, the position of the major peak for the 10 °C-acclimated carp myosin does not change even after removal of the large S1 part, but the transition of the second peak shifts to a lower temperature by about 3 °C. The myosin and rod from carp acclimated to 30 °C show three distinct peaks at 35.9, 39.7, and 49.1 °C and 34.5, 39.7, and 46.7 °C, respectively. The position of the largest peak for myosin remained unchanged, and the shift of the peak position of the highest temperature is about 3 °C, as obtained for the 10 °C-acclimated carp. The same tendency was obtained with carp myosin and rod dissolved in 0.5 M KCl at pH 6.5.

The assignment of these endotherms to structural units of myosin could not be performed because of a lack of information about endotherms of detailed units such as isolated LMM and S2. We had tried to isolate S2 and LMM from carp but have not yet obtained pure materials which can be used for the fine DSC analysis. However, putative endotherms in a preliminary experiment at pH 8.0 in 0.6 M KCl for crude LMM from the 10 °C-acclimated carp occurred at 32.5 °C, whereas crude LMM from the 30 °C-acclimated carp showed a transition tempeerature of 39.7 °C. These temperatures almost coincide with those for corresponding rod and myosin of both types. Therefore, it is very likely that the largest endotherm observed for myosin and rod corresponds to that for LMM, which is a coiled coil of two α-helices. If the above assignment is correct, the endotherm for S2 would be the smaller endotherm observed at higher temperatures than those for LMM. The shift of the temperature by about 3 °C might be due to some loosening of the structural unit of the S2 part by removing the large unit of S1.

Myosin rod from rabbit skeletal muscle has the lowest stability domain at the LMM/S2 junction which splits into two parts, one associated with S2 and the other with LMM (Bertazzon & Tsong, 1990b). The endotherms for rabbit LMM were very complex: three major peaks were observed at pH 6.45, two being most prominent at 41.7 and 56.2 °C. The largest endotherms for S2 were found at around 48.5 °C, thus locating between the two main endotherms of LMM.

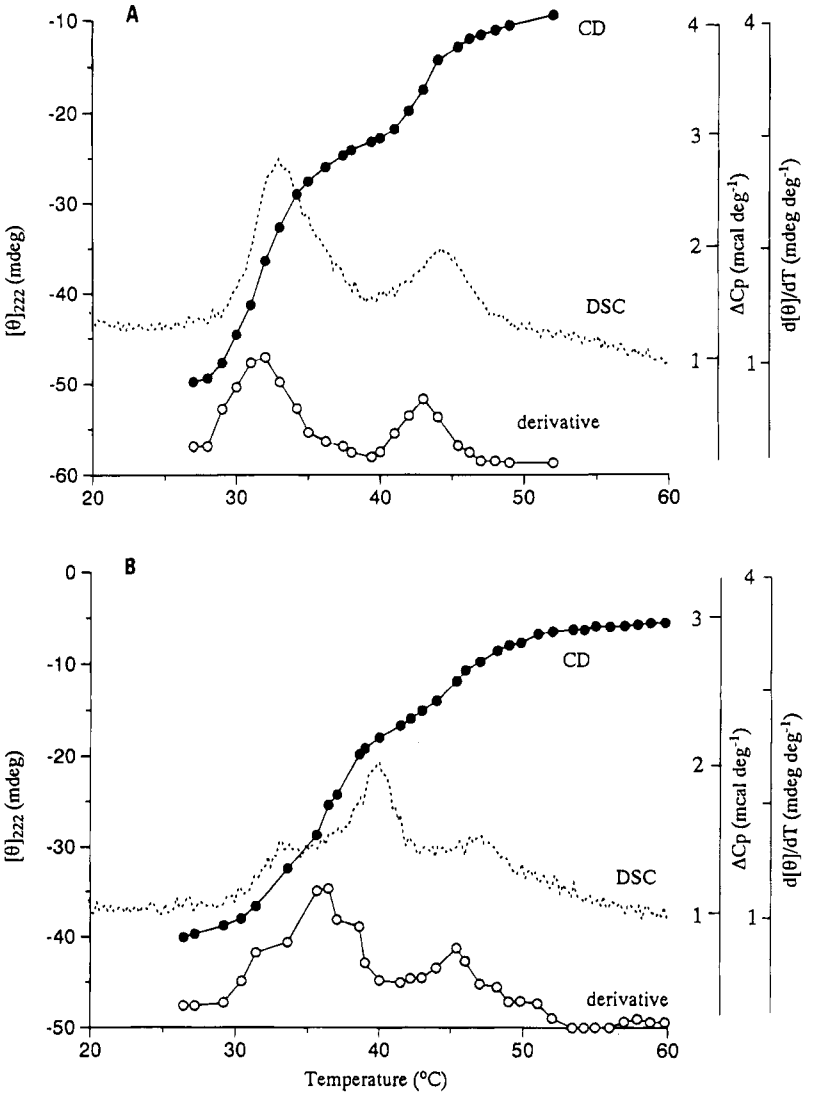

FIGURE 5: Comparison of DSC scans and the decreasing rate derivatives of the mean residue ellipticity, $[\theta]$, against temperatures for myosin rods from carp acclimated to 10 (A) and 30 (B) °C. DSC scans (dotted lines) are the same as in Figure 3. The decreasing rate derivatives ($d[\theta]/dT$) (○) were calculated from the increment of $[\theta]$ at 222 nm (●) per unit change of temperature (°C).

These endotherms are distinctly higher than those of carp. The smaller peak height of the endotherms for S2 corresponds to a lower helical content of S2 as reported for rabbit S2 (Lowey et al., 1969). The T_m values at pH 8.0 of the myosin and rod even from the 30 °C-acclimated carp were 33−47 °C, which is considerably lower than those of rabbit (42−56 °C), suggesting the low thermal stability of carp myosin.

Experiments of chymotryptic digestion of the rod for the preparation of LMM suggested marked changes in the S2 part or hinge region following temperature acclimation. The 30 °C-acclimated carp produced three LMM fragments, whereas the 10 °C-acclimated carp gave only one type of LMM (see Figure 1). Preliminary experiments in microsequence analysis revealed that some amino acids are changed even in the extreme N-terminal amino acid sequence of LMM, which will be published elsewhere. These results suggest a functional significance of the hinge region for acclimation temperature-dependent changes of myosin properties in the regulation of muscle contraction.

It has been reported that sharp bends are observed in the tail part of the skeletal myosin molecule at well-defined positions 44, 75 (hinge), and 135 nm from the head/tail junction (Offer, 1990; Rim et al., 1989). Harrington and Ueno (1987) claimed that a conformational change in the α-helical myosin S2 region of the cross-bridge occurs when this portion of the bridge is released from the thick filament surface. Harrington et al. (1990) demonstrated that rabbit skeletal skinned fibers treated with antibody against S2 markedly reduced the isometric force and suggested that the S2 region as well as the myosin head contributes to force generation in actively contracting muscle. Sugi et al. (1992) further revealed that Mg^{2+}-ATPase activity of rabbit skinned fibers remained unchanged upon a treatment of anti-S2 antibody, despite the reduction of isometric force even to zero. It is interesting to compare such effects on carp muscle fibers from different acclimation temperatures. The changes of the rod structure could affect myosin interaction with actin, thus regulating carp muscle contraction to compensate for seasonally fluctuating environmental temperatures.

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