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Uniquely Stable 40 kDa Subfragment-2 in Carp Myosin

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Digestion of carp myofibrils at 30 °C in 0.5 M KCl medium with calcium ion generated unique 135 kDa heavy meromyosin (HMM). The HMM was not produced when digested at 10 °C. A further digestion of the 135 kD HMM isolated in the absence of calcium ion generated uniquely short subfragment-2 (S-2) with a size of 40 kDa (40 kDa S-2) together with subfragment-1 (S-1). The 40 kDa S-2 was identified by N- and C-end sequencing, and demonstrated to locate the amino end of the rod portion. The unfolding temperature for the 40 kDa S-2 was around 52 °C as studied by circular dichroism measurement. The same unfolding peak was also detected with the intact rod together with a large unfolding peak at around 36 °C coming from the rest of the rod portion, light meromyosin. The unfolding peak for the 40 kDa S-2 in myosin was a little lower (48 °C) than that in free form, suggesting the involvement of the head portion in the stability of the 40 kDa S-2 in the structure.

KEYWORDS: Myosin; subfragment-2; thermal denaturation; myofibril; unfolding; chymotryptic digestion

INTRODUCTION

One of the characteristic properties of fish myosin is its unstable nature (*1*, *2*). The myosin molecule has a unique structure consisting of two 200 kDa heavy chains (HCs) and two sets of two types of light chain (LC) components with sizes of about 20−27 kDa. The amino terminal 100 kDa of a single HC together with two types of LC forms water-soluble globular subfragment-1 (S-1), and the rest of the two HC subunits form a salt-soluble coiled-coil rod structure. The head portion contains ATPase and F-actin binding sites, and the tail region assembles to form filaments under physiological conditions of low salt. The rod portion is almost completely α-helical in structure (*3*−*5*). The amino terminal half of the rod termed subfragment-2 (S-2) is water-soluble, and the rest of the rod (light meromyosin, LMM) is water-insoluble. It is well established that thermal stability of the rod portion as an S-1 portion is species-specific (*3*), and the myosin rod from cold-water species is much more unstable than that from warm-water species by circular dichroism (CD) measurement (*5*). The same conclusion was obtained by studying the thermal denaturation of LMM from carp acclimated at 10 and 30 °C by using differential scanning calorimetry (DSC) (*6*). However, the information on the thermal stability of the S-2 region seemed limited.

In the present study, we prepared uniquely stable 40 kDa S-2 from carp myosin by raising the temperature for chymotryptic digestion. The stable nature of S-2 was proved by showing a high unfolding temperature compared with that of LMM. We proposed a new concept on the rod structure that the rod consists of three parts: the most stable amino terminal 40 kDa S-2 region, the intermediate region, and the most unstable carboxyl terminal 40 kDa LMM region (*7*). The unfolding temperature for the 40 kDa S-2 in the rod was the same as its isolated form, but clearly lower in myosin, indicating that the head region reduced the stability of the 40 kDa S-2. Consequently, the cooperative denaturation of the head and tail was demonstrated.

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MATERIALS AND METHODS

Myofibrils were prepared from the dorsal muscle of carp *Cyprinus carpio* as Katoh el al. described (*8*). Minced muscle was homogenized in 0.1 M KCl, 20 mM Tris−HCl (pH 7.5) and washed repeatedly with the same buffer. Washed myofibril suspension was finally filtered through two layers of gauze to remove connective tissues, and the filtrate was used as a myofibril suspension. Myosin was isolated from the myofibrils as described earlier (*9*) by collecting the pellet between 40% and 55% saturation of ammonium sulfate in the presence of Mg-ATP. The myosin rod was isolated from myofibrils by digestion with 1/250 (w/w) chymotrypsin over myofibrils at 20 °C in the digestion medium of 0.05 M KCl, 20 mM Tris−maleate (pH 7.0), and 1 mM EDTA, and ammonium sulfate fractionation (*10*). The 40 kDa LMM was prepared from the chymotryptic digest of the myosin rod as described earlier (*7*) using DEAE-Toyopearl 650M ion exchange chromatography. Chymotryptic 135 kDa HMM was isolated from the myofibril digest performed at 30 °C in 0.5 M KCl, 20 mM Tris−HCl (pH 7.5), and 1 mM $CaCl_2$. The detailed procedures are described in the Results.

SDS−PAGE was performed according to Laemmli (*11*, *12*) using 7.5% polyacrylamide gel.

CD measurement was performed on JASCO spectropolarimeter J 725 (JASCO, Tokyo). The measurement was done in a cell with a 1 mm light path length, and the change in the ellipticity at 220 nm upon heating at a rate of 1 °C/min was measured for detecting unfolding (*13*). The measurement was repeated several times to obtain the unfolding profiles for the samples.

Gel filtration was performed on Sepharose CL 4B (1.6 × 80 cm) in a medium of 0.1 M KCl, 20 mM Tris−HCl (pH 7.5) (*14*).

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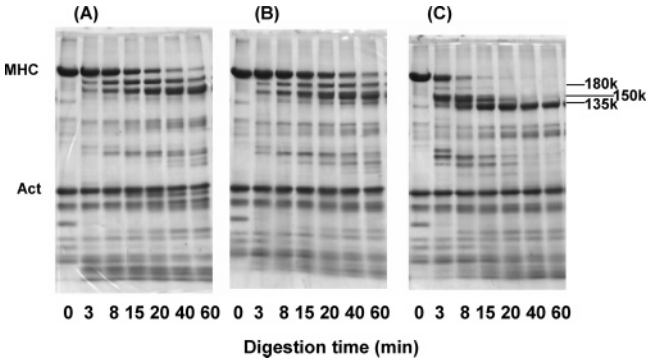

Figure 1. Chymotryptic digestion patterns of carp myofibrils at various temperatures. Carp myofibrils dissolved in 0.5 M KCl, 20 mM Tris–HCl (pH 7.5), and 1 mM $CaCl_2$ were digested with chymotrypsin. The amounts of chymotrypsin used at various temperatures were 1/100 (w/w) at 10 °C (**A**), 1/500 (w/w) at 20 °C (**B**), and 1/1000 (w/w) at 30 °C (**C**). HC and Act are myosin heavy chain and actin bands. 180k, 150k, and 135k are heavy chain components of HMM produced.

The N-terminal amino acid sequence was determined routinely. Protein bands separated on SDS−polyacrylamide gel were transferred to poly(vinylidene difluoride) membranes. Protein bands visualized by staining with Coomassie Brilliant Blue were cut, and were sequenced with a Parkin-Elmer Applied Biosystems model 473 A protein sequencer (*15*).

The C-terminal amino acid sequence was identified by using carboxylpeptidase Y digestion routinely by identifying the released amino acid upon digestion (*16*).

RESULTS

Chymotryptic Digestion of Myofibrils at Elevated Temperature. It is well-known that myosin in an isolated form or in actomyosin complex dissolved in high salt is cleaved at the HMM/LMM junction by chymotrypsin when the medium contains divalent cations to protect the cleavage at the S-1/rod junction (*17*). This is commonly observable with skeletal muscle myosin including fish myosin (*18*). We reported several digestion sites within the carp myosin rod, and uniquely short LMM, termed 40 kDa LMM, was isolated from the digest. The unique site on the rod was the 95 kDa S-2/40 kDa LMM junction. The site was found by analyzing the digestion products at 20 °C. We studied how myosin digestion is affected when the temperatures is raised from 10 to 30 °C. The digestion was performed in the medium of 0.5 M KCl with 1 mM $CaCl_2$. The digestion patterns are presented in **Figure 1**. When myofibrils were digested at 10 °C, 180 kDa HMM was prominent in the products. The counterpart of the HMM was the 40 kDa LMM (*15*), which can be detected just below the actin band in the pattern. Production of such a long HMM and short LMM has not been reported with other myosins. The 40 kDa LMM was accumulated in the digests. When the digestion was conducted at 20 °C, the production of 180 kDa HMM became less, and the 150 kDa HMM was a major product. The 40 kDa LMM was hard to see in the pattern. Elevation of the digestion temperature to 30 °C changed the pattern significantly. The 180 kDa HMM was no longer produced, the 150 kDa HMM was gradually converted into 135 kDa HMM, and the product was kept unchanged for up to 60 min. On the other hand, bands corresponding to LMM gradually disappeared by prolonging the digestion. Probably they were degraded into short fragments. The pattern for the digest for 60 min showed the 135 kD HMM and actin bands as major bands. It was suggested that the 135 kD HMM contains no cleavage sites within it under the conditions. We usually use 20 or 10 °C as the digestion temperature because of the unstable structure of fish myosin. The results showed that the ordinary or conventional HMM (150 kDa HMM) still contains an additional cleavage site within it.

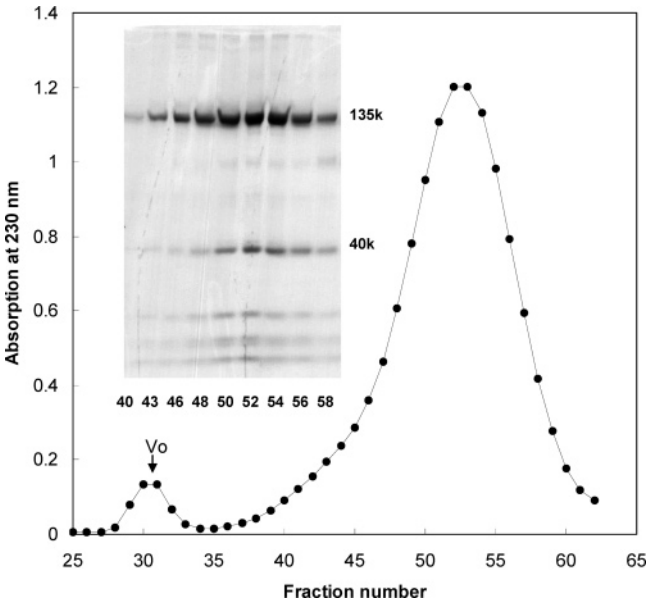

Figure 2. Gel filtration profile of the 135 kDa HMM. The HMM prepared was applied to the Sepharose CL-4B gel column (1.6 × 80 cm) equilibrated with 0.1 M KCl, 20 mM Tris–HCl (pH 7.5). V_0 is the void volume of the column. The inset is SDS–PAGE patterns of the fractions separated. 135k and 40k are fragments detected in the fractions.

Isolation of the 135 kDa HMM. As the production of uniquely short HMM was suggested in **Figure 1C** in the digest at 30 °C, the HMM was isolated from the digest. The preparative procedures were practically the same as employed for S-1 preparation from the myofibril digest (*19*). First, acto-HMM complex was collected as a pellet at 40% saturated ammonium sulfate in the absence of Mg-ATP. HMM in the complex was detached from F-actin upon addition of 2 mM Mg-ATP. F-actin was removed as a pellet at 40% saturated ammonium sulfate, leaving HMM in the supernatant. The HMM was collected as a pellet by raising the saturation to 55%. After dialysis against 0.1 M KCl, 20 mM Tris−HCl (pH 7.5), and the centrifugation at 20000*g* for 20 min, the soluble fraction was collected as crude HMM. HMM prepared was applied to a Sepahrose CL 4B gel filtration column to study whether the 135 kDa HMM is monomeric. The elution profile is shown in **Figure 2**. The 135 kDa HMM was eluted as a single peak at around fraction number 52 with a very small peak at V_0, indicating that almost all of HMM was monomeric. The SDS−PAGE analysis of the peak showed that the peak contained the 135 kDa and three light chain components, indicating that the 135 kDa HMM contained both types of light chains as myosin. We found the 40 kDa fragments in the same fractions. As the density ratio of the 40 kDa fragment to the 135 kDa fragment was identical, the 40 kDa fragment was suggested to be present as a bound form to the HMM molecule under the conditions. Subunit interaction at the S-2 region forms a coiled-coil structure in HMM. Thus, it was suggested that the 40 kDa S-2 is present as a coiled-coil structure in the 135 kDa HMM. The 40 kDa fragment would be produced from the 135 kDa fragment by tearing off the S-1 region during the digestion at high temperature. Accepting the idea, the 135 kDa HMM preparation contains single-headed HMM species, although the population seemed not large judging from the 40 kDa fragment content in the preparation.

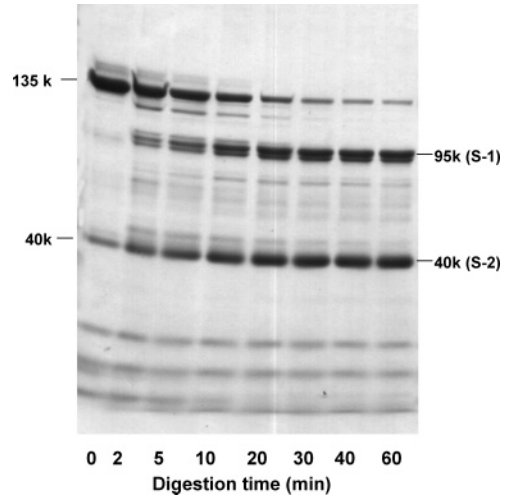

Figure 3. Chymotryptic cleavage of the 135 kDa HMM into S-1 and the 40 kDa S-2. The 135 kDa HMM was further digested with chymotrypsin at 20 °C in the medium of 0.1 M KCl, 20 mM Tris–HCl (pH 7.5), and 1 mM EDTA by using 1/100 (w/w) enzyme. 95k(S-1) and 40k(S-2) are the S-1 heavy chain and subunit of the 40 kDa S-2, respectively.

To confirm that the 40 kDa fragment came from the S-2 region, the 135 kDa HMM was further digested with chymotrypsin in the presence of EDTA, allowing chymotrypsin to cleave at the S-1/rod junction. The chymotryptic digestion was studied by SDS−PAGE (**Figure 3**). The 135 kDa fragment was gradually converted into 95 and 40 kDa fragments. The former one was S-1 HC. The 40 kDa band gradually increased, and no further cleavage occurred. It was concluded that the 40 kDa fragment was the fragment derived from the S-2 portion of the 135 kDa HMM molecule. The 40 kDa fragment present in the 135 kDa HMM from the beginning showed the same mobility as the newly produced 40 kDa fragment from the 135 kDa HMM. As will be described later, only one N-end sequence was identified with the isolated 40 kDa S-2; these two fragments are identical. As the ordinary S-2 has a mass of roughly 60 kDa, the 40 kDa S-2 was much shorter than this.

The digest of the 135 kDa HMM for 60 min was applied to the gel filtration on Sepharose CL 4B in expecting the separation of the 40 kDa S-2 from S-1. The elution pattern is presented in **Figure 4A**. Elution of protein was monitored by reading the absorption at 230 nm. Only a single peak at fraction number 67 was detected by the measurement. SDS−PAGE analysis of the peak revealed the peak for S-1. The elution peak for the 40 kDa S-2 was not detected by reading the absorption at 230 nm. However, the 40 kDa S-2 was detected at a fraction number of around 58 when the elution of protein by SDS−PAGE was studied. The elution profile of the 40 kDa S-2 was produced by the densitometric estimation of the 40 kDa S-2 on SDS−PAGE. The elution volume was smaller than that of S-1, which demonstrated that the 40 kDa S-2 has an elongated structure in the native form, although the 40 kDa S-2 has a shorter fragment than S-1 on SDS−PAGE. No detection of the 40 kDa S-2 by reading the absorption at 230 nm suggested a small content of aromatic amino acids in the primary structure.

Isolation of the 40 kDa S-2. Although Sepharose CL 4B gel filtration separated 40 kDa S-2 from S-1, the separation was not sufficient for the isolation of the 40 kDa S-2. We tested several methods for the isolation of the 40 kDa S-2. We found the separation of S-1 from the 40 kDa S-2 was simply achieved by applying the thermal treatment. The digest containing S-1 and 40 kDa S-2 was heated at 30 °C for 60 min. The heating treatment denatured S-1 almost completely resulted in aggregate formation (*20*). The turbid solution was centrifuged at 20000*g* for 20 min to remove aggregated materials. We found no 40 kDa S-2 sedimented by the procedures. The supernatant was applied to the same gel column of Sepahrose CL 4B for the removal of the remaining LC components in the soluble fraction. The elution pattern of the protein as measured by the absorption at 230 nm and by SDS−PAGE is presented in **Figure 4B**. A large peak at 67 disappeared for the supernatant due to the removal of S-1 from the digest. The absorption measurement gave a new peak at around fraction number 58. The peak was of the 40 kDa S-2 as revealed by SDS−PAGE analysis. As the elution peak for the S-2 in the above supernatant was the same as in the intact chymotryptic digest, the heating procedures to remove S-1 did not denature the 40 kDa S-2. This would be reasonable because the parent 135 kDa HMM was obtained by the digestion at 30 °C. The fractions containing the 40 kDa S-2 were pooled for further study as the purified 40 kDa S-2.

As the 135 kDa HMM generated S-1 and 40 kDa S-2, the 40 kDa S-2 was expected to occupy the N-terminal region of the rod. To identify the fragment, we sequenced the N- and C-ends of the fragment (**Figure 5**). The N-end was exactly the same as that of the rod, namely, Leu 897. The C-end of the fragment was routinely determined by using the carboxylpeptidase Y digestion. The released amino acid by the digestion was Leu, Glu, and Glu from its C-end as presented in **Figure 5**. The sequence was searched for carp myosin HC (*21*). Such residues were found in Lue1117 to Glu1115 in the sequence. The obtained sequence is shown in **Figure 5**. It was concluded that the 40 kDa S-2 spans from Leu897 to Leu1117, 221 residues. Only three Phe residues were found in the sequence for the fragment, and neither Trp nor Tyr was found in it. This was the reason for a low absorption of the fragment at 230 nm. Although the C-end of the 135 kDa HMM was not identified, the same Leu1117 would be the residue for it because the 135 kDa HMM was produced by the chymotryptic digestion at 30 °C, while the 40 kDa S-2 was produced by digestion of the 135 kDa HMM at 20 °C.

Thermal Stability of the 40 kDa S-2. As the myosin rod is almost completely α-helical in structure, unfolding of the structure upon heating could be applicable for studying the thermal stability of the 40 kDa S-2 (*13*). As a reference, the unfolding profile of the parent rod was also studied. As we have isolated the 40 kDa LMM occupying 1/3 of the carboxyl end of the rod, its stability was also compared. The N-terminal end of the 40 kDa LMM was Arg1569 (see **Figure 5**), and its assumed C-end was Tyr1878. The ellipticity at 220 nm was the index to detect the unfolding of the helix structure, and the negative value at 20 °C was taken as 100% for all the samples. A gradual increase in the temperature led to a gradual decrease in the negative magnitude of the ellipticity at 220 nm as a result of unfolding of the helix structure for all of the samples (**Figure 6**). However, the unfolding profiles were different among the three samples. The 40 kDa LMM unfolded at the lowest temperature, and reached the lowest value at around 40 °C. On the other hand, the 40 kDa S-2 did not unfold up to 40 °C, and the unfolding was observed above 50 °C, showing the minimal value at 70 °C. The unfolding profile for the parent rod gave two transient steps. The unfolding achieved at around 40 °C occupied about 2/3 of the whole change. The rest of the unfolding occurred around 55 °C. The unfolding temperature for the higher one was very similar to that of the 40 kDa S-2. The remaining negative ellipticity at 220 nm at around 70 °C was about 20% for the three samples. This value would come

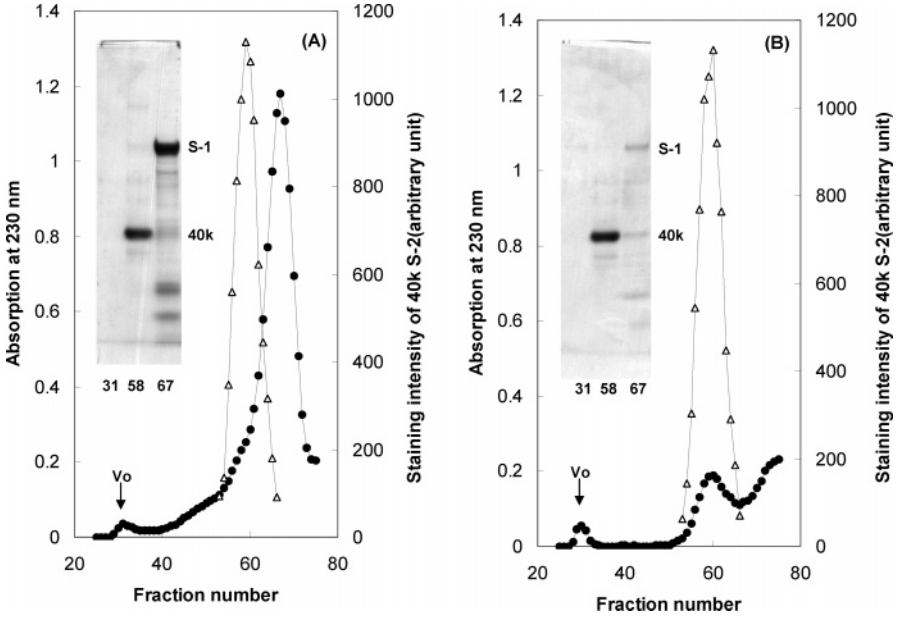

Figure 4. Gel filtration profile of the 40 kDa S-2. (**A**) The chymotryptic digest of the 135 kDa HMM prepared under the same conditions as in **Figure 3** with a digestion time of 60 min was applied to the Sepharose CL-4B as in **Figure 2**. Protein elution was monitored either by reading the absorption at 230 nm (closed circles) or by SDS–PAGE (open triangles). The insets are the SDS–PAGE patterns of the fractions. V_0 denotes the void volume. (**B**) The chymotryptic digest used in (**A**) was heated at 30 °C for 60 min. Its supernatant after centrifugation at 20000*g* for 20 min was applied to the same gel column. Protein was monitored by the same methods as in (**A**). SDS–PAGE patterns are of the fractions separated. The same abbreviations as in (**A**) were used.

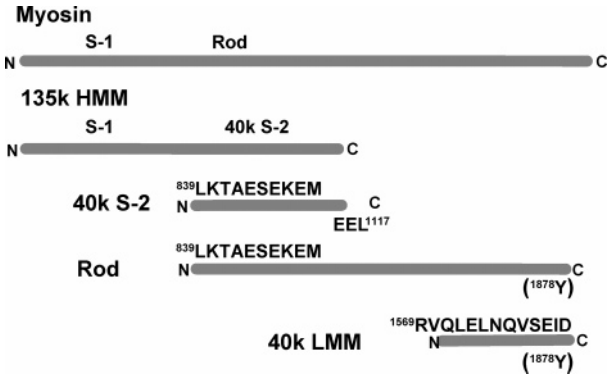

Figure 5. Identification of the 40 kDa S-2 by determination of its amino and carboxyl ends. The amino and carboxyl ends of the 40 kDa S-2 were sequenced. Its alignments in the myosin, rod, and 135 kDa HMM are schematically presented. The 40 kDa LMM in the rod structure was also aligned.

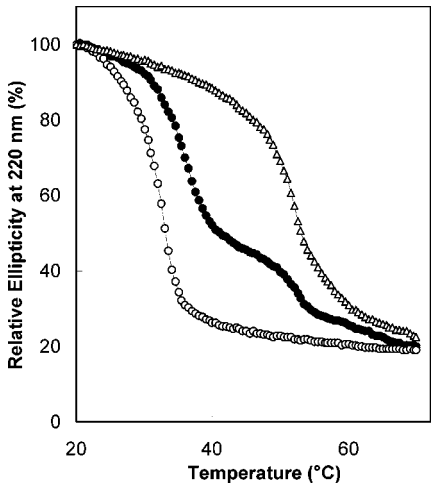

Figure 6. Unfolding profile of the 40 kDa S-2 as compared with the rod and 40 kDa LMM. A change in the ellipticity of the 40 kDa S-2 at 220 nm was followed upon increasing the temperature at a rate of 1 °C/min in 0.5 M KCl, 20 mM Tris–HCl (pH 7.5). Its negative value was plotted against the temperature reached, and the negative ellipticity at 220 nm at 20 °C was taken as 100% for all of the samples as the index of the helix content. The 40 kDa LMM (open circles), rod (closed circles), and 40 kDa S-2 (open triangles) were analyzed.

from the completely unfolded proteins. To compare the unfolding temperatures for the three samples, the derivatives of the relative ellipticity were estimated (**Figure 7**). The unfolding peak for the 40 kDa S-2 was at 52 °C, while that for the 40 kDa LMM was at 32 °C. The rod showed two unfolding peaks at 52 and 36 °C. The unfolding peak for the 40 kDa S-2 was detected with the parent rod, and that for the 40 kDa LMM was detected as a small peak at the ascending half of a large peak. A large unfolding peak for the rod at around 36 °C indicated that a majority of the LMM structure including 40 kDa LMM is relatively less stable than that of the 40 kDa S-2. Studies on the structural stabilities of LMM by using DSC provided much detailed information (*22*). However, our CD measurement could not detect such a small difference in the stability within the LMM portion. Nevertheless, CD measurement clearly demonstrated that the N-terminal 40 kDa region is uniquely stable in the rod structure.

We further studied the thermal unfolding of the 40 kDa S-2 region in the whole myosin molecule. The unfolding derived from the S-1 portion in addition to that from the rod was expected to be detected in the profile. The derivative of the unfolding profile of myosin is shown in **Figure 8**. A large broad peak at around 35 °C and a small peak at about 48 °C were found in the profile. Although the identification was not done, the peak found at high temperature would come from the unfolding of the 40 kDa S-2. This unfolding of the region in myosin (about 48 °C) occurred at a little lower temperature than

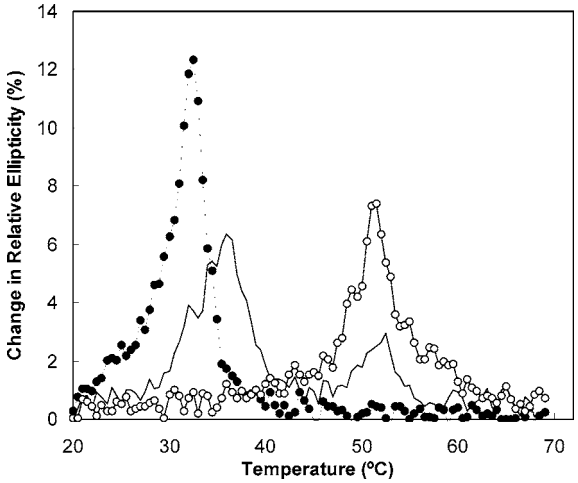

Figure 7. Comparison of the thermal stability of the 40 kDa S-2 with the rod and the 40 kDa LMM. The unfolding profiles of the 40 kDa S-2 (open circles), rod (solid line), and 40 kDa LMM (closed circles) in **Figure 6** were analyzed by taking their derivatives. The same symbols as in **Figure 6** were also used for the three samples.

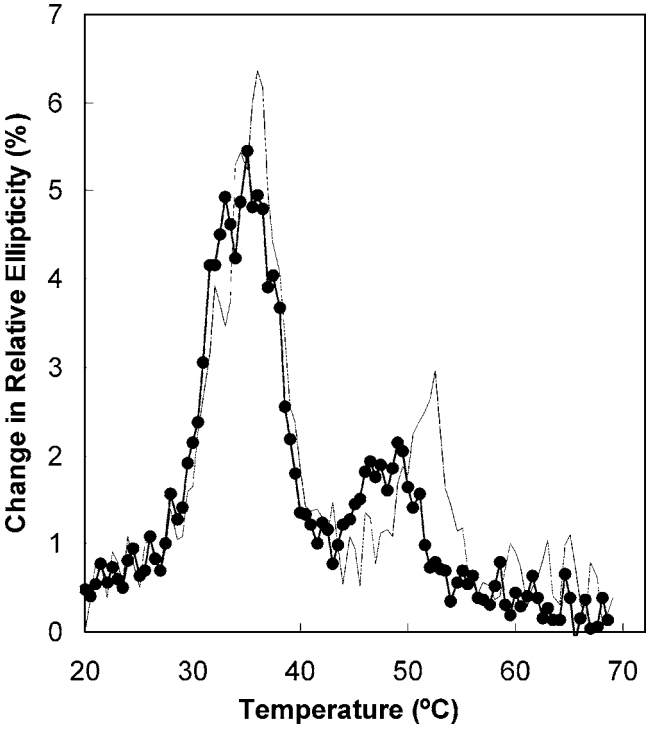

Figure 8. Unfolding of the 40 kDa S-2 when heated as myosin. The unfolding profile of the myosin molecule was compared with that of the rod under the same conditions as in **Figure 6** and analyzed as in **Figure 7**. Myosin (closed circles) and the rod (solid line) were analyzed.

that in the rod or with isolated 40 kDa S-2 (52 °C). Elevation of the temperature to 70 °C surely denatures the S-1 portion of myosin, which probably induced the aggregate formation by associating at the S-1 region; namely, the unfolding of the 40 kDa S-2 for myosin is that for the aggregated myosin. The aggregation seemed to promote the unfolding of the 40 kDa S-2. A specific unfolding peak coming from the S-1 portion was not detected in the pattern. Probably, the unfolding of S-1 could not be distinguished from that of the LMM portion; namely, the thermal stability of the S-1 region as studied by unfolding of the helix structure was rather similar to that of the LMM portion of the rod.

DISCUSSION

It is well-known that the thermal stability of the myosin rod is species-specific as studied by the unfolding of the helical structure of the region upon heating (*5*, *23*). In the studies, the thermal stability of the rod portion was discussed by comparing the unfolding temperature with an assumption that the unfolding occurred monophasically; namely, LMM and S-2 have the same stability. A study on the stability of carp myosin LMM revealed that even the LMM region contains various regions with different stabilities (*22*). We also showd that the LMM region is not homogeneous by isolating filament-forming short 40 kDa LMM from carp and tilapia myosin (*4*, *10*). However, there is little information on the structure of the S-2 region in the rod whether the region is homogeneous or not. This is probably because the S-2 portion has no attractive biochemical function such as filament formation. We have proposed the important role of the amino terminal region of the rod, the S-2 region, in the aggregate formation by heated myosin.

In the present study, we studied the structural characteristics of the S-2 region by isolating S-2. Usually, digestion of fish myosin is conducted at low temperature to avoid undesired cleavages due to myosin denaturation (*18*). For this reason, no one has attempted to digest fish myosin at high temperature. We conducted the digestion of carp myosin by varying the temperatures to study the structural stability of the carp myosin rod. As we expected, digestion at high temperature degraded myosin into short peptides. However, the new short 135 kDa HMM was found in the digest at 30 °C. The 135 kDa HMM was the shortest HMM derived from fish myosin ever. As the LMM portion as the counterpart of the HMM was degraded into short peptides at this temperature, the 135 kDa HMM was found to be a uniquely stable product. The 135 kDa HMM is supposed to consist of S-1 and S-2. F-actin was very stable and resistant to the digestion at 30 °C (**Figure 1C**). Binding to the stable F-actin resulted in the stabilization of the S-1 portion, and the region reasonably became resistant to the digestion. However, the S-2 region has no factor for its stabilization. Accordingly, it was suggested that the S-2 region in the 135 kDa HMM is intrinsically very stable, and resistant to the digestion. Sequencing of the amino and carboxyl ends of the isolated 40 kDa S-2 showed that it spans from the amino end of the rod (Leu 897) to Leu1112, 221 residues. The 40 kDa S-2 was hard to detect by reading the absorption at 280 nm, which is reasonable because the region contained no Trp residue. There was no report on the cleavage of myosin at the corresponding site with any other species of myosin. Although the data were not presented, chymotryptic digestion of rabbit myosin as dissolved myofibrils at 40 °C generated a similar 135 kDa HMM. Thus, skeletal myosin seems to contain a stable 40 kDa S-2 region in its structure commonly. Thermal stability of the 40 kDa S-2 was characterized by comparison with that of the 40 kDa LMM, carboxyl end region of the rod. Assuming the size of the rod to be 130 kDa, the S-2 and LMM roughly occupy 1/3 of the rod of the amino and carboxyl ends, respectively. The 40 kDa S-2 and the 40 kDa LMM were proved to be the most stable and unstable regions in the rod, respectively. The difference in the unfolding temperature between them was about 20 °C. This would be the first report to demonstrate that the rod consists of three different regions with such a large difference in the stability. As the unfolding peak of the 40 kDa S-2 was similarly detected with the parent rod, the region unfolded independently in the rod. However, the unfolding temperature for the region in myosin was reduced by 5 °C. Heating of carp myosin forms aggregates at the head

region as observed with an electron microscope (*14*). It was suggested that the aggregated head region surely reduced the stability of the neighboring S-2 region. We have demonstrated that aggregation of myosin S-1 is essential for rod aggregate formation; namely, rod aggregation occurred with myosin but not with myosin digest, a mixture of S-1 and the rod. The reduced stability of the 40 kDa S-2 in myosin and rod aggregation induced by S-1 aggregation are in good agreement. To understand the thermal denaturation of myosin, it is necessary to consider the myosin structure having two heads and a long tail, and that neighboring regions, especially the head region, affect the denaturation of the rest of it, the tail portion. Accordingly, studies with the isolated myosin fragments and with intact myosin are both necessary for a understanding of myosin denaturation. Moreover, when we try to understand the myosin denaturation in muscle, the stabilizing effect of F-actin on myosin S-1 upon binding should be considered.

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