Oligomerization of Carp Myosin Which Retains Its ATPase Activity

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Upon heating of carp myosin, actin-activated Mg-ATPase activity decreased much faster than its Ca-ATPase activity. Sepharose CL 4B gel filtration revealed that myosin formed oligomer, which retained its Ca-ATPase activity; oligomer at void volume (Vo) on Sepharose CL 4B exhibited ATPase activity. However, the Mg-ATPase/Ca-ATPase activity ratio for the Vo fraction was very low. Myosin filament formed by the oligomeric myosin at Vo was irregular. It was concluded that the low actin activated Mg-ATPase activity is responsible for the filament formed by oligomeric myosin. Oligomers were easily salted-out at 40% saturated ammonium sulfate, leaving monomers in the soluble fraction.

Key words: fish myosin, thermal denaturation, oligomerization, aggregation, myosin filament

Fish myosin is similar to rabbit myosin in terms of its structure consisting of a double-headed globular portion termed subfragment-1 (S-1) which has regions for binding to actin, and for the hydrolysis of ATP and a long coiled-coil α-helical portion termed rod that forms thick filaments at a physiological concentration of salt.1,2) Fish myosin is generally considered to be very labile with species-specific thermal stability. The thermal stability of fish myosin has mainly been discussed by in terms of the ATPase inactivation rate.3,4) It has also been reported that the rod portion of fish myosin is less stable than that of the rabbit studied by differential scanning calorimetric measurement.5,6) The unstable nature of fish rod has also been suggested by measuring its conformational change induced by urea or guanidine HCl addition.7,8)

It is generally believed that the subfragment-1 (S-1) portion is more unstable than rod, which has nearly a 100% helical structure. However, differential scanning calorimetry studies have demonstrated that the subfragment-2 (S-2) portion unfolds more easily than S-1 upon heating.9) Moreover, it has been reported that the change in filament formation precedes Ca-ATPase inactivation,10) suggesting that S-1 denaturation does not necessarily precede rod denaturation.

In this paper, we demonstrated that oligomer formation precedes ATPase inactivation during the heating of carp myosin, suggesting a selective denaturation of the rod portion in myosin molecules.

Methods

Myosin was prepared from carp Cyprinus carpio myofibril by the method described by Koseki et al.11) Myofibril dissolved in 0.5 M KCl and 20 mM Tris-HCl (pH 7.5) was added with 5 mM Mg-ATP to dissociate myosin from actin, then saturated ammonium sulfate was added to give 40% saturation to remove actin as precipitate. Myosin in the supernatant was collected by raising the saturation to 55%. Myosin dissolved in and dialyzed against 0.5 M KCl and 20 mM Tris-HCl (pH 7.5) was used as the sample.

Carp myosin in the above medium was heated at 28°C. Changes in the Ca-ATPase and actin-activated Mg-ATPase activities upon heating were followed. Ca-ATPase was assayed in a medium of 0.5 M KCl, 25 mM Tris-maleate (pH 7.0), 5 mM CaCl2, and 1 mM ATP. Mg-ATPase was also assayed in a medium of 25 mM KCl, 25 mM Tris-maleate (pH 7.0), 1 mM MgCl2, 1 mM ATP in the presence of rabbit F-actin (1/2 by weight relative to myosin). In some experiments, K-ATPase instead of Ca-ATPase was assayed, in which case 5 mM CaCl2 was replaced by 5 mM EDTA.

Oligomerization of myosin upon heating was studied by monitoring its elution profile on a Sepharose CL 4B equilibrated with 0.5 M KCl and 20 mM Tris-HCl (pH 7.5). Myosin oligomer was removed by salting-out at 40% saturated ammonium sulfate, and the myosin remaining soluble was referred to as a monomeric myosin.12) Filament formed by myosin was negatively stained with 1% uranyl acetate, and was examined on a Hitachi H-7000 electron microscope.12)

Actin was routinely prepared from acetone-dried muscle powder of rabbit by the method of Spudich and Watt.13) Sodium dodecylsulfate-gel electrophoresis (SDS-PAGE) was performed using 10% polyacrylamide gel containing 0.1% SDS.14)

Results and Discussion

Carp myosin dissolved in 0.5 M KCl and 20 mM Tris-HCl (pH 7.5) was incubated at 28°C and the decrease in the Ca-ATPase and actin-activated Mg-ATPase activities were followed (Fig. 1). The heating temperature was set to achieve a proper ATPase inactivation profile. Mg-ATPase activity decreased much faster than Ca-ATPase activity.
Fig. 1. Changes in Ca- and Mg-ATPase activities of carp myosin upon heating.

Carp myosin in 0.5 M KCl and 20 mM Tris-HCl (pH 7.5) was heated at 28°C. Its Ca-ATPase (△) and actin activated Mg-ATPase (○) activities were measured.

Incubation for 10 min reduced the actin-activated Mg-ATPase activity to 30%, while its remaining Ca-ATPase activity was approximately 80%. These characteristic decreasing profiles of Ca- and Mg-ATPase activities upon heating were essentially the same as those reported by Kimura et al.10)

We investigated how myosin underwent a conformational change upon heating. We examined the aggregate formation by heated myosin on a Sepharose CL 4B. As presented in Fig. 2, unheated myosin was eluted as a symmetric single peak at fraction number 36. When the myosin heated for 5 min was applied, the peak at void volume (Vo) appeared at fraction number 28. The Vo peak gradually became obvious with duration. The third peak appeared at fraction number 32 between two peaks for the myosin heated for 10 or 20 min. Although heating myosin for 20 min only reduced the Ca-ATPase activity roughly to 60% (see Fig. 1), the elution profile of the monomer peak was negligible. These results indicated that active myosin is present in an oligomeric form. We noticed that myosin solution heated for 20 min was still clear and no sediment was formed after centrifugation at 50,000 × g for 30 min. The extent of oligomerization seemed low, so we termed the myosin eluted at Vo as oligomer rather than aggregate.

Since practically no monomer peak was detected in the elution profile of myosin heated for 20 min, a much faster oligomerization than the ATPase inactivation was suggested. To examine whether the oligomer truly retains ATPase activity, we measured the K-ATPase activity of the eluted fractions. Myosin heated for 10 and 20 min whose residual activities were 80 and 62%, respectively were employed. As shown in Fig. 3, the Vo fraction clearly showed Ca-ATPase activity as in Fig. 1 in which K-ATPase was assayed. An estimated Ca-ATPase activity of the Vo fraction was about 65% that of the monomer peak. The Ca-ATPase activity of the middle peak was similar to that of the monomer peak. Actin-activated Mg-ATPase was also detected for all three peaks (Fig. 4B). However, the activity of the Vo was clearly lower than that of the monomer peak. The relative activity of the Vo fraction was only 22% that of the monomer peak. The middle peak had a quite high activity of 88%. The activity ratio, (Mg-ATPase/Ca-ATPase), was 0.53, 1.48 and 1.57 for the Vo, the middle, and the monomer peak, respectively. These values indicated that active myosin at the Vo fraction interacts with actin with an efficiency of roughly 1/3 that of monomer.

Kimura et al.10) proposed that irregular filament formation precedes Ca-ATPase inactivation upon heating of myosin.
myosin, and the irregular filament formed by the myosin heated is responsible for the reduced actin-activated Mg-ATPase activity. We examined the filament structure formed by the oligomer eluted at Vo and by monomer myosin by electron microscopy. As presented in Fig. 5, oligomers at the Vo fraction formed a huge aggregate by associating irregular small filaments, whereas myosin at the monomer peak formed a well-characterized filament possessing a central bare zone.15) These were consistent with the results reported by Kimura et al.,10 in which they observed the filament structure formed by whole heated myosin.

We have already reported that upon heating of carp myofibril, a quick decrease in the myosin extractability defined as the amount of myosin recovered in the soluble fraction at 40% saturated ammonium sulfate in the presence of ATP-Mg is followed by a slow Ca-ATPase inactivation.12) We applied the same principle to the thermal denaturation of myosin. Myosin was heated at 28°C, added with ammonium sulfate to give 40% saturation, centrifuged at 20,000 × g for 20 min, and the myosin recovered in the supernatant (myosin recovery) was estimated by measuring the staining intensity of myosin heavy chain of the supernatant on SDS-PAGE. The results are shown in Fig. 6. The myosin used in this paper was prepared by ammonium sulfate fractionation; a precipitate between 40 and 55% saturation was collected as myosin. Consequently, unheated myosin should contain no precipitate at 40% saturation. Myosin recovery for unheated myosin was about 95%. The recovery gradually decreased with duration, but the decrease was much faster than Ca-ATPase inactivation. For instance, upon heating for 20 min, myosin recovery decreased to 25%, while the residual activity...
Fig. 6. Ammonium sulfate fractionation of thermally treated myosin. 
Myosin heated at 28°C was added with ammonium sulfate to give a with 40% saturation and centrifuged. The amount of myosin recovered in the supernatant (△) was estimated. The decrease in the Ca-ATPase activity for whole heated myosin (●) is also shown.

was about 65%. Therefore, it was demonstrated that 40% saturated ammonium sulfate sedimented active myosin as well as inactive myosin. The myosin sedimented by ammonium sulfate is likely to be oligomeric myosin. To verify this, myosin heated for 20 min at 28°C, and its soluble fraction at 40% saturated ammonium sulfate were both applied to the Sepharose CL 4B column. As shown in Fig. 7, a huge Vo peak for the heated myosin completely disappeared in the elution profile for the soluble fraction, and only a small monomer peak was detected. Ammonium sulfate fractionation, therefore, is a useful technique for separating myosin monomer from oligomers or aggregates.

Studying the thermal denaturation mechanism of S-1, it has been reported that ATPase inactivation is accompanied by S-1 oligomerization, and is accompanied by a light chain release. We noticed that the oligomeric myosin eluted at Vo on the Sepharose CL 4B (Fig. 2) still retained both types of light chains. Thus, the S-1 portion is unlikely to be the site responsible for their association to form ATPase retaining oligomer. Investigating the thermal denaturation of myofibril, we have demonstrated that the loss of myosin solubility and myosin extractability both precede ATPase inactivation. As the rod portion is responsible for solubilization into a high-salt medium, the rod portion preferentially undergoes conformational change upon heating. Thus, the rod portion is likely to be the region responsible for the oligomerization of myosin molecules.

As the myosin molecule consists of two distinct structures, spherical S-1 and helical long rod, a single indicator does not provide sufficient information about conformational changes occurring in myosin molecules during the heating. It was necessary to monitor the conformational change occurring on the rod portion as well as the S-1 portion in order to understand the thermal denaturation of myosin. Ammonium sulfate fractionation was an excellent technique for monitoring the oligomerization, probably reflecting the conformational change of rod.

References


