BEHAVIOR OF CHICKEN BREAST MUSCLE MYOSIN SOLUBILIZED IN NEUTRAL AND LOW IONIC STRENGTH SOLUTIONS

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Background

Meat, an essential food for humans, is a very rich source of proteins and contains all essential amino acids. However, its usage is limited because it is maintained in a solid state even after cooking. If the proteins in meat could be solubilized in water or a solution of low ionic strength, utilization of meat could be extended in various ways, as a liquid diet for elderly people. We have already established a method to solubilize more than 80% of chicken breast muscle myofibrillar proteins in water (Ito *et al*., 2003). To accomplish solubilization, it is essential to maintain myofibrillar suspensions at neutral pH with L-histidine (L-His) and low ionic strength and to disrupt the high-ordered structures of myofibrils by ultrasonication. Furthermore, we have shown that myosin prepared from chicken skeletal muscle, one of the major myofibrillar proteins, is also solubilized in a solution of low ionic strength by ultrasonication (Ito *et al*., 2002). However, it has not been determined whether ultrasonication and presence of L-His are essential for solubilization of myosin in a solution of low ionic strength.

Objectives

The objective of this study was to examine the behavior of myosin solubilized in neutral and low ionic strength solutions without ultrasonication.

Materials and methods

Myosin was prepared from chicken breast muscle (Perry, 1955) and solubilized in 0.6 M KCl, pH 6.5. Myosin was dialyzed against neutral and low ionic strength solutions containing 1 mM KCl and various concentrations of L-His. Each dialyzed myosin suspension was centrifuged for 120 min at 100,000×g, and the obtained supernatant was defined as water-soluble myosin. The solubility of myosin in low ionic strength solutions was determined.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli *et al*. Water-soluble myosin molecules stained by the method of negative staining and subjected to rotary shadowing were observed under a transmission electron microscope. Gel-formation ability of water-soluble myosin was tested by heating for 10 minutes in a water bath at 70 °C.

Results and discussion

In the presence of L-His, myosin was solubilized in neutral and low ionic strength solution without ultrasonication (Figure 1). As shown in the SDS-PAGE patterns (Figure 2), water-soluble myosin contained intact heavy chains and light chains. The solubility increased with increase in the concentration of L-His to 7 mM. However, the solubility decreased when the concentration of L-His exceeded 10 mM. This indicates that the solubility is dependent on L-His concentration. The solubility was also dependent on protein concentration of myosin. When a water-soluble myosin solution was dialyzed against a solution of physiological ionic strength, myosin molecules aggregated and were precipitated by centrifugation. Therefore, it is concluded that water-soluble myosin maintains the ability of self-assembly under physiological conditions.

Using the method of negative staining, we observed that water-soluble myosin formed a very thin filament-like structure (Figure 3A, B). This structure clearly differed from the thick filament observed under the physiological condition. Also, using the method of rotary shadowing, we confirmed the existence of myosin monomers that have two heads and a long rod as do native myosin molecules (Figure 3C). The rod of water-soluble myosin was longer than that of native myosin soluble in a solution of high ionic strength (Figure 4). These observations indicate that water-soluble myosin in neutral and low ionic strength solutions

exists in two forms, separated monomers and very thin filament-like structures. Heating could not induce any gelation of water-soluble myosin.

Conclusions

Myosin is soluble in a solution of low ionic strength in the presence of L-His without ultrasonication. In such a condition, myosin molecules exist in two forms, separated monomers and very thin filament-like structures. The rod of the separated monomer is longer than that of the native myosin molecule. These results suggest that low ionic strength and L-His cause some conformational changes in myosin molecules resulting in lengthening of its rod portion. Water-soluble myosin could not be changed into gel by heating.

Acknowledgements

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References

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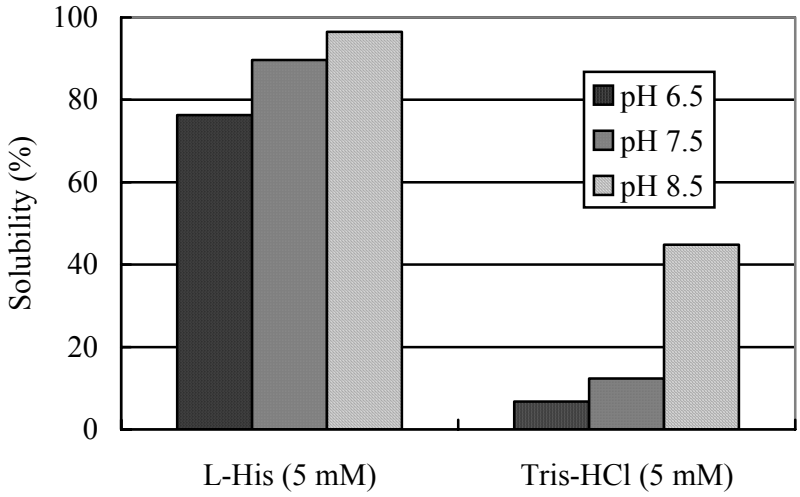

Figure 1. Effect of L-His on solubilization of myosin in neutral and low ionic strength solutions. Myosin (5 mg/ml) was dialyzed against 1 mM KCl, 5 mM L-His or Tris-HCl.

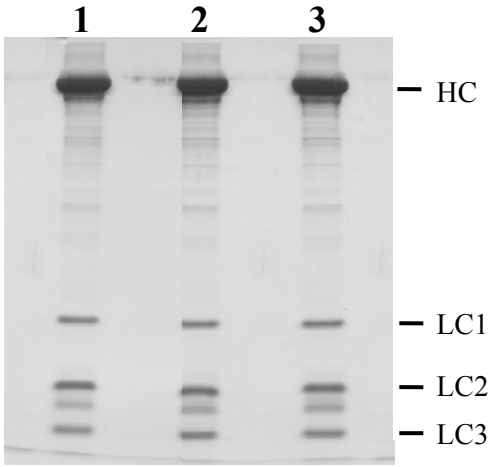

Figure 2. SDS-PAGE patterns of dialyzed myosin suspension (lane 1), water-soluble myosin (lane 2) and precipitation (lane 3). HC: myosin heavy chain, LC: myosin light chain

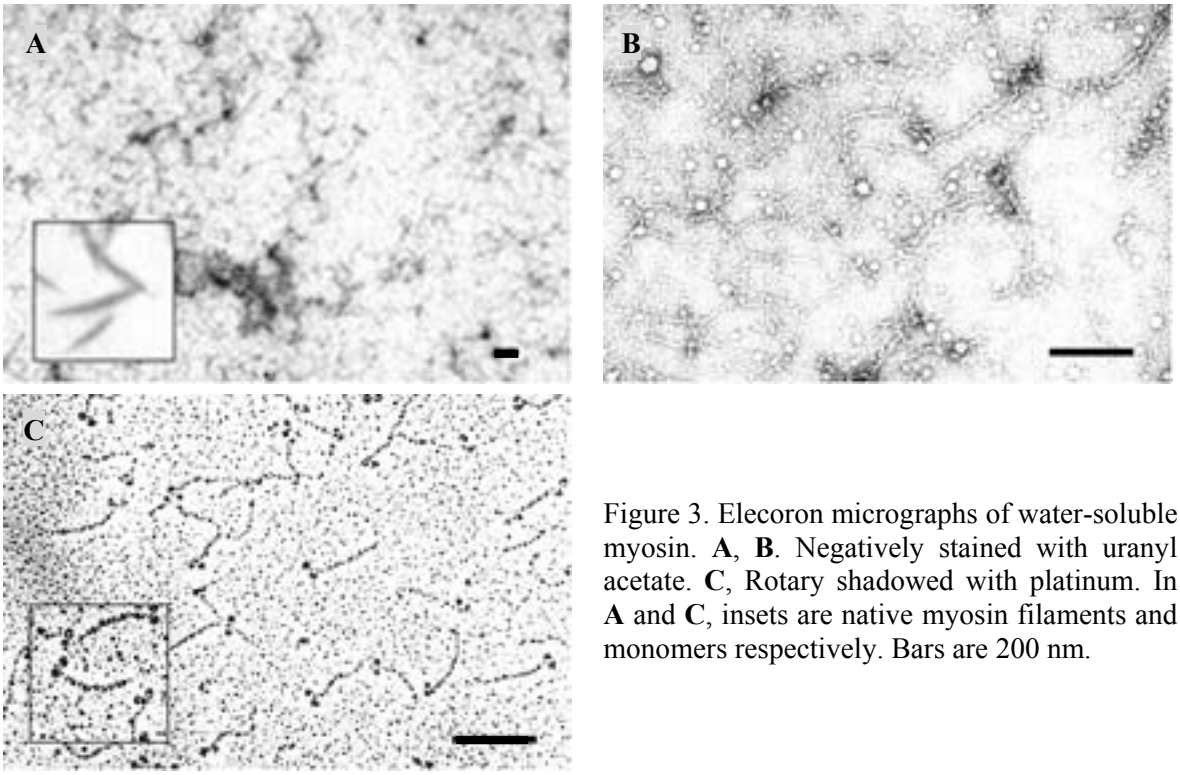

Figure 3. Elecoron micrographs of water-soluble myosin. **A**, **B**. Negatively stained with uranyl acetate. **C**, Rotary shadowed with platinum. In **A** and **C**, insets are native myosin filaments and monomers respectively. Bars are 200 nm.

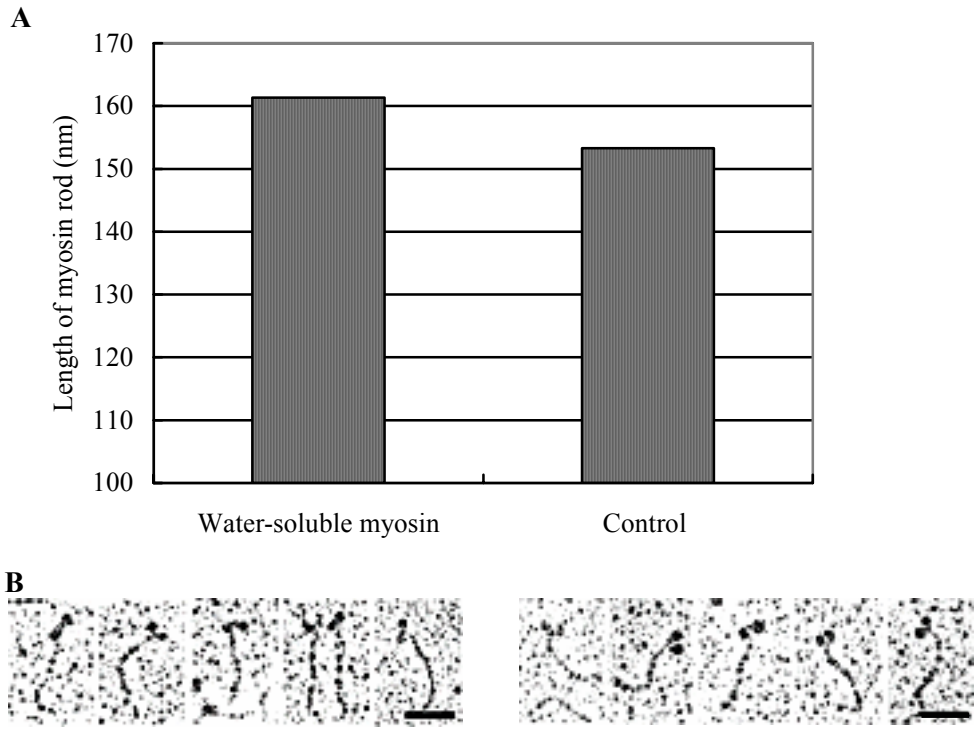

Figure 4. Length of water-soluble myosin rod. **A**, The length of the rod was determined by measurements in electoron micrographs of rotary shadowed water-soluble myosin. **B**, Electron micrographs of water-soluble myosin (left) and native myosin (right). Both are rotary shadowed with platinum. Bars are 100 nm.