PE7.17 Myosin filament depolymerizes in a low ionic strength solution containing L-histidine
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***Abstract*—Myosin, one of the major myofibrillar proteins, forms a filamentous polymer and is insoluble in physiological and low ionic strength solutions. We have shown that myosin is soluble in a low ionic strength solution containing L-histidine. In this study, to clarify the role of L-histidine on the solubilization of myosin, we investigated the morphological changes of myosin under the physiological and low ionic strength conditions with L-histidine. In the presence of L-histidine, myosin was dispersed in a low ionic strength solution and formed a filamentous polymer in the physiological ionic strength solution. Transmission electron microscopy showed that the light meromyosin (LMM) paracrystal had the wider periodicity in the presence of L-histidine as compared with that in the absence of L-histidine. These results suggest that the solubilization of myosin in a low ionic strength solution is caused by the elongation of LMM region by L-histidine.**

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I. INTRODUCTION

MEAT is rich in high-quality proteins containing all essential amino acids for humans. However, meat has not been utilized as a supplementary protein food to the extent of milk or soybean products, due to the low degree of solubility of myofibrillar proteins that comprise approximately 50% of meat proteins. Myofibrillar proteins are generally considered to be insoluble in solutions of low ionic strength, and a relatively high concentration of salt is required to solubilize them. If myofibrillar proteins could be solubilized in a low ionic strength solution or water, meat could be used in various ways, such as a liquid diet for elderly people.

Myosin is one of the major myofibrillar proteins and forms thick filaments in myofibrils of striated muscle. Myosin assembles and forms thick filament-like polymer under physiological and low ionic strength conditions *in vitro*, and is insoluble in a low ionic strength solution. In the previous paper, we demonstrated that myosin is solubilized in a neutral and low ionic strength solution containing L-histidine [1], however the reason for this is still unclear.

In this study, to clarify the role of L-histidine on the solubilization of myosin, we investigated the morphological changes of myosin in a low ionic strength solution containing L-histidine. Moreover, we investigated the structural change in the rod region of myosin, which is essential for filament formation.

II. MATERIALS AND METHODS

A. Protein preparations

Myosin and light meromyosin (LMM) were prepared from chicken breast muscle according to the methods of Perry [2] and Margossian & Lowey [3], respectively.

B. Solubilization of myosin and LMM in a low ionic strength solution

Myosin and LMM were solubilized in a solution of low ionic strength contiaining L-histidine by dialysis against solutions of various concentration of KCl (1, 10, 50, 150, 600 mM) and 5 mM L-histidine. The dialysed myosin suspension was ultracentrifuged (100,000*g*, 120 min), and the obtained supernatant was defined as myosin solubilized in a low ionic strength solution containing L-histidine. The solubility was determined by the protein concentration of each respective dialysed suspension and supernatant. Protein concentration was measured using biuret method [4].

C. Transmission electron microscopy

For negative staining, sample proteins, dialysed against solutions of various concentration of KCl with/without 5 mM L-histidine, were dropped onto a copper grid coated with collodion and carbon and stained with 2% uranyl acetate. Samples were observed under transmission electron microscope (H-800, Hitachi, Japan).

D. Statistical analysis

To determine significant difference between the

two groups, comparisons were made using a Student's *t*-test. Data were presented as means ± SE.

III. RESULTS AND DISCUSSION

Figure 1 shows the effects of L-histidine on the solubility of myosin in high (0.6 M KCl), physiological (0.15 M KCl) or low (1 mM KCl) ionic strength solutions. Myosin dialysates against 0.6 M KCl solutions with/without L-histidine were transparent. The dialysates against 0.15 M KCl solutions were opaque in the presence/absence of L-histidine. Dialysates against 1 mM KCl solution had different results depending on presence/absence of L-histidine: dialysates were opaque without L-histidine, whereas with L-histidine were transparent. The solubilities of myosin, after ultracentrifugation, are shown in Figure 2. More than 70% of myosin was solubilized in high ionic strength solutions with/without L-histidine. In physiological ionic strength solutions, both with/without L-histidine, the solubility of myosin was very low. In a low ionic strength solution, there was a difference in solubility of myosin depending on the presence/absence of L-histidine: without L-histidine solubility of myosin was very low, with L-histidine the solubility of myosin was more than 70%. These results suggest that myosin polymerizes under the physiological ionic strength and depolymerizes under the low ionic strength in the presence of L-histidine.

Next, we investigated the morphology of myosin in low ionic strength solutions with/without L-histidine by transmission electron microscopy (Fig. 3). In the absence of L-histidine, myosin formed a filamentous polymer in solutions of 1-150 mM KCl. On the other hand, in the presence of L-histidine, myosin was dispersed with a decrease of ionic strength, although myosin formed a filamentous polymer under the physiological ionic strength. In a solution of 1 mM KCl and 5 mM L-histidine, filamentous polymers were not observed. These results suggested that L-histidine could not inhibit the filament formation of myosin under the physiological ionic strength. Thus, we speculated that L-histidine might participate in the depolymerization of the myosin filament. To investigate this assumption, we examined whether myosin filaments that formed in physiological ionic strength solution without L-histidine dissociate in a low ionic strength solution containing L-histidine. The results showed that myosin filaments depolymerized and dispersed in a low ionic strength solution containing L-histidine (Fig. 4).

Myosin rods play an important role in the formation of muscle filaments, and previous research shows that light meromyosin (LMM) self-assembles to form ordered paracrystals under physiological ionic strength *in vitro*. Therefore, we investigated the effects of L-histidine on the morphology of LMM paracrystal in low ionic strength solutions with/without L-histidine with the results shown in Figure 5. In the absence of L-histidine, LMM polymerized to form ordered paracrystals in solutions of 1-150 mM KCl. In the presence of L-histidine, LMM dissociated in lower ionic strength solutions (less than 50 mM), although LMM formed an ordered paracrystal in 150 mM KCl solution. Previous research indicates that LMM forms ordered paracrystal with periodicity of 43 nm *in vitro* [5, 6], therefore we measured the periodicity of LMM paracrystals formed under the physiological ionic strength with/without L-histidine. Figure 6 shows that the paracrystal had wider periodicity in the presence of L-histidine than in the absence of L-histidine. We have shown the elongation of myosin rod by L-histidine in a previous study [1]. These results indicated that L-histidine would lengthen myosin rod and widen the periodicity of LMM paracrystal. Myosin polymerizes to form a filament by the electrostatic interaction between charge clusters in the LMM region [7]. Thus, the elongation of the LMM region by L-histidine may cause the change in electrostatic forces between molecules contributing to the weakening of myosin filament.

IV. CONCLUSION

In the presence of L-histidine, myosin could form filamentous polymer under physiological ionic strength, although myosin dispersed and solubilized in a low ionic strength solution containing L-histidine. This suggests that L-histidine has a role of depolymerization of myosin filament in a low ionic strength solution. Moreover, we showed that L-histidine caused elongation of the LMM region of myosin. These results suggest that the solubilization of myosin in a low ionic strength solution is caused due to the elongation of LMM region by L-histidine.

ACKNOWLEDGEMENT

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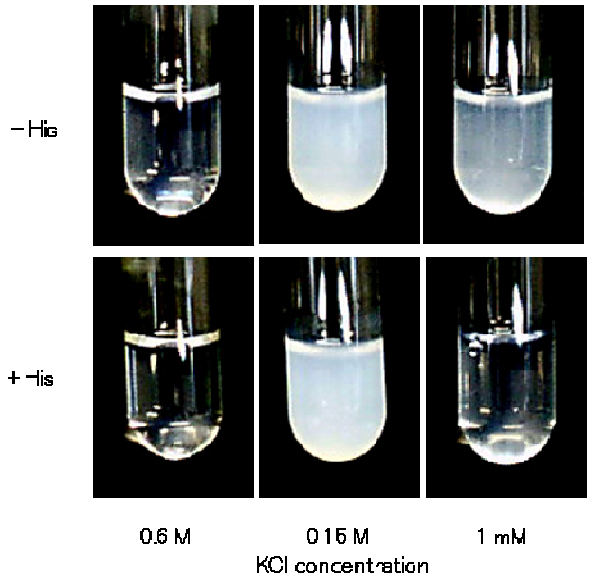

Fig.1. Effect of L-histidine on the aspects of myosin dialysates
Myosin (0.6 M KCl pH 6.5) was dialysed against solutions of various concentrations of KCl with or without L-histidine (His).

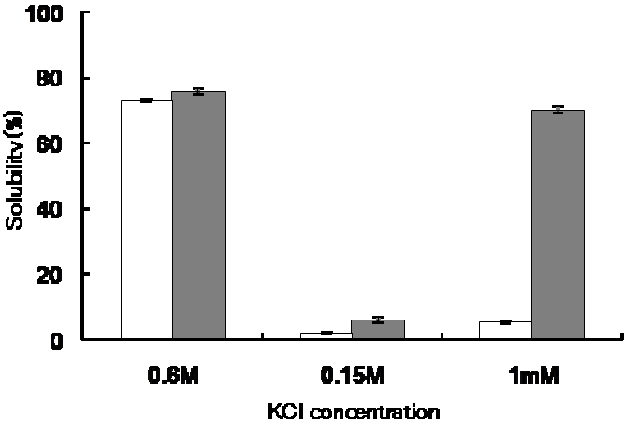

Fig.2. Effect of L-histidine on the solubility of myosin in the various concentrations of KCl solutions
Myosin dialysates were ultracentrifuged at 100,000g for 120 min, and the supernatants were obtained. The solubility was determined by the protein concentrations of dialysate and supernatant. Open columns: without His; closed columns: with His. Values are means ± SE.

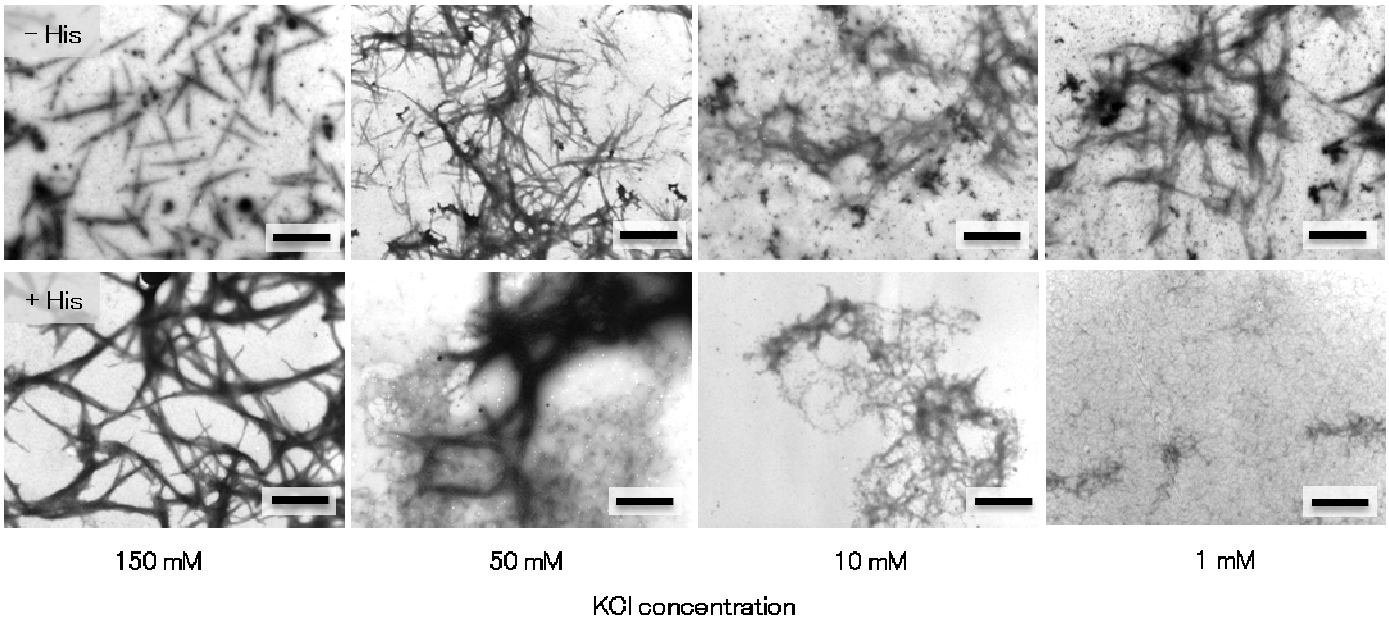

Fig.3. Effect of L-histidine on the morphology of myosin in solutions of physiological and low ionic strength.
Myosin (0.6 M KCl pH 6.5) was dialysed against solutions of 1, 10, 50 and 150 mM KCl with or without 5 mM His. Obtained dialysates were stained with 2% uranyl acetate and observed in a transmission electron microscope. Upper row: without His; lower row: with His. Bars indicate 1 μm.

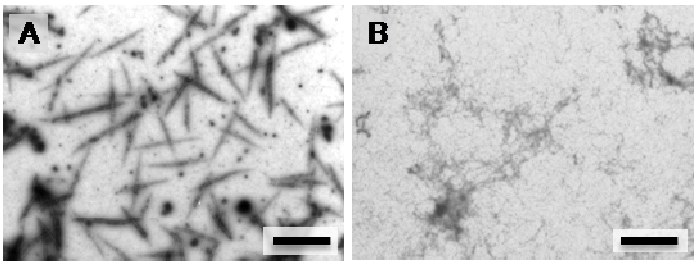

Fig.4. Effect of L-histidine on the dissociation of myosin
(A) Myosin filaments in a 150 mM KCl solution. (B) Myosin was dispersed when myosin filaments (A) were dialysed against a solution of 1 mM KCl, 5 mM His. Obtained dialysates were stained with 2% uranyl acetate and observed in a transmission electron microscope. Bars are 1 μm.

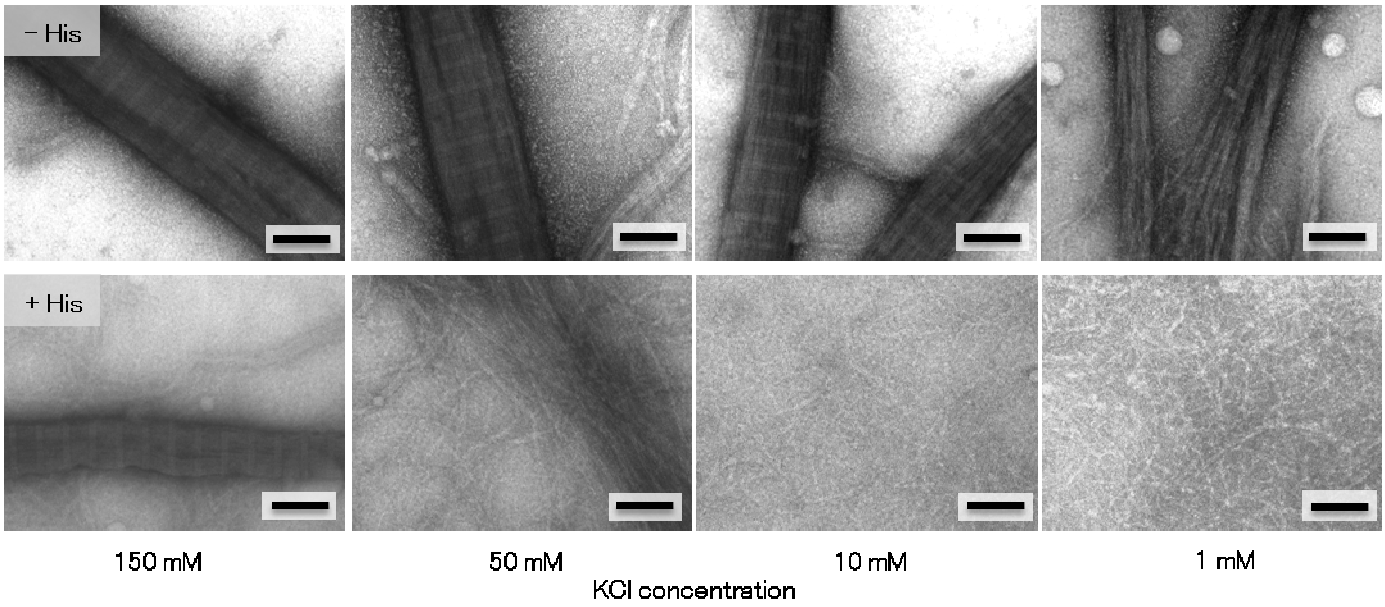

Fig.5. Effect of L-histidine on the morphology of LMM in solutions of physiological and low ionic strength.
LMM (0.6 M KCl pH 6.5) was dialysed against solutions of 1, 10, 50 and 150 mM KCl with or without 5 mM His. Obtained dialysates were stained with 2% uranyl acetate and observed in a transmission electron microscope. Upper row: without His; lower row: with His. Bars indicate 100 nm.

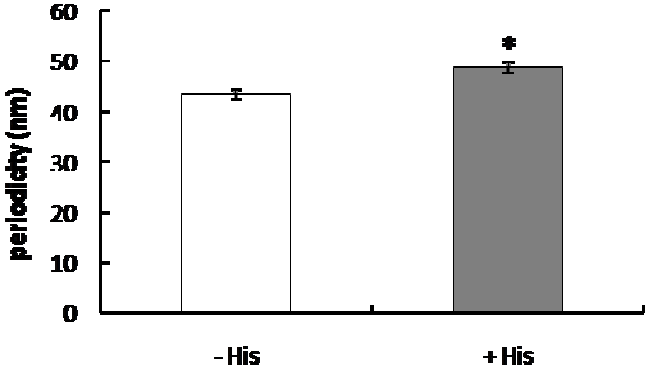

Fig.6. Effect of L-histidine on the periodicity of ordered paracrystal under the physiological ionic strength
LMM was dialysed against solutions of 150 mM KCl, with or without 5 mM His, and the dialysate was stained with 2% uranyl acetate and observed by transmission electron microscope. The width of paracrystal periodicity was measured on electron micrographs. * There were significantly different from LMM without His ($P < 0.05$). Values are means ± SE.