EFFECT OF L-HISTIDINE AND IONIC STRENGTH ON DISASSEMBLY OF MYOSIN FILAMENTS

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***Abstract*— Myosin assembles and forms a filamentous polymer under physiological and low ionic strength conditions *in vitro* and is insoluble in a low ionic strength solution. We showed that myosin is solubilized in a neutral and low ionic strength solution containing L-histidine. However, L-histidine did not inhibit the polymerization of myosin at the physiological ionic sterngth. In this study, to clarify the mechanism of the solubilization of myosin, we investigated the effect of L-histidine and ionic strength on the disassembly of myosin filaments. The decline of ionic strength did not cause the disassembly of myosin filaments, but swelled them. Although the addition of L-histidine did not disassemble myosin filaments at the physiological ionic strength, L-histidine caused the disaggregation of myosin filaments at the low ionic strength. Thus, both of the presence of L-histidine and the decline of ionic strength are essential for the disassembly of myosin filaments and for the solubilization of myosin in a low ionic strength solution.**

***Index Terms*—L-histidine, myosin, solubilization of protein, thick filament.**

I. INTRODUCTION

Meat is rich in high-quality proteins containing all amino acids essential 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 physiological and low ionic strength, and relatively high concentration of salt is requred to solubilized them. If myofibrillar proteins could be solubilize in a low ionic strength solution or water, meat could be used in various ways, such as a liquid diet for elderly people. We have reported that more than 80% of myofibrillar proteins are solubilized in water by washing with low ionic strength solutions containing L-histidine and ultrasonication of muscle tissues (Ito, Tatsumi, Wakamatsu, Nishimura & Hattori, 2003). The ultrasonication is essential for disruption of the highly-ordered structure of the myofibrils and solubilization of myofibrilar proteins.

Myosin assembles and forms a filamentous polymer under physiological and low ionic strength conditions *in vitro* and is insoluble in a low ionic strength solution. However, we demonstrated that myosin is solubilized in a neutral and low ionic strength solution containing L-histidine by dialysis (Hayakawa, Ito, Wakamatsu, Nishimura & Hattori, 2009) and that myosin forms a filamentous polymer in a physiological ionic strength solution containing L-histidine and depolymerizes in a low ionic strength solution containing L-histidine (Hayakawa, Ito, Wakamatsu, Nishimura & Hattori, 2010). Although these reports suggested that L-histidine could play an important role in the dissociation of myosin filament in low ionic strength solutions, it is unclear how L-histidine affects the polymerization and depolymerization of myosin. In this study, to clarify the mechanism of the solubilization of myosin in a low ionic strength solution containing L-histidine, we investigated the effect of L-histidine and ionic strength on the assembly of myosin.

II. MATERIALS AND METHODS

A. Myosin preparation

Myosin was prepared from chicken breast muscle according to the method of Perry (1955). Briefly, minced muscle was extracted with modified Guba-Straub solution (300 mM KCl, 50 mM EDTA, 100 mM KH_2PO_4, 50 mM K_2HPO_4) for 15 min and centrifuged at 1,200*g* for 10 min. The supernatant was diluted with 14 volumes of cold distilled water and centrifuged at 2,470*g* for 10 min. The precipitate was dissolved in 300 mM KCl, pH 7.0 and ultracentrifuged at 100,000*g* for 60 min. The supernatant was diluted with 9 volumes of cold distilled water and centrifuged 1,200*g* for 30 min. The precipitate was dissolved in 600 mM KCl, pH 6.5 and dialyzed against the same solution. After dialysis, the solution was ultracentrifuged at 180,000*g* for 120 min. The obtained supernatant was used as myosin.

B. Transmission electron microscopy

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

III. RESULTS AND DISCUSSION

Previously, we demonstrated that myosin is solubilized in a low ionic strength solution containing L-histidine (Hayakawa et al., 2009) and that myosin filament depolymerizes with the decreasing of ionic strength in the presence of L-histidine (Hayakawa et al., 2010). At the physiological ionic strength with L-histidine, myosin formed filamentous polymers, which were not like thick filaments as observed *in vivo*. Thus, we speculated that L-histidine would have an effect on myosin assembly, not only in a low ionic strength solution, but also in a physiological ionic strength solution.

First, we examined the effect of ionic strength on the disassembly of myosin filaments. In a physiological ionic strength solution, myosin assembled to form filamentous polymers which showed thick-filament like structure (Fig. 1A). When these filamentous polymers were dialysed against a low ionic strength solution without L-histidine, thick-filament like structures were swelled and aggregated but were not disassembled (Fig. 1B). This result suggested that the decline of ionic strength caused the swelling and aggregation of myosin filaments. Next, we examined the effect of L-histidine on the myosin assembly under physiological ionic strength. When the thick-filament like polymers of myosin were dialysed against a physiological ionic strength solution containing L-histidine, they were swelled and aggregated but were not disassembled (Fig. 2). This result suggested that L-histidine caused the swelling and aggregation of myosin filaments at the physiological ionic strength and that L-histidine had the same effect as the decline of ionic strength had.

Next, we examined whether filamentous polymers formed under physiological ionic strength with L-histidine could be disassembled when they were dialysed against a low ionic strength solution with L-histidine. The filamentous polymers were disassembled and dispersed in a low ionic strength solution containing L-histidine (Fig. 3B). Since it was still unclear that the disassembly was caused by either the presence of L-histidine or the decline of ionic strength, we examined whether the filamentous polymers were disassembled when they were dialysed against a low ionic strength solution without L-histidine. The filamentous polymers could not be disassembled and were further swelled (Fig. 3C). The swelled filamentous polymers under low ionic strength without L-histidine were disassembled and dispersed when they were dialysed against a low ionic strength solution containing L-histidine (Fig. 4). These results suggested that L-histidine played a role in swelling filamentous polymers of myosin under physiological ionic strength and also in dispersing myosin under low ionic strength. Thus, both of the presence of L-histidine and the decline of ionic strength could be essential for the disassembly of myosin filaments.

Myosin assembles to form thick filament by the electrostatic interaction between charge clusters in its rod region (McLachlan & Kahn, 1982; Nakasawa et al., 2005; Rosenberg, Straussman, Ben-Ya'acov, Ronen & Ravid, 2008). Our previous papers showed that myosin rod and light-meromyosin (LMM) are lengthened by L-histidine under low ionic strength (Hayakawa et al., 2009, 2010). The elongation of the rod and LMM region by L-histidine might cause changes in the surface charges of those regions, contributing to incorrect assembly of myosin under physiological ionic strength and to disassembly of myosin filaments under low ionic strength.

IV. CONCLUSION

The decline of ionic strength did not cause the disassembly of myosin filaments, but swelled them. Although the addition of L-histidine did not disassemble myosin filaments at the physiological ionic strength, L-histidine caused disaggregation of myosin filaments at the low ionic strength. Thus, both of the presence of L-histidine and the decline of ionic strength could be essential for the disassembly of myosin filaments and for the solubilization of myosin in a low ionic strength solution.

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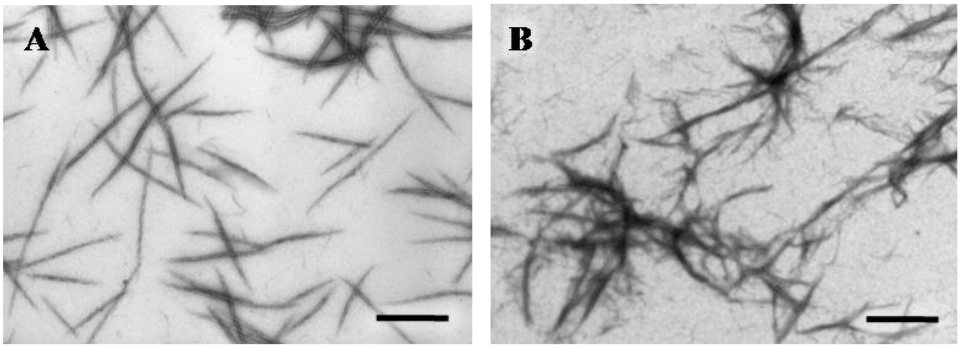

Fig. 1. Effect of the decline of ionic strength on myosin filaments. (A) thick filament-like polymers in a solution of 150 mM KCl. (B) filamentous polymers were observed when thick filament-like polymers (A) were dialysed against a solution of 1 mM KCl. Bars are 1 µm.

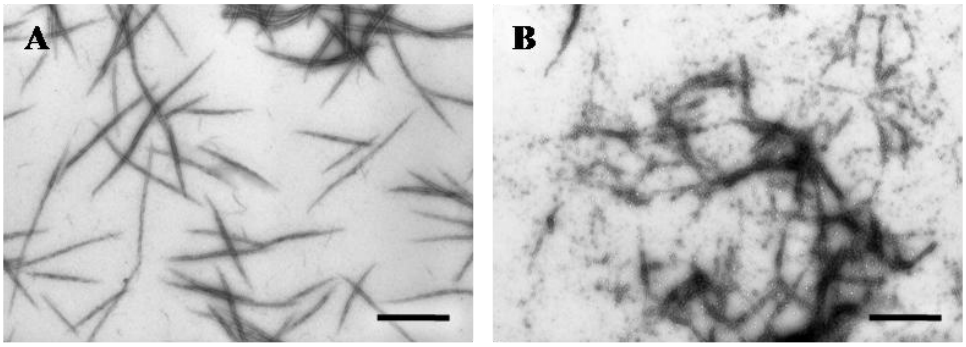

Fig. 2. Effect of L-histidine on myosin filaments at the physiological ionic strength. (A) thick filament-like polymers in a solution of 150 mM KCl. (B) filamentous polymers were observed when thick filament-like polymers (A) were dialysed against a solution of 150 mM KCl and 5 mM L-histidine. Bars are 1 µm.

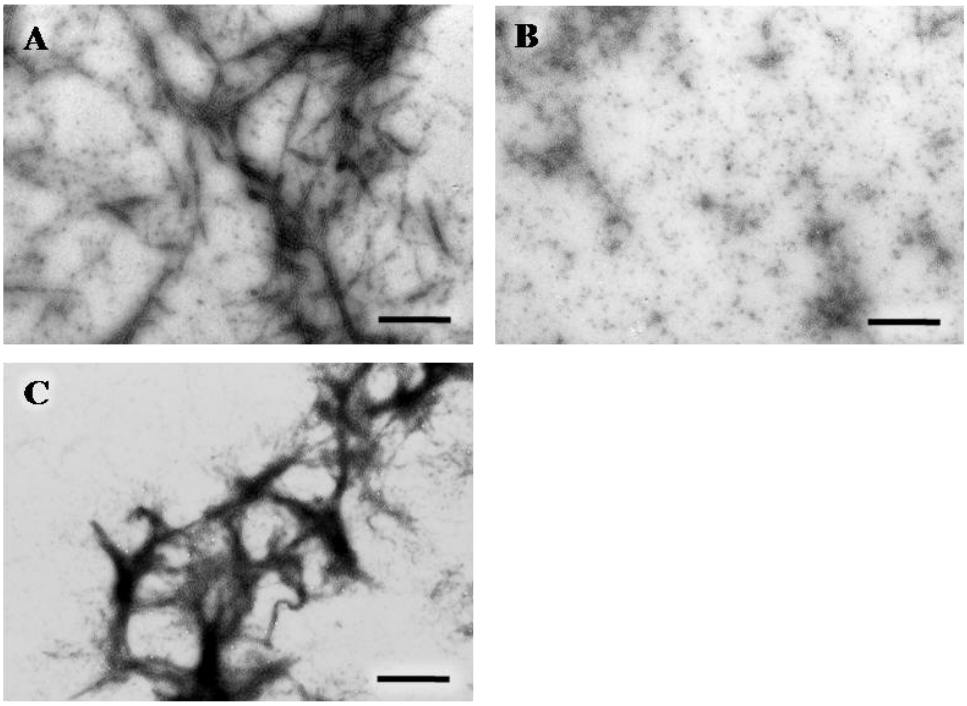

Fig. 3. Effect of L-histidine on myosin filaments at the low ionic strength. (A) swelled filamentous polymers in a solution of 150 mM KCl and 5 mM L-histidine. (B) filamentous polymers were disassembled when filamentous polymers (A) were dialysed against a solution of 1 mM KCl and 5 mM L-histidine. (C) swelled filamentous polymers were observed when filamentous polymers (A) were dialysed against a solution of 1 mM KCl. Bars are 1 μm.

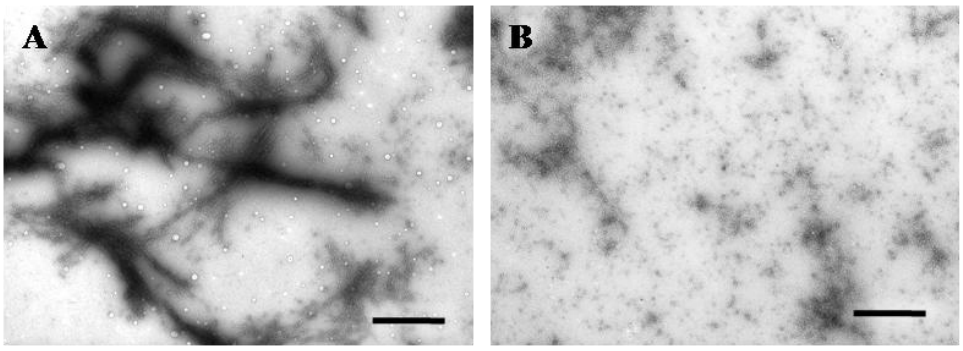

Fig. 4. Effect of L-histidine on myosin filaments at the low ionic strength solution. (A) swelled filamentous polymers in a solution of 1 mM KCl. (B) filamentous polymers were not observed when filamentous polymers (A) were dialysed against a solution of 1 mM KCl and 5 mM L-histidine. Bars are 1 μm.