Significance of imidazole ring in L-histidine on solubilizing myosin in a low ionic strength: The particle property study

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Abstract – For expanding the utilization of meat, previous studies have reported that myosin, one of its major proteins, can be soluble in a low ionic strength solution containing L-histidine (His). To elucidate chemical constituents in His responsible for the solubilization of myosin, the effects of 5 mM His, imidazole (Imi), L-α-alanine (Ala), 1-methyl-L-histidine (M-his) and L-carnosine (Car) on particle properties of myosin suspensions in low ionic strength solution (1 mM KCl, pH 7.5) were investigated. His, Imi and Car, all containing the imidazole ring, could induce a myosin suspension with small particle size species and high absolute zeta potential, thus increasing its solubility. Therefore, imidazole ring of His appeared to be the significant chemical constituent in solubilizing myosin.

Key Words –Myosin; L-histidine; Imidazole ring; Solubility; Chemical constituent.

I. INTRODUCTION

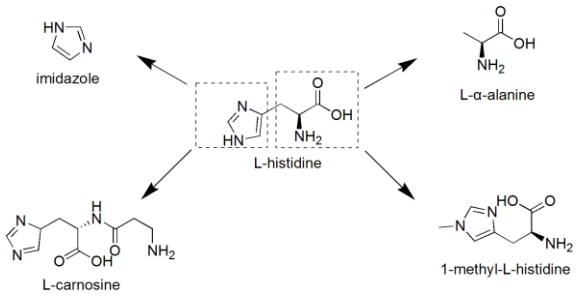
Meat which contains high-quality proteins plays an important role as a supplementary protein source for humans. However, meat has not been fully utilized to the same extent than milk or soybean products due to the low solubility of myofibrillar proteins. It is of interest to determine whether the myofibrillar proteins could also be solubilized at low ionic strength, thus meat might be developed as a liquid diet for humans, especially the elderly people and infant. Previous studies have reported that myosin, one of its major proteins, can be soluble in a low ionic strength solution (1 mM KCl) containing 5 mM L-histidine (His)[1; 2]. However, myosin was not soluble in a low ionic strength solution containing other amino acids like arginine or glycine[3]. It seemed that His might have a specific role in the solubilization of myosin in a low ionic strength solution rather than other amino acids. Thus, we speculated that a specific chemical constituent of His might be responsible for the solubilization effect on myosin in a low ionic strength solution (1 mM KCl). To clarify the role of chemical constituents in L-His for the solubilization of myosin at a low ionic strength, the effects of 5 mM L-His, imidazole (Imi), L-α-alanine (Ala), 1-methyl-L-histidine (1-M-his) and L-carnosine (Car) on solubility, size distribution, zeta potential of myosin suspension in a low ionic strength solution (1 mM KCl, pH 7.5) were investigated.

II. MATERIALS AND METHODS

Myosin was extracted from chicken breast muscle (*musculus pectoralis major*) by using a modified procedure originally reported by Hayakawa et al.[1] Myosin (0.6 KCl, pH 7.5) was dialyzed against low ionic strength solutions (1 mM KCl, pH 7.5) containing 5 mM different additives (His, Imi, Ala, 1-M-his and Car, respectively in Figure 1) over 24 h. After dialysis, the myosin suspensions were used for measurements of solubility, particle size distribution, and zeta potential. The solubility of each treatment was determined using the protein concentrations of each dialysed suspension and supernatant according to Hayakawa *et al.*[2]. Dynamic light scattering (DLS) measurement of particle size distribution was performed according to Shimada *et al.*[4] with a slight modification by using Zetasizer Nano ZS 90 (Malvern Instruments, Worcestershire, UK) equipped with a 4 mW He-Neion laser (λ = 633 nm). The zeta potential was measured by laser doppler electrophoresis using a Zetasizer Nano ZS 90 (Malvern Instrument Ltd., Malvern, Worcestershire, U.K.) equipped with a 4 mW He-Neion laser with an output of 633 nm.

The zeta potential was calculated from the electrophoretic mobility using the Smoulokowski model. All data given were mean ± SD (standard deviation) values of three independent experiments. A $P < 0.05$ significance level was used to determine the differences between the treatments.

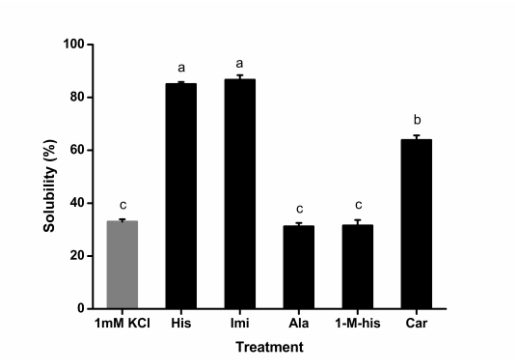
Figure 1. Chemical structure of L-his, imidazole, L-α-alanine, 1-methyl-L-histidine and L-carnosine.



III. RESULTS AND DISCUSSION

As shown in Figure 2, more than 80% of myosin was solubilized in a low ionic strength solution In the presence of 5 mM His, which was consistent with the study by Hayakawa et al.[1]. Since His was an amino acid that contained two major chemical constituents: Imi and Ala, our immediate interest was in testing the two chemicals separately. Results showed that the same solublization effect with His was solely observed in the presence of Imi (Figure 2). Methylation at the N-1 position of imidazole in 1-M-his (Figure 1) abolished its solublization property to myosin at low ionic solution (Figure 2). It seemed that the imidazole ring was the functional group contributing to the solublization effect of His. Next we examined whether other amino acid contained the imidazole ring had the same effect with His, we discovered that Car, which, like His, had an imidazole ring (Figure 2), exhibited solublization effect. Thus, it could be concluded that the imidazole ring of His played an important role for the solubilization of myosin in a low ionic strength solution.

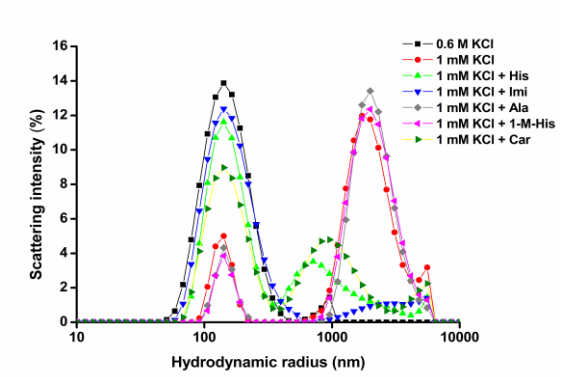
Fugure 2. The solubility of myosin in 1mM KCl solutions (pH 7.5) containing 5 mM different additives: His, Imi, Ala, 1-M-his and Car.



In figure 3, one major peak around 141 nm was observed for myosin solubilized in 0.6 M KCl, indicating that myosin was largely monomeric in the high ionic strength solution[5]. At low ionic strength of 1 mM KCl, myosin polymerized to form filaments through rod-rod electrostatic interaction, so the intensity distribution curves of myosin suspension presented two peaks with larger particle size. It was believed that the filaments were the predominant species at 1 mM ionic strength solution in this study. Therefore, our DLS data strongly supported that monomer myosin assembled to filament species with lager particle size during the dialysis process from 0.6 M to 1 mM. In the presence of His, the smaller size distribution at around 156 nm for myosin suspension became dominant comparing with that of myosin suspensions at 1 mM KCl solution in the absence of His, indicating that the contribution of monomer or small size myosin was more pronounced in 1 mM solution containing His. Hence we hypothesized that His could affect the myosin monomer-filament transition. Next we examined the effects of functional groups in His (Imi, Ala, 1-M-his and Car) on the monomer-filament transition in myosin suspensions during dialysis. The PSD of myosin suspensions in Imi solution at low ionic strength was almost similar to that in 0.6 KCl solution as shown in Figure 3. However, the presence of Ala and 1-M-his might seldom have influence on the monomer-filament transition in myosin suspension during dialysis as

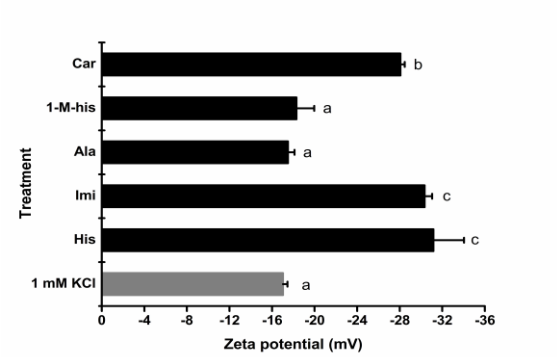
we observed a similar particle size distribution in myosin suspensions with that of 1 mM KCl solution without additives. Car, which also contained the imidazole ring, could inhibit the mono-filament transition during dialysis, probably by disocciation of myosin filaments[2]. Therefore, it could be proposed that the imidazole ring in His might be indipensable for affecting the mono-filament equilibrium in myosin suspensions during dialysis, resulting small particle size species in myosin suspensions at low ionic strength solution.

Figure 3. Size distributions of myosin suspensions in 0.6 M KCl, 1 mM KCl, 1 mM KCl + different additives (pH 7.5), respectively, monitored by DLS



Compared to the 1 mM KCl without test additives, myosin suspensions in the addition of His, Imi or Car possessed significantly higher ($P < 0.05$) absolute zeta potential (around 30 mV in figure 4). His and Imi rendered myosin suspensions a significantly higher ($P < 0.05$) absolute zeta potential than Car (Figure 4). It was well known that increasing the absolute zeta potential on colloidal particles could strengthen the inter-particle electrostatic repulsion and disrupt existing protein aggregates and discourage further aggregate formation[6]. The high negative zeta potential (around -30 mV) in myosin suspensions containing His, Imi or Car indicated very stable myosin dispersions. As a result, the solubility of myosin suspensions in His, Imi, or Car would increase (Figure 2). However, the addition of either Ala or 1-M-his presented a lower absolute zeta potential comparing with that of His, Imi or Car, meaning that the electrostatic repulsion among myosin particles was weaker. Then it was expected that the solubility of myosin suspension in Ala or 1-M-his solution was low (Figure 2).

Figure 4. Zeta potential of myosin suspensions in 1 mM KCl solutions (pH 7.5) containing 5 mM different additives: His, Imi, Ala, 1-M-his and Car.



IV. CONCLUSION

The results on particle properties of myosin suspensions suggested that the imidazole ring in His might be the significant functional group in affecting the mono-filament equilibrium in myosin suspensions during dialysis from 0.6 M KCl to 1 mM KCl solutions. It could induce a myosin suspension with small particle size species (most likely monomeric myosin) and high absolute zeta potential, thus increasing its solubility.

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