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# Solubilisation of myosin in a solution of low ionic strength L-histidine: Significance of the imidazole ring

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ABSTRACT

Myosin, a major muscle protein, can be solubilised in a low ionic strength solution containing L-histidine (His). To elucidate which chemical constituents in His are responsible for this solubilisation, we investigated 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 and conformational characteristics of soluble myosin at low ionic strength (1 mM KCl, pH 7.5). His, Imi and Car, each containing an imidazole ring, were able to induce a myosin suspension, which had small particle size species and high absolute zeta potential, thus increasing the solubility of myosin. His, Imi and Car affected the tertiary structure and decreased the α-helix content of soluble myosin. Therefore, the imidazole ring of His appeared to be the significant chemical constituent in solubilising myosin at low ionic strength solution, presumably by affecting its secondary structure.



## 1. Introduction

Meat contains high-quality proteins that provide a wide range of peptides and amino acids for humans. Meat proteins, unlike those proteins from beans and whole wheat, contain all the essential amino acids, with none limiting, and are all highly digestible (Pereira & Vicente, 2013). However for component extraction, meat has not been utilised to the same extent as milk or soybean products because of their low solubility. Although myofibrillar proteins comprise approximately 50% of the total meat proteins, they are not readily soluble in low ionic strength solutions or water, and a relatively high concentration of salt (>0.3 M NaCl or KCl) is required for their solubilisation (Krishnamurthy et al., 1996). It is therefore of interest to determine whether, under certain conditions, the myofibrillar proteins can be solubilised at low ionic strength. Such a treatment might enable development of a liquid-meat protein diet for humans, which would be especially beneficial for the nutrition of elderly people and infants.

In order to accomplish the solubilisation of myofibrillar proteins in a low ionic strength solution, washing with a sodium chloride solution buffered with L-histidine (His) followed by dialysis against water is necessary. It has also been reported that more than 80% of chicken breast myofibrillar proteins were solubilised in a low ionic strength solution containing 5 mM His by washing and ultrasonication of muscle tissues (Ito, Tatsumi, Wakamatsu, Nishimura, & Hattori, 2003). The ultrasonication was essential for disruption of the highly-ordered structure of the myofibrils and their solubilisation.

The low solubility of myofibrillar proteins is largely the result of the spontaneous formation of myosin filaments that occurs *in vitro* or at low ionic strength. Recently, it was suggested that 5 mM His might affect the secondary structure of myosin (Guo, Peng, Zhang, Liu, & Cui, 2015) and cause elongation of light meromyosin (LMM), resulting in the inhibition of native myosin filament formation. This enabled solubilisation of 80% chicken breast myosin in a low ionic strength solution (1 mM KCl) (Hayakawa, Ito, Wakamatsu, Nishimura, & Hattori, 2009; Hayakawa, Ito, Wakamatsu, Nishimura, & Hattori, 2010). However, myosin was shown not to be soluble in a low ionic strength solution containing other amino

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acids, such as arginine or glycine (Takai, Yoshizawa, Ejima, Arakawa, & Shiraki, 2013). It therefore seems that His might have a specific role in the solubilisation of myosin in a low ionic strength solution, unlike other amino acids. Thus, we speculated that a specific chemical constituent of His might be responsible for the solubilisation effect on myosin in a low ionic strength solution (1 mM KCl). Identification of the critical chemical component in L-His may provide a new insight into the mechanism of enhanced myosin solubility by His in low ionic strength solutions.

His is a neutral amino acid with no net charge at the experimental pH of 7.5 (Takai et al., 2013), and its structure consists of an imidazole ring and an L-α-alanine (Fig. 1). To clarify the role of the chemical component in L-His that is responsible for the solubilisation of myosin at a low ionic strength, we investigated the effects of 5 mM L-His, imidazole, L-α-alanine, 1-methyl-L-histidine and L-carnosine on solubility, size distribution, zeta potential of myosin suspension and conformational characteristics of soluble myosin in a low ionic strength solution (1 mM KCl, pH 7.5).

## 2. Materials and methods

### 2.1. Materials

L-Histidine (His), L-α-alanine (Ala) and L-carnosine (Car) were purchased from Aladdin Industrial Corporation (Ontario, CA), imidazole (Imi) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and 1-methyl-L-histidine (1-M-his) was obtained from Sigma–Aldrich Corporate (St Louis, MO). All chemicals (Fig. 1) were of reagent grade.

### 2.2. Extraction of myosin

Myosin was extracted from chicken breast muscle (*musculus pectoralis major*) immediately after the animal was sacrificed by using a modified procedure originally reported by Hayakawa et al. (2009). All steps were performed at 0–4 °C in a cold chamber. Briefly, minced muscle was extracted with 3 volumes of modified Guba–Straub solution (0.3 M KCl, 100 mM $KH_2PO_4$, 50 mM $K_2HPO_4$, 5 mM $EDTANa_2$, 4 mM sodium pyrophosphate, dissolved in Milli-Q water, pH 6.5) for 15 min and centrifuged at 1200*g* for 10 min. The supernatant was filtered through three layers of cheesecloth and diluted with 14 volumes of cold distilled water. After dilution it was stored for 4 h and then the floating material was removed *via* siphoning. The precipitated protein was then collected by centrifugation at 10,000*g* for 15 min. After centrifugation, the precipitate was solubilised in 0.3 M KCl, pH 7.0. The solution was centrifuged at 20,000*g* for 30 min and the supernatant diluted with 10 volumes of cold distilled water. After centrifugation (10,000*g*, 15 min), the precipitate was dissolved in 0.6 M KCl, pH 7.5. The dissolved solution was then dialysed with a dialysis bag (diameter: 36 mm, MW: 3500) against 0.6 M KCl, pH 7.5 solution for 24 h. After centrifugation (20,000*g*, 20 min) of dialysed solution, the resulting supernatant was used as the stock myosin solution in 0.6 M KCl, pH 7.5. As previously reported, myosin was highly soluble in 0.6 M KCl (pH 7.5) solution and largely present in the monomer structure (Niederman & Pollard, 1975; Sinard, Stafford, & Pollard, 1989; Takai et al., 2013).

The protein composition of the extracted myosin was validated by SDS–PAGE with a gel imaging system (GT-800 F; Epson, Nagano, Japan). The purity of the myosin was greater than 90% (Supplementary Fig. 1) as determined by densitometry (Quantity One 1-D analysis software, Bio-Rad Co., Hercules, CA).

### 2.3. Preparation of myosin suspensions in low ionic strength solutions

Myosin suspensions were prepared in low ionic strength solutions according to the procedure of Hayakawa et al. (2009). Myosin (0.6 M KCl, pH 7.5) was dialysed against low ionic strength solutions (1 mM KCl, pH 7.5) containing 5 mM of different additives (His, Imi, Ala, 1-M-his or Car) over 24 h. After dialysis, the myosin suspensions were used for further treatment or measurements of solubility, particle size distribution, and zeta potential. Myosin dialysed against 1 mM KCl containing 5 mM Tris–HCl (pH 7.5) was regarded as the control.

### 2.4. Myosin solubility

The dialysed myosin suspension was centrifuged at 20,000*g* for 20 min (Takai et al., 2013), and the obtained supernatant was defined as myosin solubilised in a low ionic strength solution containing the corresponding additive. The solubility was expressed as percent of protein concentration in the supernatant, with respect to that of dialysed myosin suspension before centrifugation, according to Hayakawa et al. (2010).

### 2.5. Particle properties of myosin suspensions

#### 2.5.1. Dynamic light scattering (DLS) measurement of particle size distribution (PSD)

DLS measurement was performed according to Shimada, Takai, Ejima, Arakawa, and Shiraki (2015) with a slight modification, by using a Zetasizer Nano ZS 90 (Malvern Instruments Ltd, Great Malvern, UK) equipped with a 4 mW He–Neon laser ($\lambda$ = 633 nm). The

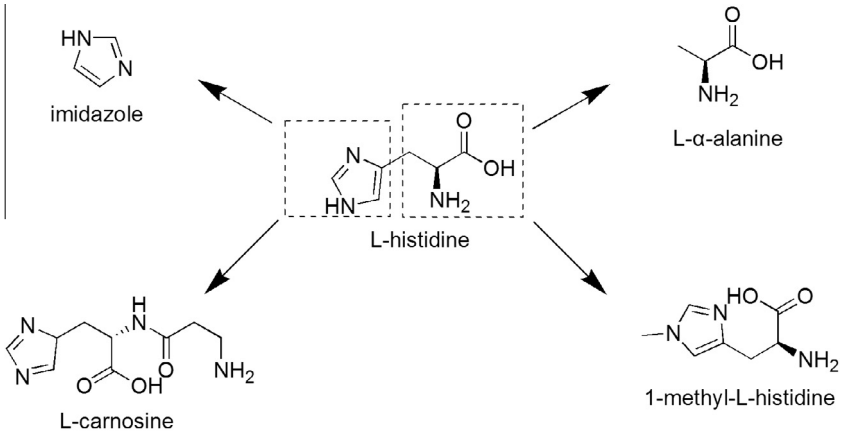


**Fig. 1.** Chemical structures of L-his, imidazole, L-α-alanine, 1-methyL-L-histidine and L-carnosine. L-His, imidazole, and L-carnosine contain an imidazole ring group whereas α-alanine was the functional component in L-α-alanine and 1-methyl-L-histidine.