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High-pressure effects on the molecular aggregation and physicochemical properties of myosin in relation to heat gelation

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ABSTRACT

Myosin was extracted from the *M. psoas* muscle of rabbits, and dissolved in 0.6 M KCl buffer (pH 6.5). Effects of high-pressure (HP, 100 to 300 MPa, 9 min, 25 °C) treatment on myosin solubility, molecular traits (molecular weight and morphology), flow behavior and strength of heat-induced myosin gels were studied and compared with the untreated controls. Myosin subjected to 200 MPa HP treatment had lower solubility than samples treated at other pressures ($P < 0.05$). Molecular dimerization and morphological swelling of myosin was observed using gel-permeation chromatography and atomic-force microscopy. Additionally, the shear-thinning behavior of myosin solutions (10 mg/mL) was improved by HP treatment (≥ 200 MPa), and a positive trend in gel-strength enhancement was inferred. It is postulated that significant morphological changes in myosin accounted for changes in its functional properties, by the influence of HP treatment on protein-protein and/or protein-water interactions. There is a relationship between molecular morphology and the coalescing behavior of myosin, since significant changes of both attributes were observed at pressures ≥ 200 MPa.

1. Introduction

Myosin is among the most important proteins in meat, accounting for nearly a quarter of the total muscle proteins. It has the ability to form a three-dimensional gel network driven by heating (Asghar, Samejima, Yasui, & Henrickson, 1985). Myosin monomer is a hexamer, consisting of two large polypeptides (~205 kDa each) and four light chains (~16 to 25 kDa). Typically, a myosin monomer has two "heads" and a rod-like α-helical tail (Mclachlan & Karn, 1982). It has been demonstrated that physicochemical changes in myosin, such as exposure of hydrophobic sites, uncoiling of the α-helix and modifications of protein-protein interactions, impart predominant effects on the functionalities of thermally-induced meat gels (Cao, Xia, Zhou, & Xu, 2012; Liu, Zhao, Xiong, Xie, & Qin, 2008). Functional properties of gel-type meat products, especially myosin, are improved by means of chemical (e.g. phosphorous salts), biological (e.g. microbial-origin enzymes), and physical treatments (e.g. non-thermal technologies) and/or their combinations (Marques, Maróstica, & Pastore, 2010; Trout & Schmidt, 1986; Troy, Ojha, Kerry, & Tiwari, 2016).

High pressure (HP) is a physical modification technique that is readily used to improve the functionality of meat proteins. In addition to its ability to prolong the shelf life of food (Heinz & Buckow, 2009), many studies have verified that moderate HP intensities (100 to 300 MPa) can impart positive influences on the gelation properties. Studies have reported that HP intensities of 200 MPa as being a tentative threshold for effective HP treatment in modifying the functional properties of gel-type meat products, leading to better moisture retention ability and/or better textural properties (Sikes, Tobin, & Tume, 2009; Yang, Han, Wang, et al., 2015). This is particularly the case in those products with low salt and/or low fat contents (Chen et al., 2014; Sikes et al., 2009; Yang, Han, Bai, et al., 2015). Moreover, our previous study with rabbit meat batter containing 2% sodium chloride, confirmed that water-holding capacity (WHC), water mobilities and distributions, as well as textural properties of cooked rabbit meat batter were significantly improved by treatment at 200 MPa (9 min, 25 °C) (Xue, Wang, et al., 2017; Xue, Yu, et al., 2017). However, a significant impairment of WHC was observed with myosin gels when pre-treated at 200 MPa (Wang et al., 2017). Therefore, a myosin-based perspective of the mechanism underlying these changes would be potentially useful.

In the 1990s, Yamamoto and coworkers found that myosin

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oligomers were formed by head-to-head association following pressure treatment at 200 MPa. When the pressure intensity reached 300 MPa, myosin formed clumps of daisy wheel-like structures (Yamamoto, Yoshida, Morita, & Yasui, 1994). As a result, enhancement in hydrophobicity, involvement of various non-covalent bonds, and a reduction in α-helix content were observed. HP-induced myosin aggregation led to improvement in gelation properties (Cao et al., 2012; Yamamoto et al., 1994). Since then, only a few studies (Chen et al., 2014; Wang et al., 2017) have focused on exploring the effects of HP treatment on the molecular traits of myosin in addition to tertiary and secondary structural changes.

Thus far, HP-induced molecular attributes of myosin, such as morphological changes and patterns of molecular aggregation remain obscure, despite their potential for the underlying functionalities of thermally-induced gels. Therefore, the aim of this study was to investigate HP-induced changes in the molecular structure of myosin by means of gel-permeation chromatography (GPC) and atomic-force microscopy (AFM). We have also attempted to elucidate the role of HP-induced changes in the alteration of gelation properties.

2. Materials and methods

2.1. Materials

A total of twelve male New Zealand white rabbits, each weighing approximately 3.0 kg, 3-months old and in similar physical conditions, were purchased from the Livestock Institute of Jiangsu Academy of Agricultural Sciences (Nanjing, China). *M. psoas* muscles were collected immediately after slaughter. All animal-related procedures were reviewed and approved by the Animal Care and Use Committee at the Chinese Academy of Agricultural Sciences. The muscles were cooled in an ice bath (0 °C) for 20 min to minimize enzyme activity.

2.2. Methods

2.2.1. Myosin extraction

Visible adipose and connective tissues were trimmed from the pre-cooled muscles. Myosin was extracted according to the protocol described by Chen et al. (2014) and Guo (2015) with some modifications. Muscles (100 g) were ground for 12 s (with a pause every 3 s to minimize temperature rise) in a chilled cutter (Grindomix GM 200, Retsch, Germany) at the speed of 3000 rpm. The batter was mixed with 400 mL cold buffer solution (0.1 M KCl, 20 mM potassium phosphate, 2 mM $MgCl_2$, 1 mM EGTA, pH 7.0) containing 1 mM dithiothreitol and then initially homogenized in a blender (Waring Products Division, Dynamics Corp., New Hartford, CT, America). These mixtures were further homogenized at 4 °C for 3 min at 7500 rpm using a homogenizer (Ultra Turrax T25 BASIS, IKA, Labortechnik, Germany). The resulting batters were then centrifuged (Beckman Avanti J-E, Beckman Coulter, Fullerton, CA, USA) at 4 °C and 5000 g for 10 min. The pellets were extracted with 3 volumes of a modified Guba-Straub solution (0.3 M KCl, 0.15 M potassium phosphate, 1 mM EGTA, 4 mM sodium pyrophosphate, 20 mM $MgCl_2$, pH 6.5) and stirred for 15 min at 4 °C with a magnetic agitator. The extract was centrifuged at 4 °C and 8000 g for 15 min. The supernatants were diluted with ten volumes of chilled 1 mM EDTA-2Na solution, and stored for 8 h at 4 °C. The precipitated protein was collected by centrifugation at 4 °C and 10,000*g* for 15 min after siphoning the floating material, and suspended using one volume of 0.6 M KCl buffer (0.6 M KCl, 20 mM potassium phosphate, pH 6.5). The protein was diluted by magnesium chloride solution (1 M) and sodium pyrophosphate solution (0.1 M) up to 5 mM final concentration. The final mixture was stirred at 4 °C for 30 min without foaming and centrifuged at 4 °C and 10,000*g* for 20 min. The supernatants were re-diluted in ten volumes of cold solution (1 mM EDTA-2Na) and allowed to precipitate for approximately 12 h. The supernatant and pellets were re-subjected to the same treatment as described above. The solution was dialyzed against the 0.6 M KCl buffer (pH 6.5). The resulting supernatants were regarded as the myosin extract. Protein concentration in the extract was measured using the Biuret method, and the final concentration was adjusted to 10 mg/mL using the 0.6 M KCl buffer (pH 6.5) prior to HP treatment. Subsequently, myosin solutions (10 mg/mL) were sealed in 12 individual aseptic bags (three bags for each treatment) with a heat sealer (Bleuets, FR-300A, Shanghai, China). Each bag contained 50 mL of myosin solution, and air bubbles were excluded prior to sealing. Samples were then stored at 4 °C until further testing.

2.2.2. High-pressure (HP) treatment

HP treatment was performed in a 0.3 L capacity 850 Mini Food Lab high-pressure vessel (Stansted Fluid Power Ltd., UK). The inner diameter and the length of the HP champer are 37 mm and 300 mm respectively. A mixture of propylene glycol and distilled water (30:70) was used as the pressure-transfer medium and the medium temperature was kept below 25 °C by means of a thermostated jacket. Three different HP intensities (100, 200 and 300 MPa, 9 min, 25 °C) which would likely lead to identifiable and significant effects on protein functional properties were selected, based on previous studies (Xue, Wang, et al., 2017; Xue, Yu, et al., 2017). Myosin, not subjected to HP treatment, was set as the control group. Prior to pressure treatment, myosin solutions were pre-incubated in a 25 °C water bath for 15 min to reach an equivalent temperature to pressure-transfer medium. Three bags (150 mL of myosin solution in total) were processed for each defined HP treatment. During the processing, the pressurization rate was 20 MPa/s and the pressure-release rate was 12 MPa/s. The maximum temperature as a consequence of pressurizing was 30 °C. Upon release of pressure and removal from the vessel, the samples were kept at 4 °C for 8 h.

2.2.3. Protein solubility

Myosin solubility was determined based on the protocol described by Chen, Xu, and Zhou (2015) with slight modifications. The processed myosin solutions were diluted to 5 mg/mL, followed by centrifugation at 20,000*g* for 20 min (4 °C). The protein concentrations of supernatants were determined by the Biuret method and were compared with the initial concentration (5 mg/mL).

2.2.4. Gel-permeation chromatography (GPC)

GPC was conducted to estimate the molecular weight of myosin based on the protocol described by Lange, Rulli, and Crestini (2016) with slight modifications. Twenty microliter (μL) of myosin sample was injected into an analytical GPC column (TSK gel, G3000 swxl, 6.5 × 300 mm) for separation on Waters 1525 (Waters Corporation, US). Phosphate buffer (0.05 M of sodium phosphate, pH 6.7) as a flow rate of 0.5 mL/min was used as the mobile phase. Calibration was performed using a series of standard proteins, i.e. Bacillus enzyme (Mw = 1.422 kDa), carbonic anhydrase (Mw = 29 kDa), bovine albumin serum (Mw = 66 kDa), and β-amylase (Mw = 200 kDa). Detection of molecular sizes was achieved using a UV-detector at a wavelength of 220 nm. Both number-average molecular weight (Mn) and weight-average molecular weight (Mw) were calculated based on the following equations:

$$Mn = (\sum h_i M_i)/(\sum h_i) \quad (1)$$

$$Mw = (\sum h_i M_i^2)/(\Sigma h_i M_i) \quad (2)$$

where M_i represents the molecular weight at a certain measurement spot i depending on a calibration, and h_i is the signal intensity of a certain log M at a measurement spot i.

2.2.5. Atomic-force microscopy (AFM)

Morphology of HP-treated myosin and the control was compared by