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Effects of different NaCl concentrations on self-assembly of silver carp myosin

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ABSTRACT

The effect of NaCl (0.1 − 3.0 M) on self-assembly of silver carp myosin at 4 °C was investigated in terms of microstructure, conformation, intermolecular interactions, and particle size distribution. During setting at 4 °C, the self-assembly of myosin showed an obvious concentration dependence. At low concentrations (< 0.3 M), myosin assembled into filaments mainly through rod-rod ionic linkages, and showed significantly ($P < 0.05$) higher turbidity and lower solubility than at ≥0.3 M. The addition of NaCl (0.3 − 0.6 M) led to the extension and dissolution of myosin by interfering with electrostatic interactions. The myosin assemblies gradually became smaller and more uniform. The solubility and Ca^{2+}-ATPase activity reached a maximum at 0.6 M NaCl. When the concentrations increased to > 1.0 M, myosin further assembled into filaments dominated by hydrophobic interactions. Both rod-rod and head-head interactions contributed to the myosin filaments at high concentrations (1.0 − 3.0 M). The solubility and uniformity of the myosin assemblies decreased as the NaCl concentration increased from 1.0 to 3.0 M.

1. Introduction

Salted surimi can set at ≤ 40 °C, leading to the formation of translucent and elastic gels (Lee, Lanier, Hamann, & Knopp, 1997). Myosin, the predominant muscle protein, is largely responsible for the gelation properties of surimi. Salt concentration (ionic strength) affects the amount of extracted myosin, and therefore, has an effect on protein gelation (Tahergorabi & Jaczynski, 2012). Surimi gels are generally produced with 2 − 3% NaCl (0.34 − 0.51 M) (Lanier, Yongsawatdigul, & Carvajal-Rondanelli, 2014). Decreases in salt content depressed protein solubility, water-holding capacity and the strength of surimi gels, as well as influencing organoleptic properties of the final surimi products (Pietrasik & Li-Chan, 2002). However, excessive salt ions promote protein intermolecular interactions because of their stronger hydration ability than protein. Therefore, proteins tend to precipitate after addition of excessive salt (Baxter & Skonberg, 2008). A set before cooking could improve the gel strength and elasticity of surimi gel as well as its water holding capacity (Kamath, Lanier, Foegeding, & Hamann, 1992; Liu, Zhao, Xie, & Xiong, 2011a). The optimal setting temperature varied with the thermal stability of myosin, e.g., myosin derived from cold water fish species undergoes setting at 4 °C, while that from tropical fish species typically set at 40 °C (Lanier et al., 2014).

Silver carp is a widely cultured and processed freshwater fish in China (Shi, Luo, Shen, & Li, 2014), and has become a potential raw material for surimi as marine fish resources decrease. Silver carp is a temperate water fish. The strongest gels could be obtained with 40 °C setting (Liu et al., 2011a). Silver carp surimi could also set at 4 °C, and showed higher fracturability than that set at 40 °C for 1 h (Zhang, Fan, Zhang, Xiong, & Liu, 2017b). However, the reasons for the difference were not determined. It was reported that the melting temperature of silver carp myosin was 40 °C (Liu, Zhao, Liu, Xie, & Xiong, 2007; Qiu, Xia, & Jiang, 2014). Heating surimi up to the 40 °C could induce myosin unfolding followed by formation of abundant ε-(γ-Gln)-Lys bonds and other covalent bonds (Liu et al., 2011a). However, myosin is relatively stable at 4 °C. Most reactive groups remain inside the myosin molecules. It could be hypothesized that the intermolecular bonds of myosin at 4 °C would be different from those at 40 °C. According to Whitesides and Seto (1991), molecular self-assembly is defined as the spontaneous association of molecules into stable, structurally well-defined aggregates through non-covalent bonds. Extracted myosin from Atlantic cod is usually found as aggregates of between 8 and 20 myosin molecules (Brenner, Jóhannsson, & Nicolai, 2009). Therefore, it was presumed that myosin molecules could spontaneously associate with each other to form assemblies even at a low temperature. This could contribute to a

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set gel at low temperatures (e.g., 4 °C). Electrostatic interactions are thought to be the most important forces involved in the myosin assemblies (Miroshnichenko, Balanuk, & Nozdrenko, 2000). Addition of salt (usually NaCl) could disturb electrostatic interactions, influencing the myosin assemblies. Therefore, it is useful for exploring the mechanism behind low-temperature setting to study self-assemble properties of myosin with different salt concentrations at low temperature.

In this study, myosin was extracted from silver carp muscle. Microstructures of myosin assemblies were observed using confocal laser scanning microscopy (CLSM). Subsequently, the effects of concentration (0.1 − 3.0 M NaCl) on conformation and intermolecular interactions of myosin were investigated at low temperature (4 °C). The solubility and particle size distribution of myosin assemblies were also studied. The relationship between myosin self-assembly and physicochemical properties was analyzed. The aim was to understand the driving mechanism of NaCl concentration on the self-assembly of myosin at low temperature.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA) and rhodamine B were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 8-Anilino-1-naphthalenesulphonic acid (ANS) was obtained from Aladdin Reagents Co., Ltd. (Shanghai, China). All other chemicals used were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Materials

Live silver carp (*Hypophthalmichthys molitrix*) (about 1.0 − 1.5 kg) were bought from the Huazhong Agricultural University market (Wuhan, China), and transported to the laboratory alive, about 10 min. The live fish were immediately killed by blunt force trauma to the head, gutted by hand, washed with tap water, and then filleted manually with a knife. Only white muscle was used. Fish flesh was sliced into little pieces (about 3 × 1 × 0.3 cm) and washed 3 times using tap water with a ratio of water to fish pieces of 4–1. Each washing cycle lasted for 5 min. The muscle was minced using a food processor (Braun GmbH, Kronberg im Taunus, Germany) for less than 1 min.

2.3. Preparation of myosin assemblies

Myosin was extracted using the method of Cao, Su, Regenstein, Xiong, and Liu (2015). The mince was mixed with 10 volumes of 0.10 M KCl, 0.02% sodium azide and 20 mM Tris-HCl buffer, pH 7.5 and homogenized for 1 min using a dispersing homogenizer (Model FJ-200, Shanghai Specimen and Models Factory, Shanghai, China). The mixture was centrifuged at 5000 × *g* for 5 min at 4 °C using a high-speed refrigerated centrifuge (Avanti J-26 XP Centrifuge, Beckmen Coulter, Fullerton, CA, USA). The sediment was suspended with 5 volumes of 0.45 M KCl, 5 mM β-mercaptoethanol (β-ME), 0.2 M $Mg(CO_2)_2$, 1 mM EGTA and 20 mM Tris-maleate buffer, pH 6.8, mixed with ATP to a final concentration of 5 mM and incubated for 1 h at 4 °C. The mixture was centrifuged at 10,000 × *g* for 10 min at 4 °C. The resulting supernatant was diluted with 5 volumes of 1 mM $KHCO_3$ and kept at 4 °C for 15 min. Subsequently, the mixture was centrifuged at 12,000 × *g* for 10 min at 4 °C and the pellet was re-suspended with 2.5 volumes of 0.5 M KCl, 5 mM β-ME and 20 mM Tris-HCl buffer, pH 7.5. The re-suspended pellet was incubated at 4 °C for 15 min and diluted with 2.5 volumes of 1 mM $KHCO_3$. Meanwhile, $MgCl_2$ was also added to obtain a final concentration of 10 mM. After overnight incubation at 4 °C, the prepared solution was centrifuged at 12,000 × *g* for 15 min at 4 °C. The myosin pellet was dispersed in 20 mM Tris-HCl buffer (pH 7.0, including 0.5 M NaCl). Subsequently, this dispersion was centrifuged at 10,000 × *g* for 10 min at 4 °C. The supernatant (myosin) was kept at 4 °C for use within 2 days.

The Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) was used for protein concentration determination. Bovine serum albumin (BSA) was used as the standard (assuming 100% purity). Myosin was diluted to 1.0, 5.0 or 10.0 mg/mL, and NaCl concentrations were adjusted to 0.1, 0.2, 0.3, 0.4, 0.6, 1.0, 2.0, or 3.0 M with 20 mM Tris-HCl buffer (pH 7.0) and 20 mM Tris-HCl buffer (pH 7.0, including 5 M NaCl). The myosin solutions were incubated at 4 °C for 12 h of self-assembly. The myosin suspension (10.0 mg/mL) was used for visualizing the microstructures of myosin assemblies. Subsequently, the whole myosin suspension (5.0 mg/mL) was used for Ca^{2+}-ATPase activity measurements. The suspension with 1.0 mg/mL was used for analyzing the UV absorption, S_0-ANS, SH content, turbidity, solubility, and particle size distribution.

2.4. Confocal laser scanning microscopy (CLSM)

The microstructures of myosin assemblies were observed using a confocal laser scanning microscopy (LSM501 META, Carl Zeiss Microscope Systems, Jena, Germany). The suspensions of myosin assemblies were dripped onto a freezing microtome and sliced using a pathological slicer (Leica RM2016, Shanghai Leica Instrument Ltd., Shanghai, China). The staining solution of 10^{-3} M rhodamine B was added to the sliced samples, and then, the samples were washed with distilled water to remove excessive stain. The stained samples were immediately covered with a cover slip and observed under the microscope, using 60× magnification. The fluorescent rhodamine B was excited using a laser at 543 nm. The images were acquired in the Zeiss LSM Image Examiner software. In this experiment, rhodamine B and the samples with rhodamine B were placed and processed in the dark.

2.5. UV absorption spectra

Myosin samples (1.0 mg/mL) were placed in a 1 cm quartz cuvette. UV absorption spectra were obtained from 230 to 350 nm using a UV-721 spectrophotometer (Shanghai Precision Instrument Co., Ltd., Shanghai, China). The second derivative spectra were obtained from the UV absorption spectra using OriginPro 8.6 software (OriginLab, Northampton, MA, USA).

2.6. Ca^{2+}-ATPase activity

The Ca^{2+}-ATPase activity was measured using the method of Benjakul, Seymour, Morrissey, and Haejung (1997). The sample (1 mL) was mixed with 0.5 mL of 0.5 M Tris-maleate, pH 7.0. The $CaCl_2$ was added to obtain a final concentration of 10 mM $CaCl_2$ with the total volume being 9.5 mL. To initiate the reaction, 0.5 mL of 20 mM ATP was added. The reaction ran for exactly 10 min at 25 °C and was terminated by adding 5 mL of chilled 15% (w/v) trichloroacetic acid (TCA). The reaction mixture was centrifuged at 3500 × *g* for 5 min at 4 °C and the inorganic phosphate (P_i) liberated in the supernatant was measured using the color assay method of Fiske and Subbarow (1925) with some modifications. A 10 mL aliquot of the supernatant was poured into a 50 mL volumetric tube and mixed with 8 mL of 0.015% hydrazine sulfate and 2.0 mL of 2.5% sodium molybdate in 5 M sulfuric acid. The mixture was incubated in boiling water for 10 min and cooled using running water. Subsequently, distilled water was added to the mark, mixed and the absorbance measured at 650 nm after 10 min. A standard curve using KH_2PO_4 was prepared to obtain the phosphorus content. Ca^{2+}-ATPase activity was defined as μmol of P_i/mg protein/min at 25 °C. A blank was prepared by adding chilled 15% TCA prior to addition of ATP and its value subtracted from each sample.