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Physicochemical, conformational and functional properties of silver carp myosin glycated with konjac oligo-glucomannan: Implications for structure-function relationships

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ABSTRACT

Silver carp myosin (Ms) was glycated with konjac oligo-glucomannan (KOG) under dry reaction conditions. The physicochemical properties (amino acid composition, total sulfhydryl groups, hydrophobicity, viscosity and microstructure), conformational properties (heat changes, secondary and tertiary conformations) and functional properties (emulsifying properties) were investigated. The amino acid composition (especially relative acidic/basic charged amino acid ratio) was an intrinsic physicochemical parameter that largely determined other physicochemical and conformational properties. Formation of Ms-KOG conjugates reduced the total sulfhydryl groups content and hydrophobicity. Glycation for 12 h increased the viscosity of Ms by 35-fold. Nanoscale structure and secondary conformation, as determined by atomic force microscopy and far-UV circular dichroism spectroscopy, remarkably changed. Combined analysis of differential scanning calorimetry and intrinsic emission fluorescence indicated that Ms may undergo a tertiary conformation unfolding and subsequent rearrangement process. More importantly, we have interestingly found that KOG improved the flexibility in conformation and enhanced the steric hindrance of Ms. This specific structure of Ms-KOG eventually improved the emulsifying properties.

1. Introduction

In China, silver carp (*Hypophthalmichthys molitrix*) is a major freshwater fish with low economic value because of its strong earthy/musty taste and odor as well as containing too much intramuscular small bones (Qiu, Xia, & Jiang, 2013). Like other species of fish, silver carp proteins are thermally and chemically less stability due to its "soft flesh" condition, which easily leads to proteins denaturation during processing. Consequently, the functional properties of the silver carp proteins, such as solubility, water-holding capacity, gel-forming ability, foaming ability, and emulsifying ability can deteriorate easily (Liu, Xu, Zhang, Zhao, & Ding, 2016). Therefore, improving the functionalities of these proteins could contribute to the enlarging usage of the freshwater fishes.

It is now well-recognized that impressive application of protein and requirements for high-quality proteins in food industry can be achieved via the glycation, which can be obtained by the reaction between reducing sugars and amino groups, has attracted much attention of many researchers (Liu, Ru, & Ding, 2012). Through this reaction, the conjugation of the reducing sugar to the protein does not need chemical cross-linking, which can alter biopolymer structure and has been demonstrated in many studies to improve functional properties of food proteins (Liu & Zhong, 2013). Until now, different proteins, such as carp myofibrillar (Mf) protein (Sato, Sawabe, Kishimura, Hayashi, & Saeki, 2000), and myosin (Ms) protein (Maitena, Katayama, Sato, & Saeki, 2004), whey protein (Jimenez-Castano, Villamiel, & López-Fandiño, 2007), and milk protein (Yadav, Parris, Johnston, Onwulata, & Hicks, 2010) have been conjugated with various saccharides to improve functional

Abbreviations: Ms, myosin; Mf, myofibrillar; KOG, konjac oligo-glucomannan; KGM, konjac glucomannan; AO, alginate oligosaccharide; DP, degree of polymerization; BPB, bromophenol blue; EAI, emulsifying activity index; ESI, emulsion stability index; AFM, atomic force microscopy; DSC, differential scanning calorimetry; CD, circular dichroism; SDS, sodium dodecyl sulphate; ANOVA, analysis of variance; LSD, least significant difference; SAPP, acid-precipitated soy protein; SH, sulfhydryl; HMM, heavy meromyosin; LMM, light meromyosin; H_0, surface hydrophobicity; OVA, ovalbumin; WPI, whey protein isolate; GOD, glucose oxidase.

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properties. However, the functional properties of these proteins are closely associated with the structure of saccharides, i.e., the molecular weight, branched chains, and other substituent groups. For example, saccharides with more branched chains would contribute to higher emulsifying capacity of protiens (Oliver, Melton, & Stanley, 2006).

Konjac glucomannan (KGM) is an essential polysaccharide extracted from roots and tubers of the *Amorphophallus* konjac plant. It has the structure of a linear random copolymer of 1, 4-linked-β-D-mannopyranose and β-D-glucopyranose units in a molar ratio of 1.6:1, with approximately 1 in 19 sugar units being acetylated at the side C-6 position (Xiong, Cheng, Ye, Du, Zhou, Lin, et al., 2009). Unlike many other biopolymers, KGM has very narrow molecular weight distribution. What's more, its molecular chains were extending, linear, semi-flexible and low rigidity (Wen, Cao, Yin, Wang, & Zhao, 2009). KGM, whatever in types of hydrophilic groups, spatial distribution or steric hindrance from branched chains, is quite different from traditional polysaccharides. Konjac oligo-glucomannan (KOG) is prepared by degradation of KGM using β-mannanase. It is a new-type of oligosaccharide with abundant hydrophilic groups and a certain amount of branches. Maitena et al. (2004) reported that carp Ms conjugated with alginate oligosaccharide (AO), with the average degree of polymerization 6.1, displayed preferable functional properties. However, in molecular terms, alginates consist of a family of unbranched binary copolymers of 1-4-1inked β-D-mannuronic acid and α-L-guluronic acid (Smidsrød & Skja, 1990), which indicates that KOG might be an excellent sugar donator for glycation with Ms, in terms of branches to provide steric hindrance and enhance the above mentioned functional properties.

The objective of this study is to investigate the benefits of silver carp myosin protein glycation under dry reaction conditions. The new-type of sugar donator, KOG, was used for glycation, and the physicochemical, conformational and functional properties of the glycoconjugates were evaluated. Specific attention was paid on the relationship between structure and emulsifying properties of the glycoconjugates.

2. Materials and methods

2.1. Materials

A fresh silver carp was purchased from a local fish market. Konjac glucomannan (KGM) flour with a purity of 90% was acquired from Hubei Johnson Konjac Co., Ltd (Hubei, China). β-mannanase with an activity of 50000 U/g was purchased from Beijing Challenge Bio-Technology Co., Ltd (Beijing, China). Other chemicals were from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.2. Preparation of myosin (Ms)

Ms was prepared from dorsal muscle of silver carp according to our previously reported method (Liu et al., 2016). Silver carp muscle mince was homogenized for 30 s four times, while cooled with ice-cold water, using a homogenizer (Fluko homogenizer, Model F6/10, Germany) in chilled buffer consisting of 0.1 M KCl and 20 mM Tris-HCl (pH 7.5). The homogenate was then repeatedly washed by centrifugation at 5000 rpm for 10 min at 4 °C and suspension of the pellet. The homogenate was extracted with Guba-Straub solution (0.3 M KCl-0.1 M KH_2PO_4-0.05 M K_2HPO_4-4 mM sodium pyrophosphate -1 mM EDTA, pH 6.5) for 1 h with vigorous stirring. The extract was diluted with 3 vol of distilled water. The muscle residue was filtered through three layers of cheesecloth. The filtrate was then diluted with 6.5 vol of 1 mM EDTA with rapid stirring. The crude precipitate was spun down at 5000 rpm for 10 min after 3 h. The precipitate was resuspended in 3 M KCl solution, which was subsequently diluted with distilled water to 0.6 M KCl. Magnesium chloride and sodium pyrophosphate were added and the final concentration was 5 mM for each. The solution was stirred vigorously for 10 min, and was then diluted with distilled water to 0.3 M KCl. The solution was then centrifuged at 10000 rpm for 15 min to remove actomyosin as precipitate. And then, the solution was diluted with distilled water to 0.03 M KCl. The precipitated protein was collected by centrifugation at 10000 rpm for 10 min at 4 °C. The above steps were repeated 3 times. The precipitate was resuspended in 3 M KCl/25 mM PIPES buffer (pH 7.0), and subsequently diluted with distilled water to 0.6 M KCl. Magnesium chloride and sodium pyrophosphate were added to the final concentration of 5 mM for each. The solution was stirred vigorously for 10 min, and solid $(NH_4)_2SO_4$ was added slowly to 35% saturation with constant stirring. The solution was then centrifuged at 10000 rpm for 15 min at 4 °C and the supernatant was brought to 48% saturation. The Ms pellet was collected by centrifuged at 10000 rpm for 15 min at 4 °C and redissolved in 0.6 M KCl containing 25 mM Tris-HCl (pH 7.5). Finally, the Ms solution was dialyzed against several changes of the same solvent to remove any traces of $(NH_4)_2SO_4$. All preparations were used within 7 days.

2.3. Preparation of konjac oligo-glucomannan (KOG)

The KOG was prepared according to our previously reported method (Liu et al., 2016). Briefly, Konjac powder was added to 150 mL of 0.2 M acetate buffer to make a 5% (w/v) suspension and then mixed with β-mannanase (150 U/g) to start the reaction. The solution was incubated at 50 °C for 2 h with continuous stirring, stopped by boiling the samples for 10 min. After concentrated with a rotary evaporator, the solution was mixed with 95% ethyl alcohol. KOG was collected as a precipitate after washing and centrifuging five times. After redissolving in distilled water, the solution was membrane-filtrated (MW cut-off limit = 8000 Da) to remove undegraded KGM and then lyophilized in a freeze-dryer. The degree of polymerization (DP) of KOG was calculated as the ratio of total sugar content (milligrams per milliliter) to reducing sugar (milligrams per milliliter). According to our previous report, the average DP of KOG is approximately equal to 5.2 (Liu et al., 2015).

2.4. Glycation of myosin with konjac oligo-glucomannan

Ms and KOG were fully solubilized in 50 mM NaCl at protein/KOG molar ratios of 1:2 and then lyophilized using a freeze-dryer (FD-1-50, Bo Yikang Co. Ltd., Beijing, China). The lyophilized samples were then incubated at 50 °C and 75% relative humidity for 0–96 h in a humidity cabinet (model PR-1G, Tabai Espec Co., Tokyo, Japan). After that, the samples were collected, dispersed in 30 mL of 0.1 M NaCl and 40 mM phosphate buffer (pH 7.4) and then centrifuged at 8000 rpm for 20 min. The glycated samples were collected as a precipitate after washing and centrifuging five times. Finally, the samples were lyophilized and stored at −20 °C prior to further analyses.

2.5. Amino acid composition

The amino acid composition of the protein samples was determined by an automatic amino acid analyzer (M510, Waters, Milford, MA), using Pico-Tag column. The determination was carried out at 38 °C, at a detection wavelength of 254 nm and flow rate of 1.0 mL per minute. The samples were hydrolyzed with 6 M HCl for 24 h at 110 °C in a sealed tube. Results were expressed as percentage (%) of amino acid. The amino acid tryptophan was not determined.