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Microstructure and inter-molecular forces involved in gelation-like protein hydrolysate from neutrase-treated male gonad of scallop (*Patinopecten yessoensis*)

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ABSTRACT

Neutrase-hydrolyzed male gonad of scallop (*Patinopecten yessoensis*) displayed a gelation-like profile, whereas the underlying mechanism was unknown. In this study, the microstructure and inter-molecular forces involved in the gel network of scallop (*P. yessoensis*) male gonad hydrolysates (SMGHs) were investigated, by monitoring changes of its gel strength in presence of different chemicals. The results showed that SMGHs chiefly consisted of peptides both below 1000 Da (46.86%) and above 10,000 Da (30.30%), and exhibited a porous, three-dimensional network, with firmness of 40.49 ± 1.96 g, cohesiveness of 479.02 ± 37.04 g·s and adhesive force of 23.69 ± 1.92 g, which were similar with that of 1.0–1.5% guar gum, 1.5% carrageenan, 1.0–2.0% xanthan gum and 0.5–1.5% gelatin. Addition of urea, and propylene glycol (PG) decreased the gel strength of SMGHs with the increase of concentration, and only a very weak gel was observed when the concentration of urea reached 8 M. The overall gel properties of SMGHs were improved in the presence of 0.3 M NaCl, KCl, CH_3COONa and NaSCN ($p < 0.05$). However, elevated salt concentration led to decreased gel properties of SMGHs, especially the inhibiting effect of NaSCN at 3 M. In addition, inclusion of dithiothreitol (DTT), 2-mercaptoethanol (2-ME), and *N*-ethylmaleimide (NEM) also diminished the gel properties at high concentration ($p < 0.05$), but not severely, and their addition did not change the gelation-like profiles of SMGHs. These results suggest that the gel network of SMGHs was primarily maintained by hydrophobic, electrostatic interactions and hydrogen bonds.

1. Introduction

Yezo giant scallop (*Patinopecten yessoensis*) is an economically important shellfish, widely cultured and landed in Eastern Asia. The main edible and processing part is adductor muscle, which has high nutritive value and is traditionally regarded as delicacy. During the processing of scallops, large amounts of the internal organs and hard tissues are usually discarded and underutilized as industrial wastes (Oyamada, Kaneniwa, Ebitani, Murata, & Ishihara, 2008). The quantity of scallop landings reaches about 1.4 million tons per year in China (Ministry of Agriculture of China, 2011, pp. 33–34). On a wet basis, scallops are composed of shell (52%), adductor muscle (13%), mantle lobe (9%), and gonad (ovary and testis, 3–9%). Thus, the internal organs and hard tissues such as shells, mid-gut glands, and gonads, accounting for more than 60% of the scallop body, are underutilized as wastes (Oyamada et al., 2008). However, these wastes contained many useful materials such as carotenoid (Suhnel, Lagreze, Ferreira, Campestrini, & Maraschin, 2009; Zheng et al., 2010), lipid (Zhou et al., 2010), lysozyme (Lee, Kim, & Kim, 2008), polysaccharide (Zhu et al., 2009), and mycosporine-like amino acid (Oyamada et al., 2008). To reduce the burden on the environment and increase the added value, new utilization approaches of these wastes are needed.

Abbreviations: SMGHs, scallop male gonad hydrolysates; PG, propylene glycol; 2-ME, 2-mercaptoethanol; DTT, dithiothreitol; NEM, *N*-ethylmaleimide; DH, degree of hydrolysis; MW, molecular weight.

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In recent years, preparation of protein hydrolysates by enzymatic hydrolysis from food by-products is growing, which has led to the production of many novel food ingredients with improved functionalities and biological activities such as antioxidant activity, anticancer activity, antihypertensive activity and antimicrobial activity (Di Bernardini et al., 2011; Hmidet et al., 2011; Jamdar et al., 2010; Jin, Wu, Zhu, & Ran, 2012; Klompong, Benjakul, Kantachote, & Shahidi, 2007; Sheih, Fang, Wu, & Lin, 2010). In this field, our previous studies have successfully obtained protein hydrolysates from abalone (*Haliotis discus hannai* Ino) viscera and sea urchin (*S. nudus*) gonads, which exhibited strong antioxidant capacity (Qin et al., 2011; Zhou et al., 2012). As scallop gonads contain high contents of protein and are also edible somewhere, they could be potential material for preparation of protein hydrolysates. In our attempts for preparing scallop (*P. yessoensis*) gonad hydrolysates, we found that neutrase-treated male gonad hydrolysate showed a unique characteristic of gelation, and the gelation-like protein hydrolysates of scallop male gonad exhibited improved functionalities (Jin, Wu, Zhu, Liu, & Xu, 2011; Jin et al., 2012), but the underlying mechanism of the gelation remains unknown.

Several reports showed that enzyme/protease-induced aggregation and gelation of proteins could occur, but are subjected to specificity of enzyme or substrate used. For instance, the hydrolysis of a whey protein isolate by alcalase or *Bacillus licheniformis* proteinase led to gelation (Doucet, Gauthier, Otter, & Foegeding, 2003; Doucet, Otter, Gauthier, & Foegeding, 2003; Ju, Otte, Madsen, & Qvist, 1995; Ju, Otte, Zakora, & Qvist, 1997). The gelation properties of sunflower protein hydrolysate obtained from trypsin have also been reported by Sanchez and Burgos (1997a,b). Creusot and Gruppen (2007) reviewed enzyme-induced gelation of protein, and summarized the possible mechanism of *B. licheniformis* protease and Alcalase-induced gelation. On one hand, these two enzymes both had glutamyl endopeptidase activity, responsible for peptide aggregation; on the other hand, they proposed that upon hydrolysis, peptides containing clusters of hydrophobic amino acids could link other peptides by hydrophobic interaction and thus form aggregation.

Generally, protein gelation is the cross-linking of its polypeptide chains to form a three-dimentional network, which contains initial denaturation to cause protein unfolding, protein–protein interactions and aggregation (Riebroy, Benjakul, Visessanguan, Erikson, & Rustad, 2009). It is chiefly maintained by different inter-molecular forces including hydrogen bonds, ionic attraction, hydrophobic interactions, disulfide bonds or a combination of the above (Riebroy et al., 2009; Sun & Arntfield, 2012). The involvement of different forces in formation of protein gels can be deducted from the effects of pH, salts, reducing agents and dissociating agents (Sun & Arntfield, 2012; Utsumi & Kinsella, 1985; Xiong & Kinsella, 1990). Some reagents have been proposed and used to investigate inter-molecular forces of protein gels, for example, urea and guanidine hydrochloride (GuHCl) can destabilize hydrogen bonds and hydrophobic interactions in protein (Sun & Arntfield, 2012; Xiong & Kinsella, 1990); dithiothreitol (DTT), β-mercaptoethanol (β-ME or 2-ME) and *N*-ethylmaleimide (NEM) has been used in sulfhydryl/disulfide interchange during protein gelation (Hua, Cui, Wang, Mine, & Poysa, 2005; Sun & Arntfield, 2012; Zou, Habermann-Rottinghaus, & Murphy, 1998). When electrostatics forces are involved in gel formation of protein, gel strength is affected by pH and salts (Britten & Giroux, 2001; O'Riordan, Mulvihill, Kinsella, & Morrissey, 1988; Sun & Arntfield, 2012; Takenaka, Arll, & Masui, 2011; Zou et al., 1998).

In spite of these chemical reagents can be used for targeting at inter-molecular forces of protein gel, many researches are mainly focused on heat-induced gelation of protein. To the best of our knowledge, no similar report using these chemicals for targeting at forces of enzyme-induced gelation could be found. The chemicals and reagents targeting at inter-molecular forces of protein gel provide a simple and feasible way of probing its gel properties (Sun & Arntfield, 2012; Utsumi & Kinsella, 1985; Xiong & Kinsella, 1990). Therefore, the objective of present work was to investigate the microstructure and inter-molecular forces in the gelation-like protein hydrolysates from neutrase-treated male gonad of *P. yessoensis*, with the hope of understanding its gelation mechanism.

2. Materials and methods

2.1. Materials and chemicals

Scallop (*P. yessoensis*) was provided by Hailin Aquatic Products Foodstuff Co., Ltd (Dalian, China) in March 2010. After dissection, the male gonads with milk-white color were collected, vacuum freeze-dried, crushed and the gonad powder was stored at −20 °C before use. Neutrase (*Bacillus subtilis* var. *amyloliquefaciens* strain 1398) was purchased from Pangbo Biological Engineering Co., Ltd (Nanning, China) and stored at 4 °C until it was used for the hydrolysis experiments. Gelatin, guanidine hydrochloride (GuHCl), sodium thiocyanate (NaSCN), urea, propylene glycol (PG) and dithiothreitol (DTT) were purchased from Sangon Biotech Co., Ltd (Shanghai, China); xanthan gum and *N*-ethylmaleimide (NEM) were purchased from Hualan Chemistry Co., Ltd (Shanghai, China); guar and carrageenan were purchased from Aladdin Chemistry Co., Ltd (Shanghai, China). All other reagents were of analytical grade.

2.2. Preparation of scallop male gonad hydrolysates (SMGHs)

Lyophilized male gonad of *P. yessoensis* (SMG) powder was mixed with deionized water at a ratio of 1:20 (*w*/*v*). Several reports have indicated that heat-denatured proteins (higher than 70 °C) are susceptible to enzymatic hydrolysis (Bax et al., 2012; Mutilangi, Panyam, & Kilara, 1996). Therefore, the mixture was heated in boiled water for 10 min (material temperature reached to 95 °C), and designed as denatured gonad proteins (Jin et al., 2011, 2012). After cooling, the mixture was adjusted to a required pH using 0.5 M NaOH, and pre-incubated at 50 °C for 10 min. The enzymatic hydrolysis was initiated by adding neutrase at a dose of 3000 U/g protein with continuously stirring at 50 °C. The pH was maintained at 7.0 by adding 0.5 M NaOH during the reaction. After 60 min of hydrolysis, the enzyme was inactivated by heating at 95 °C for 10 min, and the hydrolysates of scallop male gonad (SMGHs) were immediately poured into 100-mL beakers (approximately 50 mL each). At the same time, chemicals (salts, urea, GuHCl, PG, DTT, 2-ME and NEM) were also immediately dispersed and soluble in SMGHs at different concentrations, respectively. The beakers were transferred into a chromatography freezer at 4 °C overnight prior to measurement of gel properties.

2.3. Measurement of gel properties

The gel properties of SMGHs were evaluated with a TA-XT2i Texture Analyzer (Stable Micro Systems, Godalming, UK). Briefly, sample was compressed using a type A/BE acrylic cylinder probe. The equipment was set as follow: pre-test speed: 1.0 mm/s; test speed: 1.0 mm/s; post-test speed: 10 mm/s; compression distance: 15 mm; trigger force: 0.5 g. Parameters like firmness (g), cohesiveness (g·s) and adhesive force (g) of the sample were immediately obtained through the station software after the test was finished. For each sample, the test was performed in three independent experiments.