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Heat-induced denaturation and aggregation of actomyosin and myosin from yellowcheek carp during setting

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ABSTRACT

Thermal inactivation kinetics of Ca^{2+}-ATPase, changes in turbidity and rheological properties of actomyosin and myosin from yellowcheek carp during setting at different temperatures were investigated. Actomyosin and myosin setting at 40–45 °C exhibited greater extent and more rapid Ca^{2+}-ATPase inactivation compared to at 25–30 °C. Formation of protein aggregates and three-dimensional network structures of actomyosin and myosin at 25–30 °C was far less than those at 40–45 °C. Thermal stability of actomyosin was higher than that of myosin as revealed by its higher activation energy for the inactivation of Ca^{2+}-ATPase. Actomyosin and myosin also exhibited different dynamic rheological properties, especially when the setting temperatures were 40 and 45 °C.

1. Introduction

Among fish muscle proteins, myofibrillar proteins, especially actomyosin and/or myosin play the major roles in the gelation properties of fish meat (Yongsawatdigul & Park, 2003). Thermal gelation properties of surimi are generally governed by denaturation and aggregation behaviors of actomyosin and myosin (Hermansson, 1979; Yongsawatdigul & Park, 2003). It is known that the denaturation and aggregation properties of actomyosin and myosin are influenced by intrinsic factors (fish species, freshness of fish, fishing season, etc.) and extrinsic factors (protein concentration, pH, temperature, ionic strength, type of salts present, pressure, additives, etc.) (Hayashi, Azuma, Koseki, & Konno, 2007; Takahashi, Yamanoto, Kato, & Konno, 2005; Tao, Kobayashi, Fukushima, & Watabe, 2005). Therefore, in order to manipulate the gel forming properties of surimi, it is necessary to understand the denaturation and aggregation behaviors of actomyosin and myosin.

Gel properties of surimi could be improved by subjecting surimi sol to setting at a lower temperature (below 40 °C) prior to cooking at a higher temperature (Benjakul, Visessanguan, & Chantarasuwan, 2004; Liu, Zhao, Xie, & Xiong, 2011). Setting phenomenon of fish surimi can be attributed to the formation of the cross-linking between myosin heavy chains catalyzed by endogenous transglutaminase (endo-TGase) (Benjakul et al., 2004). Moreover, the unfolding of α-helix and the formation of disulfide-bonds and hydrophobic interactions were also responsible for the aggregation of actomyosin and/or myosin during setting (Hemung & Yongsawatdigul, 2005; Ogawa, Nakamura, Horimoto, An, Tsuchiya, & Nakai, 1999a, 1999b). Setting response of actomyosin and myosin varied with the fish species and setting temperatures, and might be determined by their thermal stability.

Yellowcheek carp (*Elopichthys bambusa*) is considered as one of the best freshwater fish materials for high-grade surimi-based products by Chinese chefs, especially in inland of China. Kamaboko and fish ball produced from yellowcheek carp possessed superior gel-forming ability, good flavor and mouthfeel. Currently, its wild populations have declined to a threatening level. However, captive breeding and artificial cultivation of yellowcheek carp have developed rapidly. Large-scale production of yellowcheek carp demands effective ways of preservation and processing. However, the application of yellowcheek carp into surimi production industry is scarce. In order to utilize efficiently yellowcheek carp there is a need to understand the physicochemical properties of its proteins. In our previous studies with the dynamic rheological properties of actomyosin from yellowcheek carp and grass carp, we found that the thermal gelation profiles of yellowcheek carp actomyosin and grass carp actomyosin solutions were remarkably different, especially as heating proceeded from 10 to 45 °C (Ding, Liu, Rong, Zhao, & Xiong, 2012). These findings have encouraged us to investigate the thermal stability and the response to setting of actomyosin

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and myosin from yellowcheek carp. The objective of this study was to investigate the heat-induced denaturation and aggregation properties including thermal inactivation kinetics of Ca^{2+}-ATPase, changes in turbidity and rheological properties of actomyosin and myosin from yellowcheek carp during setting at various temperatures.

2. Materials and methods

2.1. Materials

Yellowcheek carp (*Elopichthys bambusa*) with a weight of 3500–4500 g/fish were caught off the Danjiang reservoir, Hubei province in China in September in 2010. The fish was headed and gutted. The white muscle was separated manually from skin and bone, and chopped finely. The minced meat was mixed with 50% (w/w) glycerol to prevent actomyosin denaturation, and then stored at −80 °C until use. All chemicals used in this work were of analytical grade.

2.2. Myosin preparation

Myosin was extracted according to the method of Park and Lanier (1989) with some modifications. All solutions used for the myosin preparation were kept cold at 4 °C to minimise proteolysis and protein denaturation. After thawing at 4 °C for 3–4 h, fish mince was centrifuged at 9820*g* for 10 min at 4 °C using a refrigerated centrifuge (Avanti J-26 XP Centrifuge; Beckman Coulter, Fullerton, CA, USA), and the supernatant was discarded. Then the fish mince was mixed with 10 volumes of solution A (0.10 M KCl, 0.02% sodium azide and 20 mM Tris–HCl buffer, pH 7.5) and homogenized for 1 min using an inline dispersing homogenizer (Model FJ-200, Shanghai specimen and models factory, China). The mixture was incubated for 15 min at 4 °C and then centrifuged at 2455*g* for 10 min. The supernatant was discarded. The precipitates was suspended with 5 volumes of solution B (0.45 M KCl, 5 mM β-mercaptoethanol, 0.2 M $Mg(COO)_2$, 1 mM EGTA and 20 mM Tris–maleate buffer, pH 6.8), mixed with adenosine triphosphate (ATP) to a final concentration of 5 mM and incubated for 60 min at 4 °C. The mixture was then centrifuged at 12,000*g* for 10 min. The resulting supernatant was diluted with 5 volumes of 1 mM $KHCO_3$ and kept at 4 °C for 15 min. Then the mixture was centrifuged at 12,000*g* for 10 min and the pellet was re-suspended with 2.5 volumes of solution C (0.5 M KCl, 5 mM β-mercaptoethanol 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. The mixture was kept overnight at 4 °C prior to centrifugation at 12,000*g* for 15 min. The myosin pellet was dissolved in 0.5 M NaCl-20 mM Tris–HCl buffer (pH 7.0) and then centrifuged at 10,000*g* for 10 min. The supernatant was stored at 4 °C and used within 2 days of preparation. Myosin purity was determined using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) (Laemmli, 1970). The electrophoretic profile of the extracted myosin was illustrated in Fig. 1. The purity of the myosin samples was greater than 92% as determined by densitometry (Gel Logic 200 Imaging System, Kodak Co., USA), although a little actin did remain. The extracted myosin was stored at 4 °C and used within 2 days of preparation. The protein concentration was determined by Lowry method (Lowry, Rosebrough, & Randall, 1951) using serum albumin as standard.

2.3. Actoymyosin preparation

Actomyosin from yellowcheek carp was prepared from mince according to a modified procedure of Ogawa et al. (1999a, 1999b) with some modifications as described earlier by Ding et al. (2012). The extracted actomyosin was stored at 4 °C and used within 2 days of its preparation. Fig. 1 illustrates the electrophoretic profile of the extracted actomyosin. The purity of the actomyosin samples was greater than 96% as determined by densitometry (Gel Logic 200 Imaging System, Kodak Co., USA). The extracted actomyosin was stored at 4 °C and used within 2 days of preparation. The protein concentration was determined by Lowry method (Lowry et al., 1951) using serum albumin as standard. The values were the means of three measurements.

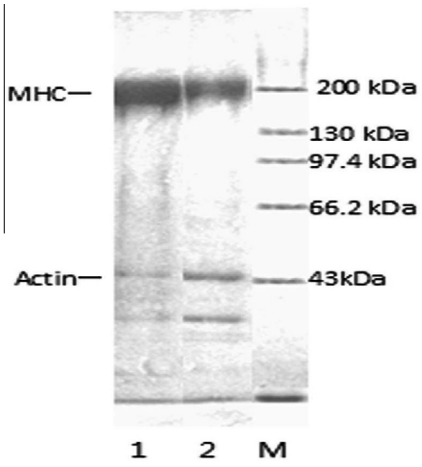

Fig. 1. SDS–PAGE of actomyosin and myosin from yellowcheek carp 1-myosin; 2-actomyosin; M-molecular weight markers.

2.4. Measurement of Ca^{2+}-ATPase activity

Five milliliters of myosin or actomyosin were incubated at different temperature (25, 30, 35, 40, 45 °C) for definite times, the temperatures of protein solutions in the test tube were monitored by an electronic thermometer with digital indication, and the designated temperatures were obtained within 1 min for all the samples. And then the samples were immediately cooled in ice water and assayed immediately at 25 °C. The sample was equilibrated at 25 °C prior to determination of Ca^{2+}-ATPase activity by the method by Ko, Tanaka, Nagashima, Taguchi, and Amano (1991). Ca^{2+}-ATPase activity was assayed in a medium of 0.5 M KCl 25 mM Tris–maleate (pH 6.8), 5 mM $CaCl_2$ and 1 mM ATP at 25 °C for 10 min after 2 min pre-incubation. And TCA solution was added to the mixture to stop the ATPase reaction. Liberated inorganic phosphate was analyzed by the method of Fiske and Subbarow (1925). The Ca^{2+}-ATPase activity was expressed as μmoles inorganic phosphate released/mg protein/min. A blank solution was prepared by adding chilled trichloroacetic acid prior to the addition of ATP.

2.5. Ca^{2+}-ATPase inactivation parameters

The kinetic data on the inactivation of myosin Ca^{2+}-ATPase were initially analyzed using a conventional first-order model (Hashimoto, Kobayashi, & Arai, 1982; Takahashi et al., 2005), i.e.,

$$\log[C/C_0] = -(K_D/2.303)t \quad (1)$$

where C is the mean residual enzyme activity at time t(s), C_0 the mean initial enzyme activity and K_D the inactivation rate constant (s^{-1}) at a given temperature. The values of K_D were obtained from the regression of log $[C/C_0]$ versus time as −slope/2.303. The temperature dependence of the rate constant K_D is often expressed by