Available online at www.sciencedirect.com
ScienceDirect
LWT 40 (2007) 1316–1320
LWT
www.elsevier.com/locate/lwt

Changes in conformation and sulfhydryl groups of tilapia actomyosin by thermal treatment

Wen-Ching Ko[a], Chi-Cheng Yu[b], Kuo-Chiang Hsu[c,*]

[a]Department of Bioindustry Technology, Da-Yeh University, 112 Shan-Jiau Road, Da-Tusen, Chang-hua 515, Taiwan, ROC
[b]Department of Food Science, National Chung-Hsing University, 250 Kuo-kuang Road, Taichung 402, Taiwan, ROC
[c]Department of Health Diet and Restaurant Management, Chung Shan Medical University, 110, Sec. 1, Chien-Kuo N. Road, Taichung 402, Taiwan, ROC

Received 13 July 2006; received in revised form 20 September 2006; accepted 23 October 2006

Abstract

Actomyosin (AM, 5 mg/ml) extracted from ordinary (white) muscle of tilapia (*Orechromis niloticus*) was subjected to thermal treatments (25–95 °C, 10–60 min) to investigate the changes of sulfhydryl groups and conformation. Aggregates of AM were found by the thermal treatments at 42 and 45 °C, and the aggregates could be removed by centrifuging at 15,000*g* for 5 min. Otherwise, the AM aggregates induced by the other thermal treatments beyond 35 °C were still soluble in 0.6 mol/l KCl-20 mmol/l Tris-maleate buffer (pH 7.0) even after centrifugation. Reactive sulfhydryl groups (R-SH) contents of AM showed the greatest amount in this study by 42 and 45 °C treatments, and those decreased almost 50% by heating at 95 °C. Total sulfhydryl groups (T-SH) contents of AM decreased with elevating temperatures. This study revealed that thermal treatments beyond 45 °C induced AM to form a cluster of aggregates with noncovalent bonds; however, those beyond 75 °C induced AM to aggregate mostly attributed by disulfide bonds. Also, thermal treatments at different temperatures would produce fish protein-related products with various characteristics.

Keywords: Actomyosin; Thermal treatment; Conformation; Sulfhydryl group

1. Introduction

Fish muscle proteins are typically heat-gelled in the manufacture for surimi seafood products. Functional and textural characteristics of fish muscle proteins depend mainly on the myofibrillar proteins (Montecchia, Roura, Roldan, Perez-borla, & Crupkin, 1997) due to its collagen content being lower than mammalian muscle (Hartshorne, Barns, Parker, & Fuchs, 1972). Actomyosin (AM) is the major protein in myofibrils and mainly contributes the gelation properties of fish proteins. Then, protein–protein interactions, known as association, aggregation and polymerization, on AM are dependent upon temperature, pH and the type of AM used (Deng, Toledo, & Lillard, 1976). Protein–protein interactions lead to the changes in the secondary and tertiary structures of the protein molecule. The change of AM conformation correlates with exposure of functional groups such as sulfhydryl groups and hydrophobic groups (Sano, Ohno, Otsuka-Fuchino, Matsumoto, & Tsuchiya, 1994), physico-chemical properties as ATPase activity (Samejima, Hashimoto, Yasui, & Fukazawa, 1969) and superprecipitation (Wolfe & Samejima, 1976) and gel strength (Ogawa, Kanamaru, Miyashita, Tamiya, & Tsuchiya, 1995). However, according to the changes of reactive sulfhydryl groups (R-SH) and total sulfhydryl groups (T-SH) contents in AM, we can understand more about the conformation change and disulfide bonds formation. Moreover, the observation of AM by transmission electron microscopy (TEM) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis was more helpful to realize the aggregation and polymerization of AM.

Some of the sulfhydryl groups of AM exist deeply inside its molecules, and disulfide bonds are formed due to the augmented interaction of intra- and inter-proteins by extrinsic energy and the change of environment. More disulfide bonds are formed due to more sulfhydryl groups

*Corresponding author. Tel.: +886 4 24730022x11836; fax: +886 4 23248188.
E-mail address: kchsu@csmu.edu.tw (K.-C. Hsu).

0023-6438/$30.00
doi:10.1016/j.lwt.2006.10.002

of AM exposed (Monahan, German, & Kinsella, 1995). Therefore, an increase in R-SH contents detected probably shows the protein unfolding; a decrease in T-SH contents shows that disulfide bonds are formed. The polymerization of protein attributes to disulfide bonds formation, which occurs between wheat glutenins (Ewart, 1968), soy protein (Saio, Kajikawa, & Watanabe, 1971), whey protein (Ewart, 1968) and fish protein (Gilleland, Lanier, & Hamann, 1997). Moreover, disulfide bonds formation is significantly affected by variously heating conditions (Li-Chan, 1983).

Tilapia muscle proteins are hard to proceed to setting progress, and the gel strength of tilapia meat paste induced by thermal treatments is weaker than that of other species (Jao, Ko, Hwang, & Hsu, 2006; Ko, Tanaka, Nagashima, Taguchi, & Amano, 1990). In this study, tilapia, used as the material focusing on its strong replication and obtained with live round fish, was adopted to extract AM from its ordinary muscle. Our objective was to investigate the changes of AM conformation as well as total and free sulfhydryl content of AM by thermal treatment ranging from 25 to 95 °C.

2. Materials and methods

2.1. Materials

Live tilapia (*Orechromis niloticus*) weighing about 600 g, were purchased from a local retail store in Taichung City. They were kept in ice and dissected immediately to obtain the ordinary muscles. The ordinary muscles were then minced in a chopper with a hole size of 4 mm and used to extract AM in 5 min. All chemicals were of reagent grade.

2.2. Preparation of AM

AM was extracted from minced ordinary muscle with 2.5 vol. of Weber–Edsall solution (0.6 mol/l KCl, 0.04 mol/l $NaHCO_3$, 0.01 mol/l Na_2CO_3) for 4 h with slow stirring at 4 °C (Ko, Tanaka, Nagashima, Taguchi, & Amano, 1991). The extract was filtered through gauze and the filtrate was diluted with 2 volumes of cold water. The precipitate collected by centrifuging at 6000*g* for 10 min was added with 2 mol/l KCl until the solution contained 0.6 mol/l KCl, and then centrifuged at 10,000*g* for 30 min. The precipitation–dissolution step was repeated once and the supernatant thus obtained was dialysed against 0.6 mol/l KCl–20 mmol/l Tris-maleate buffer (pH 7.0) at 4 °C overnight. AM thus obtained was adjusted to a protein concentration (determined by the biuret method) of 5 mg/ml for the following thermal treatments.

2.3. Thermal treatment

Five milliliters of tilapia AM solution was sealed in a 6 ml tube and then heated between 25 and 95 °C for 10–60 min in a water bath. After that, AM solutions were cooled in ice for 15 min and used for the following analyses immediately.

2.4. TEM observation

After thermal treatments, each AM solution was diluted with 0.6 mol/l KCl-20 mmol/l Tris-maleate buffer (pH 7.0) to 0.25 mol/l (Hsu & Ko, 2001). A drop of the sample was fixed for 2 min upon carbon-coated copper 400-mesh grids, which were rendered hydrophilic by glow discharge and rinsed with several drops of Tris-maleate buffer. After blotting away, the sample was negatively stained with 50 ml/l uranylacetate bihydrate in the Tris-maleate buffer (pH 7.0) for 20 s. After blotting away, the samples were washed with Tris-maleate buffer to eliminate uranylacetate bihydrate. At an accelerating voltage of 80 kV, the samples were viewed through a JEOL JEM-1200EX II TEM (× 50,000). Five or more fields were observed and photographed on film for each sample.

2.5. SDS-PAGE analysis

SDS-PAGE was done with separating gels of 100 ml/l polyacrylamide and stacking gels of 50 ml/l polyacrylamide (Weber & Osborn, 1969). Stacking gels were used to make all the samples through into the gels, and separating gels were used to separate the samples mainly attributed by molecular size. Each sample was centrifuged at 15,000*g* for 5 min to remove insoluble protein. The supernatant was added to the same volume of buffer containing 20 ml/l SDS–8 mol/l urea–20 ml/l mercaptoethanol–20 mmol/l Tris–HCl (pH 8.0) and 2 drops of tracking dye (0.01 g bromophenol/10 ml glycerol), which was incubated for 5 min at 100 °C. About 8 μl of the treated sample was applied to the stacking gels which were run at 80 and 140 V. Myosin, β-galactosidase, phsphorylase B, bovine albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme were used as protein standards.

2.6. Measurement of reactive sulfhydryl (R-SH) content

R-SH content was determined by using Ellman's reagent [5′,5′-dithio-bis(2-nitrobenzoic acid) (DTNB)] (Janatova, Fuller, & Hunter, 1968). About 0.1 ml of each sample solution was added to 2.9 ml of 6 mmol/l EDTA–0.1 mol/l KH_2PO_4 phosphate buffer (pH 4.0), which was mixed and added to 0.02 ml of 10 mmol/l DTNB. After reacting for 5 min at room temperature, the absorbance at 412 nm was measured with Hitachi spectrophotometer U-2000 (Ellman, 1959). SH content was calculated as mmol/l. Analyses were conducted for triplicate and 10 samples for each replicate.

2.7. Measurement of total sulfhydryl (T-SH) content

Total SH content was determined by using Ellman's reagent (Buttkus, 1971; Ellman, 1959). About 0.1 ml of