Food Chemistry 240 (2018) 346–353

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The effect of oxidation on the structure of G-actin and its binding ability with aroma compounds in carp grass skeletal muscle

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ARTICLE INFO

Article history:
Received 3 February 2017
Received in revised form 4 June 2017
Accepted 13 July 2017
Available online 14 July 2017

Keywords:
Hydrogen peroxide
G-actin
Oxidative modification
Secondary structures
Adsorption capacity
Volatile compounds

ABSTRACT

To investigate the influence of oxidative modifications of G-actin on its binding ability with aroma compounds, the influence of H_2O_2 treatments on G-actin structure and the absorption for alcohols and aldehydes was investigated. Raman spectroscopy and scanning electron microscopy were used to evaluate structural changes of G-actin; GC–MS was used to analyze the binding with alcohols and aldehydes. Results showed that 0–5 mM H_2O_2 enhanced the absorption of G-actin toward alcohols involved in the formation of hydrogen bonds by increasing α-helix and carbonyl values. 0–1 mM H_2O_2 caused the release of aldehydes with decreased sulfhydryl sites. 1–20 mM H_2O_2 increased the retention of aldehydes, due to the increased hydrophobic sites by G-actin rebuilding and aggregating. The aggregated G-actin favoured the hydrophobic interactions with aroma compounds, forming the protein-aroma compound complex, thus enhancing the resultant binding ability, as evidenced by scanning electron microscopy and GC/MS analysis.

1. Introduction

Acceptability of foods mainly depends on their sensory attributes, in particular, their flavour. The quality of fish and meat products, a major source of protein of high biological importance, is highly influenced by their flavour perception. Protein oxidation is currently one of the most important research topics within food science (Zhang, Xiao, & Ahn, 2013). Reactive oxygen species (ROS) include superoxide anions, hydroxyl radicals, peroxyl radicals, hydrogen peroxide, nitric oxide and lipid oxidation products derived from free radicals, and play a role in oxidation of proteins during meat maturation, storage and industrial processing (Zhou, Zhao, Zhao, Sun, & Cui, 2014). The discovery that muscle proteins are susceptible to oxidative reactions, leading to potential deleterious effects on muscle foods, greatly promotes the interest for this issue (Mercier, Gatellier, & Renerre, 1995). Oxidative damage for muscle proteins includes the alteration of protein structure, peptide chain scission, the formation of amino acid derivatives and polymers, decrease in solubility and the change of functional properties, such as water-holding capacity, texture-forming ability and absorbing capacity of volatile compounds. Four oxidizing models (protein carbonylation induced by Cu^{2+}/systems, H_2O_2-activated metmyoglobin oxidizing system, iron-catalyzed oxidizing system and linoleic acid-oxidizing system) have been introduced to study the oxidation of myofibrillar proteins in muscle foods (Utrera, Armenteros, Ventanas, Solano, & Estevez, 2012). Hydrogen peroxide could cause the discoloration of muscle food by denaturing haem proteins to release free irons and haem groups or converting haem proteins to ferryl or perferryl radicals, depending on its concentration (Baron & Andersen, 2002). In addition, hydrogen peroxide could influence the texture and binding capacity of myofibrillar proteins to volatile compounds, since it can alter the cross-linking and polymerization by attacking the thiols sites of actin and myosin (DalleDonne, Milzani, & Colombo, 1995). However, it is still not clear how the hydrogen peroxide treatments change the structure of actin.

In recent years, several model solutions, protein homogenates and peptides from skeletal muscle have been introduced and developed in the field of retention and release of volatile compounds, such as myofibril proteins, actomyosin, sarcoplasmic proteins. Some factors can affect the absorption capacity of muscle proteins to volatile compounds, such as protein concentration, protein conformation (Pérez-Juan, Flores, & Toldrá, 2007a), volatile compounds nature (Zhou, Zhao, Su, & Sun, 2014), headspace concentration (Pérez-Juan, Flores, & Toldrá, 2008) and ionic strength of the medium. Numerous research studies discovered that myofibril proteins were affected by ROS and developed a gel net-

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http://dx.doi.org/10.1016/j.foodchem.2017.07.068
0308-8146/

work (Mariana Utrera & Estévez, 2012). Previous studies have shown that the concentration (0–25 μM) of hydrogen peroxide influenced the retention and adsorption of myofibril proteins to aroma compounds by changing the spatial structure of the proteins (Zhou et al., 2014). Alcohols and aldehydes are typical volatile flavour compounds in fish products, and they have a low threshold compared with other flavour compounds. Actin is the major component of myofibrillar proteins which could not develop a gel network. In the oxidation system, it is significantly different as the ROS attack sites between G-actin and myosin. Up to now, the effect of hydrogen peroxide on the adsorption capacity of G-actin and the specific mechanism has not been evaluated.

In the present work, the influence of H_2O_2 concentrations on the adsorption capacity of G-actin binding alcohols and aldehydes was determined; the surface hydrophobicity, carbonyl content, total and reactive sulfhydryl group levels, secondary structures and microstructure of G-actin were assessed.

2. Materials and methods

2.1. Materials

1-Pentanol, 1-hexanol, 1-octen-3-ol, 1-octanol, pentanal, hexanal, octanal, nonanal were obtained from Sigma-Aldrich (Sigma, USA). The purity of the standard flavour compounds was between 98 and 99.7%. Grass carps (Catenopharyngodon idella) were purchased from local Tesco (Zhejiang, China). Grass carps with a length range from 50 to 55 cm (bodyweight 1973 ± 147 g) were transported to the laboratory under ice. Then, muscle tissue was taken and minced, vacuum-packaged and frozen (−80 °C) until use.

2.2. Preparation of G-actin

G-actin was prepared as described previously Pérez-Juan, Flores, and Toldrá (2007b), with slight modification. Five grammes samples were homogenized with 35 ml of 0.1 M Tris-HCl containing 20 mM EDTA (pH 7.0) at 10,000 rpm for 3 * 10 s while cooled on the ice with a DY89-I high speed homogenizer (Scientz co., Ningbo, China) and centrifuged at 9150*g* by a refrigerated centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Changsha, China) for 30 min at 4 °C with three repetitions. The pellet was obtained in Hasselbach-Schneider solution (0.1 M potassium phosphate buffer at pH 6.4 with 0.6 M KCl, 1 mM $MgCl_2$, 10 mM $Na_4P_2O_7$ and 20 mM EGTA) and was precipitated by diluting 1/20 with deionised water at 11,700*g* for 30 min at 4 °C. Afterwards, the pellet was successively extracted with acetone (20 vol/g pellet) for 20 min at 4 °C, three times, and further filtration through Whatman paper. The sediment was dried with liquid nitrogen. Then the acetone powder was used for the extraction with buffer A (2 mM Tris-HCl at pH 8.0 and 0.5 mM β-mercaptoethanol, 0.2 mM $ATPNa_2$, 0.2 mM $CaCl_2$ and 0.005% NaN_3) with 20 ml/g acetone powder at different extraction times. The first extraction with buffer A was shaken by a Vortex-Genie Mixers (Vortex-Genie2, Scientific Industries, Inc., Bohemia, NY, USA) for 15 min, centrifuged at 10,400*g* for 20 min at 4 °C and the supernatants discarded. The second and the third extraction was kept under agitation for 45 min and centrifuged at 10,400*g* for 20 min at 4 °C. All of the supernatants were merged. Finally, the supernatants were centrifuged at 12,737*g* for 60 min at 4 °C and filtered through Whatman paper. The concentration of G-actin was calculated using bovine serum albumin as a standard and bicinchoninic acid protein assay kit (Thermo Scientific, MA).

2.3. Protein oxidation

Chemically induced oxidation of G-actin. The concentrations (1, 2, 5 and 30 mg/ml) of G-actin were prepared for the incubated assays of oxidation. G-actin solution was incubated at 37 °C for 3 h with a hydroxyl radical-generating system (Park, Xiong, & Alderton, 2007). The hydroxyl radicals were produced by adding H_2O_2 at various concentrations (0 mM, 1 mM, 5 mM, 10 mM and 20 mM). The G-actin solution without H_2O_2 was chosen as the control. To prevent further oxidation, butylated hydroxyl toluene (1 mM final concentration) was then added to the G-actin solution after H_2O_2 incubation for 3 h.

2.4. Determination of the surface hydrophobicity of G-actin

The surface hydrophobicity of G-actin was determined according to the method described by Yongsawatdigul and Park (2003), with slight modification. The 10 μl of 8 mM 1-anilino-8-napthalenesulfonate (ANS) in 0.1 M potassium phosphate buffer (pH 7.0) was added to 2 ml of 2 mg/ml G-actin solution and shaken by a Vortex-Genie Mixers (Vortex-Genie2, Scientific Industries, Inc., Bohemia, NY, USA) for 1 min. The control (without G-actin) consisted of the addition of 10 μl of 8 mM ANS in 0.1 M potassium phosphate buffer (pH 7.0) was added to 2 ml of 20 mM Tris-HCl buffer (pH 7.0). Samples with different concentrations of H_2O_2 and control were kept under dark condition at 37 °C for 10 min. Fluorescence intensity of the mixture was continuously monitored at 370 and 480 nm excitation and emission wavelengths, using a 96-Well black Plate Reader M200 (Tecan, Austria) against a blank of 20 mM Tris-HCl buffer (pH 7.0).

2.5. Determination of the content of carbonyls

The content of carbonyls was determined by the reaction with 2, 4 dinitrophenylhy-drazine (DNPH) using a 96-Well black Plate Reader M200 (Tecan, Austria)), as described by Oliver, Ahn, Moerman, Goldstein, and Stadtman (1987) and Zhou et al. (2014) with some modification. Briefly, 200 μl of 5 mg/ml protein suspension in 20 mM sodium phosphate buffer (pH 6.0) was mixed with 800 μl of 2 M HCl (control) or 10 mM DNPH in 2 M HCl and incubated at room temperature (25 °C) for 1 h. The DNPH-reacted samples after 10% trichloroacetic acid (TCA) precipitation were recovered by centrifugation at 8000*g* for 10 min and then washed three times with 1 ml of ethanol: ethyl acetate (1:1, v/v) to eliminate free DNPH. The final protein pellets were dissolved in 1.2 ml of 6 M guanidine HCl with 20 mM sodium phosphate buffer (pH 6.0) and then centrifuged at 8000*g* for 10 min. Protein concentration in the supernatant was calculated at 280 nm in the control using BSA in 6 M guanidine as standard. The carbonyl concentration was calculated using the absorption of 21.0 mM^{-1} cm^{-1} at 370 nm against the control for protein hydrazones.

2.6. The determination of total and reactive sulfhydryl groups

Total sulfhydryls were determined according to Yongsawatdigul and Park (2003) with some modification. Briefly, 9 ml of 50 mM potassium phosphate buffer (10 mM ethylenediaminetetraacetic acid, 0.6 M KCl, 8 M urea, pH 7.0) was added to 1 ml of G-actin (2 mg/ml). 0.5 ml of 0.2 mM 5, 5′-dinitrobis (2-nitrobenzoic acid) (0.1 M potassium phosphate buffer, pH 7.0) was added to 5 ml of the resultant mixture. The mixture was incubated at room temperature for 25 min. The absorbance was measured at 412 nm to calculate the total sulfhydryl groups using the extinction coefficient of 13,600 M^{-1} cm^{-1}. Reactive sulfhydryl