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Effects of oxidative modification on gel properties of isolated porcine myofibrillar protein by peroxyl radicals

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ABSTRACT

AAPH-derived (2,2′-azobis (2-amidinopropane) dihydrochloride) peroxyl radicals were selected as representative free radicals of lipid peroxidation to investigate the effects of oxidative modifications on isolated porcine myofibrillar protein structures as well as their rheological and gelling properties. Incubation of myofibrillar protein with increasing concentrations of AAPH resulted in a gradual increase ($p < 0.05$) in carbonyl content and SH → S–S conversion. Results from SDS-PAGE indicated that medium (~1 mM) and relatively high (>3 mM) concentrations of AAPH induced aggregation of myosin and denaturation of myosin, troponin and tropomyosin, respectively. These structural changes resulted in changes on gelation of myofibrillar protein. Low level protein oxidation (AAPH ≤ 0.5 mM) had no remarkable effect ($p > 0.05$) on the viscoelastic pattern of myofibrillar protein gelation. Moderate oxidative modification (AAPH ~ 1 mM) enhanced the water-holding capacity (WHC) and texture properties of gels, while further oxidation (AAPH > 3 mM) significantly reduced the gel quality.

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1. Introduction

Consumers are becoming more demanding about meat product quality. It is well accepted that the second attribute after meat color that affects on the consumer decision at the point of purchase meat products is meat texture in terms of product firmness, meat tenderness, and the fineness of meat grain (Kawahara, Ahhmed, Ohta, Nakade, & Muguruma, 2007). Formation of protein gels in processed muscle foods is one of the most important functionalities, which influences the texture and sensory characteristics of the final products (Acton, Ziegler, Burge, & Froning, 1982).

Myofibrillar proteins, contributing 55–65% of total muscle protein, or about 12% of skeletal muscle weight, are excellent gelling agents that are largely responsible for the textural and structural characteristics of meat products (Acton et al., 1982). The thermal gelation ability of myofibrillar proteins has been well documented in terms of factors influencing heat-induced gelation properties as well as rheological properties of myofibrillar protein. The majority focus on how myosin and actin in different animal species react, and how physicochemical properties, the interactions of myofibrillar proteins with other additives, influence gel texture and rheological properties of protein itself (Sun & Holley, 2011).

With the increasing understanding that food proteins are sources and targets for reactive oxygen species (ROS), protein oxidation is currently one of the most important research topics within the Food Science field (Estévez, 2011; Lund, Heinonen, Baron, & Estevez, 2011; Zhang, Xiao, & Ahn, 2013). In recent years, how protein oxidation which occurred during handling, storage or processing of meat and meat products affected their texture and gelation properties has attracted more attention and brought about a new research area (Estévez, 2011; Xia, Kong, Xiong, & Ren, 2010). Studies have shown that the structure of myofibrillar proteins can be readily modified and altered by ROS. The protein carbonylation, principally induced by Cu^{2+}/systems, leads to a fast and severe loss of myofibrillar protein functionality, including impaired gelling capacities (Utrera & Estévez, 2012). While Xiong, Blanchard, Ooizumi, and Ma (2010) suggested that in hydroxyl radical and ferryl-generating systems, mild oxidation promoted protein network formation and enhances gelation of myofibrillar protein involving disulfide linkages. Moreover, the interaction between protein and lipid oxidation product (MDA, HNE) also had a significant influence on protein gelation (Benjakul, Visessanguan, Thongkaew, & Tanaka, 2005). It is well documented that changes of biochemical and functional properties in oxidized myofibrillar proteins are dependent upon the specific oxidizing systems (Martinaud et al., 1997; Park, Xiong, Alderton, & Ooizumi, 2006). In real meat system, it contains organic peroxides like lipoperoxides resulting from polyunsaturated fatty acid oxidation (Promeyrat, Daudin, Astruc, Danon, & Gatellier, 2013). And it is highly unlikely that the oxidation/peroxidation of lipids and proteins takes place independently. As reported, the onset of lipid oxidation would take place faster (Estévez, Kylli, Puolanne, Kivikari, & Heinonen, 2008; Mercier, Gatellier, Viau, Remignon, & Renerre, 1998) and hence, it is more likely that lipid-derived radicals and hydroperoxides promote protein oxidation (Mercier et al., 1998). And among all the free radicals generated during propagation of lipid peroxidation, peroxyl radicals (*ROO•*) are key chain-propagating species and reaction

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of peroxyl radicals constitutes propagation process in protein chain oxidation reactions by abstracting hydrogen atoms from protein (Duggan, Rait, Platt, & Gieseg, 2002). Thus, it is necessary to firstly figure out the potential effect of *ROO*•-induced protein oxidation on myofibrillar protein before better understanding the effect of lipid peroxidation on meat product.

In this study, thermal decomposition of hydrophilic azo compounds of 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) was used to generate peroxyl radicals (*ROO*•) at a known and constant rate (Liu, Yu, & Liu, 1999). And the experiment was conducted to investigate the effects of protein oxidation by AAPH-derived peroxyl radicals on protein structure as well as on rheological and gelling properties of myofibrillar proteins in order to improve our understanding of the implications of lipid and protein oxidation in meat products.

2. Materials and methods

2.1. Samples and materials

M. longissimus dorsi was purchased from a local commercial abattoir (Guangzhou, China) and the pigs (100–110 kg live weight) were slaughtered about 6 months of age following the operating procedures of pig-slaughtering GB/T 17236-2008 (Chinese National Standards). *M. longissimus dorsi* was removed within 12 h after slaughter. Fat was trimmed away and muscle was cut into cubes and frozen at −80 °C until use (used within 3 days). 2, 2′-Azobis (2-amidinopropane) dihydrochloride (AAPH) (CAS No. 2997-92-4) was purchased from Shanghai BOAO Biochemical Co. (Shanghai, China). All the other chemicals were of analytical or better grade.

2.2. Preparation of myofibrillar proteins

Myofibrillar proteins were prepared according to the method of Martinaud et al. (1997). Briefly, 10 g of raw muscle (multiple pieces) was homogenized in 100 mL of a sodium phosphate buffer solution (20 mM) at pH 6.5 containing 150 mM NaCl, 25 mM KCl, 3 mM $MgCl_2$, and 4 mM EDTA, to which a protease inhibitor (1 mM PMSF) had been added, using an Ultra-Turrax homogenizer (Beijing Jingke Huarui Instrument Co. Ltd., Beijing, China) at 11,000 rpm for 1 min in an ice water bath. The homogenate was sieved through a sifter (mesh 250 μm, Hangzhou Sansi Instrument Co. Ltd., Hangzhou, China), and collagen was eliminated by filtration on the sifter. The extract was centrifuged at 2000 ×*g* for 15 min at 4 °C using a GL-21 M centrifuge (Xiang Yi Centrifuge Instrument Co. Ltd., Changsha, China). The pellet was washed twice with 100 mL of a 50 mM KCl solution at pH 6.4 and once with 100 mL of 20 mM sodium phosphate buffer at pH 6.0. The pellet was finally suspended in 20 mM phosphate buffer containing 0.6 M NaCl (pH 6.0) and the protein concentration was adjusted to designed concentrations by the Biuret method using BSA as a standard.

2.3. Incubation of myofibrillar protein with AAPH-derived peroxyl radicals

Myofibrillar protein (30 mg/mL of final concentration containing 0.5 mg/mL NaN_3) was incubated in the dark for 24 h at 37 °C in 20 mM phosphate buffer at pH 6.0 with AAPH (*ROO*• producer) at various concentrations (from 0 to 10 mM). Phosphate was used in this study because, contrary to other buffers commonly used for protein studies, it has no marked effects on protein oxidation (Davies, 1987).

To prevent subsequent oxidation, the mixture was immediately cooled to 0–4 °C by ice-bathing and centrifuged at 4000 ×*g* for 10 min (4 °C) to remove the oxidant. The pellet was further washed twice with distilled water. Samples subjected to SDS-PAGE (see below) were lyophilized.

2.4. Determination of carbonyl content

Carbonyl groups were detected by reaction with 2,4 dinitrophenylhydrazine (DNPH) to form protein hydrazones, and their content was estimated using the method of Oliver, Ahn, Moerman, Goldstein, and Stadtman (1987) with slight modifications (Sun, Cui, Zhao, Zhao, & Yang, 2011). The amount of carbonyl was expressed as nmol of DNPH equivalents/mg of protein using an absorption coefficient of 21 mM^{-1} cm^{-1} for protein hydrazones.

2.5. Determination of total sulfhydryl groups (SH) and disulphide groups (S–S)

The SH group levels were determined by a modification of Ellman's method using 2,2′-dithiobis(5-nitropyridine) DTNP according to Sun et al. (2011). Results were expressed in nmol of SH or S–S per milligram of protein.

2.6. SDS-PAGE electrophoresis

Myofibrillar protein samples were subjected to SDS-PAGE (Flores et al., 2006) to evaluate changes during oxidation. Three milligrams of the lyophilized samples was mixed with 500 μL of 50 mM Tris buffer, pH 6.8, containing 8 M urea, 2 M thiourea, 5% of β-mercatoethanol, 3% (w/v) SDS and 0.05% bromophenol blue. The mixture was heated at 100 °C for 5 min, centrifuged (10,000 ×*g*, 10 min, 4 °C) and used for electrophoresis. Electrophoresis was run using a Mini-PROTEAN 3 Cell apparatus (Bio-Rad Laboratories, Hercules, CA, USA). An aliquot of 10 μL of samples was loaded to each well in the 5% polyacrylamide stacking gel, and individual proteins were separated in the 12% resolving gel.

Table 1
Physicochemical changes of myofibrillar protein upon the oxidation by *ROO*• generated from AAPH (mean values ± SEM[A]).

Concentration of AAPH added	Carbonyl (nmol/mg protein)	SH (nmol/mg protein)	S–S (nmol/mg protein)
0 mM (fresh)	1.53 ± 0.17^{a}	72.74 ± 3.21^{e}	7.68 ± 0.54^{b}
0 mM	4.37 ± 0.55^{b}	63.31 ± 0.06^{d}	5.35 ± 1.17^{a}
0.2 mM	4.49 ± 0.54^{b}	63.82 ± 0.31^{d}	6.36 ± 0.83^{a}
0.5 mM	4.50 ± 0.48^{b}	$60.84 \pm 0.13^{c,d}$	7.89 ± 0.28^{a}
1 mM	4.92 ± 0.21^{b}	58.58 ± 1.77^{c}	12.46 ± 0.98^{c}
3 mM	5.62 ± 0.35^{c}	$60.59 \pm 0.25^{c,d}$	13.64 ± 0.54^{c}
5 mM	5.92 ± 0.05^{c}	54.85 ± 1.82^{b}	17.19 ± 0.84^{d}
10 mM	7.07 ± 0.21^{d}	49.68 ± 0.56^{a}	20.90 ± 1.02^{e}

[A]Standard deviation obtained from measurement in triplicate.
[a–e]Means in the same column with different superscript letters are significantly different ($p < 0.05$).

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