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Biochemical and physicochemical properties of thermally treated natural actomyosin extracted from normal and PSE pork *Longissimus* muscle

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ABSTRACT

Biochemical and physicochemical properties of thermally treated natural actomyosin (NAM) from normal and pale, soft and exudative (PSE) pork were studied. About 37% and 25% of available sulphydryl groups formed disulphide bonds or other permanent chemical bonds at 70 °C in NAM from normal and PSE pork, respectively. Surface hydrophobicities of NAM from normal and PSE pork at 70 °C were 3.6 and 2.4 times greater than that at 40 °C. About 90% of the α-helical structure of NAM was lost by heating to 70 °C. The temperature at maximum α-helical content decline of NAM was in accordance with the peak 3 thermal transition obtained by differential scanning calorimetry and the lowest storage modulus (G') during thermal rheology. NAM from normal pork underwent aggregation with a higher extent of hydrophobic interaction and disulphide bonds, higher temperatures at maximum velocity for conformational change and unfolding than that from PSE pork. As a consequence, NAM from normal pork had superior rheological properties.

1. Introduction

Pork with pale, soft and exudative (PSE) characteristics continues to be a significant meat quality problem in the pork industry (Barbut et al., 2008). PSE meat has a pale colour, soft texture and low water-holding capacity, resulting in significant economic losses (Torley, D'Arcy, & Trout, 2000; Wang, Pato, & Shand, 2005). It is well-known that meat gels from PSE meat have inferior textural qualities compared with meat gels from normal meat. However, the factors influencing impaired gelation of PSE pork are still not fully understood.

Knowledge of the biochemical and physicochemical basis of thermally-induced gelation of muscle proteins is important to muscle foods because it affects sensory, textural and water-holding properties of comminuted meat products (Baier & McClements, 2003). It has been proposed that the thermal formation of protein gels involves a three-step procedure, i.e., protein denaturation, aggregation and coagulation (Asghar, Morita, Samejima, & Yasui, 1985; Ziegler & Acton, 1984; Ziegler & Foegeding, 1990). The type of molecular interactions that stabilize the gel of different protein systems can differ. These interactions may consist of multiple hydrogen bonds and disulphide linkages or peptide bonds. The formation of gels may also involve electrostatic and hydrophobic interactions (Careche, Alvarez, & Tejada, 1995).

Thermal unfolding and gelation properties of meat proteins, including myosin, salt soluble proteins and myofibrillar proteins with different species, pH and ionic strength have been intensively studied (Egelandsdal, Fretheim, & Samejima, 1986; Levitsky et al., 1998; Liu, Foegeding, Wang, Smith, & Davidian, 1996; Robe & Xiong, 1994; Xiong & Blanchard, 1994a,1994b). Myosin plays a very important role in the gelation process in meat and meat products (Asghar et al., 1985). Actin is important in reinforcing the gel structure of myosin (Yasui, Ishioroshi, & Samejima, 1982). One of the limitations of studying myosin gelation is that extraction of myosin is very time consuming and myosin is sensitive to the extraction condition which may cause its structure to change. Secondly, studying myosin alone instead of myofibrillar proteins may not reflect the real situation in pork muscle since myosin is present as an actomyosin complex in full and post rigour meat. Natural actomyosin (NAM) can be extracted under mild conditions and is thought to represent the native state of proteins in postmortem pork meat. We have previously (Rathgeber, Boles, Pato, & Shand, 2002) reported that pork with a rapid postmortem pH decline produced meat gels with lower strain values compared to those from normal pork. The cause of poor gelation may be related to denaturation and degradation of myofibrillar proteins, but the biochemical and physicochemical properties of proteins from normal and PSE

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pork during heating and their gelation mechanisms are not fully understood.

The objectives of the present study were to investigate the gelation mechanism of NAM from pork muscle and investigate biochemical, physicochemical and rheological properties of NAM extracted from normal and PSE pork. Protein conformational changes were studied by examining alterations of tertiary and/or quaternary structures (surface hydrophobicity and surface sulphydryl and disulphide contents), while protein unfolding was monitored by determining alterations of secondary structures (α-helical content).

2. Materials and methods

2.1. Identification of normal and PSE pork and preparation of samples

Pork loins from seven PSE and five normal pigs were selected based on pH at 2 (pH_2) and 24 h postmortem (pH_u) and on amount of drip loss (normal: $pH_2 > 5.8$, $pH_u < 5.8$, drip loss < 5% and PSE: $pH_2 < 5.8$, $pH_u < 5.8$, drip loss > 5%). Details of animal selection are as described by Wang et al. (2005). At 72 h postmortem, a chop adjacent to 12–13th costae from the left side of each animal was removed and vacuum packed, frozen and stored at −30 °C. Frozen samples were utilised within two months.

2.2. Extraction of pork NAM

NAM was extracted according to the method described by Wang et al. (2005).

The protein content was determined according to the bicinchoninic acid (BCA) protein assay procedure provided by PIERCE (Dartmouth NS, Canada). Bovine serum albumin provided in the PIERCE BCA protein assay reagent kit was used as the standard. Measurements were done in duplicate.

2.3. Heat treatment of NAM

About 10 mL of NAM solution (5.0 mg protein/mL in 0.6 M KCl, 20 mM KH_2PO_4/K_2HPO_4 buffer, pH 7.0) from each sample was poured into nine sterile Falcon centrifuge tubes and covered with lids to avoid dehydration during heating in a water bath. The sample temperatures were raised at a constant rate of 1.3 °C/min from 22 to 90 °C. The temperature at the centre of the tube was monitored using a copper–constantan thermocouple. The NAM solution was immediately cooled in ice-water once the central temperature of samples reached 25, 40, 50, 55, 60, 70, 80 or 90 °C. An unheated solution kept at 4 °C served as a control. To minimise potential protein conformational changes after heat treatment, the heat-treated samples were kept at 4 °C and biochemical and physicochemical properties measured within 3 h.

2.4. Determination of α-helical content of NAM

The α-helical content of thermally treated NAM was measured according to Ogawa, Kanamaru, Miyashita, Tamiya, and Tsuchiya (1995) with some modifications. NAM was diluted to 0.2 mg/mL with 0.6 M KCl, 20 mM KH_2PO_4/K_2HPO_4 buffer, pH 7.0. Circular dichroism (CD) spectra and ellipticities at 222 nm were obtained with a π*-180 circular dichroism spectrometer (Applied Photophysics Ltd., Surrey, UK) in a 1 cm path length quartz cell. A water-jacketed cell holder was used to maintain a constant temperature by circulating water at 4 °C. The instrument was calibrated by using D-10-camphorsulfonic acid ($[\theta]_{290.5}$ = 7800° cm^2 $dmol^{-1}$) before each measurement. The α-helical content was estimated as described by Wang et al. (2005).

2.5. Differential scanning calorimetry (DSC) of NAM

DSC analysis of NAM (20 mg/mL in 0.6 M KCl, 20 mM KH_2PO_4/K_2HPO_4 buffer, pH 7.0) from normal or PSE meat was performed on a TA modulated DSC thermal analyser (TA Instruments, New Castle, DE, USA). Each NAM solution (15–25 mg) was accurately weighed into aluminium pans which were hermetically sealed and then heated from 20 to 90 °C at a rate of 5 °C/min. A sealed pan containing 15 mg of the same buffer was used as a reference. Denaturation temperature (Td, also called peak transition temperature), and enthalpy of denaturation (ΔH) were computed from the thermograms using a Universal Analysis Program, Version 3.9A (TA Instruments, New Castle, DE, USA). Temperature calibration was done with indium as a standard. Each sample was analysed in duplicate.

2.6. Measurement of surface hydrophobicity of NAM

Protein surface hydrophobicity was determined with the fluorescent probe 1-anilinonaphthalene-8-sulfonic acid (ANS) according to Li-Chan, Nakai, and Wood (1985) as described by Wang et al. (2005). All fluorescence measurements were made with a SPEX Fluorolog 1680 double spectrometer (Jobin Yvon Inc., Edison, NJ, USA) with the excitation and emission wavelengths at 390 and 470 nm, respectively. The excitation and emission slit widths were 1 nm. The initial slope (S_0) of the plot of the fluorescent intensity against % protein concentration was calculated by linear regression analysis and used as an index of the protein surface hydrophobicity (S_0-ANS). To correct for instrumental fluctuations in fluorescence intensity, standardization was performed by measuring the fluorescent intensity of 10 μL ANS in 4 mL methanol and corrected to a value of 30.

2.7. Measurement of sulphydryl and disulphide contents in NAM

Total and surface sulphydryl (S-SH) (also called reactive sulphydryl) contents of heat-treated NAM were determined in the presence and absence of 8 M urea, respectively, by colorimetric assay using Ellman's reagent 5,5′-dithio-bis(2-nitrobenzoic acid). The sulphydryl plus disulphide (SH + SS) content of thermally treated NAM was measured by using 2-nitro-5-thiosulphobenzoate (NTSB) according to Damodaran (1985) with slight modifications as described by Wang, Liceaga-Gesualdo, and Li-Chan (2003). Disulphide content was calculated as the difference between (SS + SH) and total sulphydryl contents. Measurements were done in triplicate. The SH and SS contents were calculated from A_{412} values using an extinction coefficient of 13,600 M^{-1} cm^{-1} and expressed as moles SH per 10^5 g protein.

2.8. Assessment of turbidity of NAM

Turbidity was determined according to Liu et al. (1996) with modifications. The NAM solution after heat treatment was diluted to 0.5 mg/mL with 0.6 M KCl, 20 mM KH_2PO_4/K_2HPO_4 buffer, pH 7.0. Absorbance at 350 and 660 nm of the diluted protein solution was obtained using an UV/Vis spectrophotometer (Hewlett Packard 8453, Columbus, OH, USA) and expressed as absorbance of mg/mL of protein.

2.9. Dynamic rheological measurement of NAM

Dynamic rheological properties of NAM (20 mg/mL in 0.6 M KCl, 20 mM KH_2PO_4/K_2HPO_4 buffer, pH 7.0) from PSE and normal pork were measured by using an AR 1000 advanced rheometer (TA Instruments, New Castle, DE, USA), which was equipped with a parallel plate measuring system (40 mm diameter). The gap be-