Food Chemistry 234 (2017) 323–331

Contents lists available at ScienceDirect

Food Chemistry

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

Effect of mitochondrial apoptotic activation through the mitochondrial membrane permeability transition pore on yak meat tenderness during postmortem aging

Lin-Lin Wang [a], Ling Han [a,*], Xiu-Li Ma [a], Qun-Li Yu [a,*], Suo-Nan Zhao [b]

[a] College of Food Science and Engineering, Gansu Agricultural University, Lanzhou 730070, Gansu, China
[b] Haibei Institute of Animal Science, Xihaizhen 810200, Qinghai, China

ARTICLE INFO

Article history:
Received 5 September 2016
Received in revised form 25 April 2017
Accepted 29 April 2017
Available online 2 May 2017

Chemical compounds studied in this article:
Sucrose (PubChem CID: 5988)
Mannitol (PubChem CID: 6251)
EDTA (PubChem CID: 6049)
Hepes (PubChem CID: 23831)
JC-1 (PubChem CID: 5492929)
DCFH-DA (PubChem CID: 104913)
β-Mercaptoethanol (PubChem CID: 1567)
Cytochrome *c* (PubChem CID: 16057918)
DEVD-*p*NA (PubChem CID: 100953063)
Proteinase K (PubChem CID: 118855431)

Keywords:
Mitochondrial membrane permeability transition pore
Mitochondrial apoptosis
Cyclosporin A
Yak meat
Tenderness

ABSTRACT

The effect of membrane permeability transition pore dependent mitochondrial apoptotic activation on yak meat tenderness was investigated. Results indicate that MPTP opening increased significantly and the mitochondrial membrane potential decreased markedly in the early aging process ($P < 0.05$). Cytochrome *c* was released from the mitochondria to the cytoplasm via the MPTP in the early period. Meanwhile, the activation of procaspase-9 occurred earlier than that of procaspase-3. Cyclosporin A suppressed the MPTP opening, depolarization of the mitochondrial membrane potential, activities of caspase-9 and caspase-3, apoptosis rate, myofibril fragmentation index, reactive oxygen species generation, and Ca^{2+} levels. These results demonstrated that MPTP mediated the release of cytochrome *c* in the mitochondrial apoptotic pathway. Furthermore, yak meat tenderness was improved by mitochondrial apoptotic pathway during aging. MPTP opening may be influenced by the ROS generation and Ca^{2+} overloading in yak meat during postmortem aging.

1. Introduction

The postmortem tenderization of muscle is a complex process that improves meat quality, such as color, flavor, texture, juiciness, and tenderness (Ouali et al., 2006; Zhang et al., 2013). Among these properties, tenderness is a fairly important contributory factor for consumers. This factor is mainly regulated by caspases and other endogenous proteolytic enzymes (Koohmaraie & Geesink, 2006; Zhang, Pan, Cao, & Wu, 2013). Apoptosis plays a major role in postmortem meat tenderization (Chen et al., 2011). Recent studies have attempted to emphasize the molecular mechanisms of apoptosis and the influence of postmortem biochemical factors on meat quality (Cao et al., 2010; Ouali et al., 2006). However, additional evidence is necessary to evaluate the apoptotic activation and its contribution to meat quality during muscle postmortem aging.

Apoptosis refers to the highly organized programmed cell death choreographed by a set of previous dormant proteases, such as caspases, which can cleave downstream cellular substrates (Thornberry & Lazebnik, 1998). The features of apoptosis include cell shrinkage, mitochondrial depolarization, chromatin condensation, DNA fragmentation, membrane blebbing, and apoptotic body formation (Kemp & Parr, 2012; Wyllie, Kerr, & Currie, 1980). The apoptotic pathways can be broadly divided into two main categories, namely the death receptor pathway and the mitochondrial pathway. In the mitochondrial pathway, the mitochondrial

* Corresponding authors.
E-mail addresses: hlsciggyx@126.com (L. Han), yuqunlihl@163.com (Q.-L. Yu).

http://dx.doi.org/10.1016/j.foodchem.2017.04.185
0308-8146/

membrane permeability transition pore (MPTP) plays a significant role in transferring cell death signals in early apoptosis. MPTP consists of the adenine nucleotide translocase and cyclophilin D, which are situated in the inner membrane, and voltage dependent anion selective channel (VDAC). The VDAC is currently proposed mitochondrial phosphate carrier. This carrier is situated outside the mitochondrial membrane (Bernardi, 1999). However, Kathleen, Kinnally, Shin, and Laurent (2011) showed that mitochondrial permeabilization mediated by MPTP is generally closely linked with late apoptosis and necrosis. MPTP can be opened by pro-apoptotic stimuli, followed by the release of many molecules (i.e., cytochrome *c*) into the cytoplasm. This phenomenon will lead to the activation of a cascade of caspases (such as caspase-9, caspase-3, and caspase-6) (Luo et al., 2014). Meanwhile, the mitochondrial membrane potential ($\Delta\Psi m$) is disrupted in the early phase of apoptosis. Cyclosporin A (CsA) is a specific inhibitor of MPTP formation that can prevent the MPTP opening. Several studies demonstrated that Ca^{2+}, reactive oxygen species (ROS), and Bcl-2 families as well as low ATP concentration, can trigger MPTP opening by causing mitochondrial dysfunction (Hu, Zhou, & Ding, 2015). Recent studies have mainly focused on the mediation of Ca^{2+} and ROS generation during apoptosis in many kinds of cells (Gehlert, Bloch, & Suhr, 2015). However, limited evidence is available on the role of MPTP in the apoptotic pathway and whether cytochrome *c* is released via the MPTP during muscle postmortem aging.

The yak is a scarce and precious livestock living in the Qinghai–Tibetan Plateau and surrounding regions in China. Yak meat is rich in protein and minerals, and low in fat, but tough because of the animal's unusual diet (Tian, Han, Yu, Shi, & Wang, 2013). This study aims to investigate the role of MPTP in mediating the mechanisms of cytochrome *c* release. This goal was achieved by examining the MPTP opening and the changes in apoptotic factors. This study also intends to understand the effects of the mitochondrial apoptotic pathway on the tenderness of yak meat during postmortem storage.

2. Materials and methods

2.1. Sampling and treatment

Ten yaks (average age: 3.5 years old; average live weight: 200 kg) were obtained randomly from Xining City, Qinghai Province, China. Yaks were slaughtered humanely according to the Islamic practice. The experiment was conducted in accordance with the guidelines of Canadian Council on Animal Care, and animal welfare and conditions were considered in the use of experimental animals. Longissimus dorsi muscles were excised from the carcasses immediately and cut into pieces with an average weight of 30 g. A sample from each yak was immediately snap frozen in liquid nitrogen (<10 min) as 0 h sample. The remainder of each of the 30 g of muscle pieces was subdivided into two fractions and distributed to two treatments as follows: one section did not receive any treatment (as the control group) and the other samples were injected with CsA (200 mM) in the ratio of 1:1 (w/v) and then stored at 4 °C for 6, 12, 24, 72, 120, and 168 h. At the end of each storage period, the samples were obtained and stored at −80 °C until needed.

2.2. Isolation of mitochondria

Mitochondrial isolation was performed using the methods as described by Li, Tong, Xu, and Chan (1999) with minor modification. Minced muscle was homogenized in 1:10 (w/v) buffer A (containing 70 mM sucrose, 220 mM mannitol, 2.0 mM ethylenediamine tetraacetic acid (EDTA), 5.0 mM 4-morpholinepropanesulphonic acid, and 0.5% bovine serum albumin (BSA); pH 7.4). The homogenate was centrifuged at 1000*g* and 4 °C for 10 min. The supernatant obtained was centrifuged again at 1000*g* and 4 °C for 10 min. Subsequently, the supernatant was centrifuged for 20 min at 8000*g* and 4 °C with buffer B (obtained by diluting 7.5-fold buffer A without BSA, pH 7.4). The pellet and supernatant were separated. The resulting supernatant contained cytosolic-enriched protein, and the resultant mitochondrial pellets were suspended in buffer B. The protein concentration was determined using the biuret method in the report by Tian et al. (2013).

2.3. Determination of MPTP opening

The mitochondrial inner membrane shows high permeability for sucrose and mannitol because of the opening of MPTP, which lead to the decrease in the absorbance at 540 nm. The changes in the absorbance were determined to understand the opening of MPTP following the methods of Hu et al. (2015). According to the isolation method of mitochondria (Section 2.2), the separated and purified mitochondrial pellets were suspended in 3.0 mL of the cooled MPTP test medium (230 mM mannitol, 70 mM sucrose, 3.0 mM Hepes; pH 7.4), and the protein concentration of mitochondria was determined using the biuret method, then the protein concentration was adjusted to 0.3 mg/mL with the cooled test medium. The purified mitochondrial pellets suspension (1.0 mL) and cooled test medium (3.0 mL) were added into the cuvettes. The absorbance at 540 nm was determined using an ultraviolet spectrophotometer (UV2550, Shimadzu Corporation, Kyoto, Japan).

2.4. Detection of the mitochondrial membrane potential

The Changes in the mitochondrial membrane potential were monitored using a JC-1 assay kit (Beyotime, Beijing, China) according to the manufacturer's instructions. Purified mitochondrial pellets (0.1 mL) with the total protein content of 100 μg were incubated with 0.9 mL of JC-1 dyeing working solution for 20 min, and the fluorescence intensity was immediately measured using a fluorescence spectrophotometer (Shimadzu RF 5301, Kyoto, Japan). In the mitochondria with high membrane potential, the JC-1 dye mainly existed in the mitochondrial matrix with red fluorescent aggregates. Nevertheless, the green fluorescence represented the monomeric form of JC-1 and the mitochondria is with low membrane potential. The red/green fluorescence intensity ratio was used to denote the level of mitochondrial membrane potential depolarization (Ex = 525 nm and Em = 590 nm for J-aggregates; Ex = 490 nm and Em = 530 nm for JC-1 monomers).

2.5. SDS-PAGE and Western blot

Samples containing the mitochondrial protein and cytosolic-enriched protein for SDS–PAGE were boiled in 2 × sampling treatment buffer (0.6 M Tris–HCl, 2% SDS, 10% glycerol, and 5% *β*-mercaptoethanol) for 5 min. Then, the mixture was stored at −80 °C until loading. Samples containing equal amounts of protein were applied to 12% (cytochrome *c*) polyacrylamide gels and transferred to polyvinylidene fluoride membranes (Bio-Rad Laboratories, California, USA) after electrophoresis with a wet transfer apparatus (Bio-Rad Laboratories, California, USA). The membranes were blocked in blocking buffer (TBST: 10 mM Tris, 150 mM NaCl, 0.1% Tween-20, and 5% [w/v] skimmed milk power) at room temperature for 1 h. After blocking, the membranes were probed with rabbit anti-cytochrome *c* antibody (Abcam, Cambridge, UK) overnight at 4 °C. After three more washes with TBST for 10 min each time, the membranes were incubated with goat anti-rabbit IgG horseradish peroxidase at room temperature for 1 h with blocking