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Distribution of tropomyosin isoforms in different types of single fibers isolated from bovine skeletal muscles

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ABSTRACT

To clarify the relationship between myosin heavy chain (MyHC) isoforms and tropomyosin (TPM) isoforms in single fibers, 64 single fibers were isolated from each of bovine three muscles (masseter, semispinalis and semitendinosus). mRNA expressions of MyHC and TPM isoforms were analyzed by real-time PCR. All single fibers from the masseter expressed MyHC-slow. The fibers from the semispinalis expressed both MyHC-slow and 2a. The fibers from the semitendinosus expressed MyHC-slow, 2a and 2x. TPM-1 and TPM-2 were co-expressed in 2a and 2x type fibers, and TPM-2 and TPM-3 were co-expressed in slow type fibers. The expression pattern of TPM isoforms in each fiber type was similar between fibers isolated from different muscles. These results suggest that TPM-1 and TPM-3 isoforms correspond to the function of 2a or 2x type fibers and slow type fibers, respectively, with TPM-2 in common. Furthermore, the patterns of MyHC and TPM isoform combinations did not vary among single fibers isolated from the individual muscles examined.

1. Introduction

Skeletal muscles are composed of different types of muscle fibers, which affect meat quality traits such as texture and tenderness (Maltin et al., 1998; Ouali, 1990). Muscle fiber types have long been classified into type 1 (slow type) and type 2a and 2b (fast type) by histochemical techniques (Brooke & Kaiser, 1970); a new fast type fiber, 2x, was identified later (Bar & Pette, 1988). Introduction of molecular techniques into muscle fiber analysis has indicated that myosin heavy chain (MyHC) isoforms are major proteins responsible for the different fiber types (Schiaffino & Reggiani, 1996). In bovine trunk and limb skeletal muscles, MyHC-slow, MyHC-2a and MyHC-2x are expressed (Chikuni, Muroya, & Nakajima, 2004; Toniolo et al., 2005; Zhang et al., 2014), whereas MyHC-2b expression has not been observed in most cattle except for young Blond Aquitaine bulls (Picard & Cassar-Malek, 2009). In mammalian muscles, MyHC-slow, MyHC-2a, MyHC-2x and MyHC-2b are correlated with type 1, 2a, 2x and 2b fibers (Rubinstein & Kelly, 2004). The difference in ATPase activities of MyHC isoforms is fundamental in determining the speed of contraction of muscle fibers (Schiaffino & Reggiani, 1996; Toniolo et al., 2007), and may also be associated with changes in the mechanical properties during postmortem rigor development and thus affect meat quality.

Tropomyosins (TPMs) are myofibrillar proteins that regulate the calcium-sensitive interaction of actin and MyHC in association with the troponin complex (Perry, 2001). The basic TPM molecule consists of two isoforms of about 33 kDa each, which are packed in parallel forming an α-helical coiled-coil dimer. In skeletal muscles, three major isoforms, namely TPM-1 (or α-TM), TPM-2 (or β-TM) and TPM-3 (or γ-TM), are expressed (Perry, 2001). The variable expression of TPM isoforms is one determinant of the contractile properties of muscle fibers (Schiaffino & Reggiani, 1996). In a previous study we analyzed not only the composition of TPM isoforms in five different types of muscles at the protein and mRNA levels (Oe, Ohnishi-Kameyama, Nakajima, Muroya, & Chikuni, 2007), but also the expression pattern of MyHC and TPM isoforms in ten different types of muscles at the mRNA level (Oe, Nakajima, Muroya, Shibata, & Chikuni, 2009). However, the relationships between fiber types and TPM isoforms have not been sufficiently clarified by analysis of whole muscles consisting of various types of fibers.

Since studies using single muscle fibers have revealed the composition of myofibrillar protein isoforms (Bicer & Reiser, 2013), we recognized that a study using single muscle fibers would be useful to obtain more persuasive evidence of the relationships between fiber types and TPM isoforms. In this study, to clarify the composition of TPM isoforms in different fiber types, we analyzed the mRNA expression patterns of MyHC and TPM isoforms in single muscle fibers.

2. Material and methods

2.1. Animal and sample preparation

The animals were cared for as outlined in the Guide for Care and Use of Experimental Animals (Animal Care Committee of National Institute

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of Livestock and Grassland Science). Animals were anesthetized with 15 mg/kg pentobarbital and slaughtered by exsanguination. Skeletal muscle samples for isolation of muscle fibers and real-time PCR analyses were obtained from masseter, semispinalis and semitendinosus muscles of four Holstein cows aged 94, 86, 51 and 49 months. For preparation of standard DNA fragments, the longissimus thoracis muscle as well as the above-mentioned three muscles were also obtained from those cows. Five mm × 5 mm × 20 mm muscle pieces were excised within 1 h after slaughter and were immediately immersed in 1 mL RNAlater® solution (Ambion, TX, USA) and stored at −20 °C until analysis. The muscle pieces were excised from the center part of the semitendinosus muscle.

2.2. Isolation of single muscle fibers and cDNA synthesis

The single fibers were isolated manually under a stereomicroscope in RNAlater® solution. The lengths of the isolated single muscle fibers were 1–1.5 cm. Sixty-four single fibers were isolated from each muscle and a total of 192 single fibers were isolated from masseter, semispinalis and semitendinosus muscles. The isolated fiber was dissolved in 500 μL ISOGEN®, a total RNA extraction kit (NipponGene, Tokyo, Japan). Total RNA was prepared from each fiber according to the manufacturer's protocol with ISOGEN. The first-strand cDNA was synthesized in 20 μL of reaction mixture containing 100 U M-MLV Reverse Transcriptase RNase-H minus (TOYOBO, Osaka, Japan), 1 mmol/L deoxynucleotide triphosphate (dNTP) and 1 μmol/L of 5′ CTGCAGGAATTCGATATCGAAGCT TGC-(T)15(A/C/G)(A/C/G/T) 3′. The reaction mixture was incubated at 42 °C for 1 h.

2.3. Preparation of cDNA standards for quantitative real-time PCR

Total RNA was extracted from bovine masseter, longissimus thoracis, semispinalis and semitendinosus muscles. Then cDNA synthesis was conducted as described above. The cDNAs from those muscles were mixed and used as template for amplification of standard DNA fragments corresponding to MyHC-slow, MyHC-2a, MyHC-2x, TPM-1, TPM-2 and TPM-3. Those amplified DNAs were electrophoresed on 4% Nusieve® GTG® agarose gel (Canbrex Bio Science Rockland, Inc., ME, USA) and purified by a Gel/PCR™ DNA Isolation System (Viogene, New Taipei City, Taiwan). The concentrations of purified DNA fragments were analyzed by spectrophotometry with NanoVue (GE Healthcare UK Ltd. Amersham Place, Little Chalfont, Buckinghamshire HP7 9NA, England).

2.4. Analysis of MyHC and TPM isoforms expression by quantitative real-time PCR

The sequence of primers used for quantitative real-time PCR of MyHC and TPM isoforms are shown in Table 1. The sizes of amplified cDNA fragments were 167 bp, 149 bp, 152 bp, 203 bp, 153 bp and 146 bp for MyHC-slow, MyHC-2a, MyHC-2x, TPM-1, TPM-2 and TPM-3, respectively. Real-time PCR was performed with QuantiTect™ SYBR® Green PCR System (Qiagen K.K., Tokyo, Japan) using CFX96™ Real-Time PCR Detection System (Bio-Rad, CA, USA). PCR was performed first for 15 min at 95 °C, followed by 45 cycles of 15 s at 94 °C, 30 s at 60 °C and 15 s at 72 °C. To quantify the cDNA content of MyHC and TPM isoforms by real-time PCR, calibration curves were established with known concentrations of the purified DNA fragment prepared as described above. Each MyHC isoform composition was represented as a percentage of the sum of MyHC-slow, MyHC-2a and MyHC-2x. Each TPM isoform composition was represented as a percentage of the sum of TPM-1, TPM-2 and TPM-3.

In this study, fibers in which the composition of MyHC-slow or MyHC-2a or MyHC-2x was more than 90% were classified as a slow or 2a or 2x type fibers, respectively, and the fibers in which the most dominant MyHC isoform was present at less than 90% was classified as a hybrid fiber.

3. Results and discussion

3.1. MyHC isoform expression in single fibers

To classify the fiber types of isolated single fibers, real-time PCR of MyHC isoforms was conducted. In the masseter muscle, all fibers isolated were slow type fibers (Table 2, Fig. 1A). In the semispinalis muscle, slow and 2a type fibers were isolated (Table 2, Fig. 1B). Slow type fibers accounted for 54.7% of the total fibers isolated in the semispinalis, and 2a type fibers for other 45.3% (Table 2). No hybrid fibers were isolated from the masseter and semispinalis. The proportion of different muscle fiber types varied according to location within semitendinosus muscle. A parallel increase in the proportion of slow type fibers and decrease in that of fast type fibers was observed from superficial to deep layers (Totland, Kryvi, & Slinde, 1988). In this study, we used the central part of the semitendinosus muscle as the muscle samples. In the semitendinosus muscle, slow, 2a, 2x and hybrid fibers were isolated. Slow, 2a and 2x type fibers accounted for 7.8%, 42.2% and 20.3% of the total number of fibers isolated in the semitendinosus, respectively (Table 2). Hybrid fibers accounted for 29.7% of the total number of fibers isolated in the semitendinosus (Table 2). Two types of hybrid fibers, namely slow/2a and 2a/2x hybrid fibers, were present (Table 2). In 2a/2x hybrid fibers, the composition of MyHC-2a varied from 10.3 to 83.7% and that of MyHC-2x varied from 16.2 to 89.5% (Fig. 1C).

Compared to muscle fibers isolated from masseter and semispinalis, those from semitendinosus showed a large variation of MyHC isoform composition (Fig. 1C). Semitendinosus consisted of not only fibers composed of single MyHC isoform but also the hybrid fibers. Furthermore, the composition of MyHC-2a and 2x in hybrid fibers isolated from semitendinosus varied from approximately 10 to 80% (Fig. 1C). This variation might be due to the functional differences among these muscles. The function of the masseter in cattle is to chew the cud, and the function of the semispinalis is postural control. Thus, both the masseter and semispinalis are exposed to constant loads. In contrast, the semitendinosus muscle is required for hind limb contraction at various speeds and forces for locomotion. Considering that more than four MyHC isoforms were expressed in the extraocular muscles, which contract at various speeds for eye rotation (Bicer and Reiser, 2009), the presence of various fiber types in the semitendinosus might also be

Table 1
Nucleotide sequence of the PCR primers.

Isoform name	Accession no.	Forward primer sequence (‘5→’3)	Reverse primer sequence (‘5→’3)
MyHC-slow	NM_174727	AATCCCAGGTCAACAAGCTG	TGCTTTATTCTGCTTCTTCCAAA
MyHC-2a	NM_001166227	GGTTCACACAAAAATCATCAGTG	CCCTATGCTCTTTATTTCCTTTGCA
MyHC-2x	NM_174117	AGTCCCAGGTCAACAAGCTG	TGCAAAAATCATAAGTACAAAACAGA
TPM-1	NM_001013590	CATGACTTCCATATAAGTTTCTTTGCTTCA	TAGTGTTTATTTTACACTGAAGTGAAGA
TPM-2	NM_001010995	GATGACCTGGAAGATGAAGTCTATGCA	ATAGAGAAAATGGAAAGGAGAAGAGAG
TPM-3	NM_001011674	AAAGAAGCTAAGCACATTGCAGAAGAGGCA	AGCTCCTCCTCCAGCTCAGAACATTTAGA