COMPOSITION OF TROPOMYOSIN ISOFORMS AND MYOSIN HEAVY CHAIN ISOFORMS IN BOVINE SKELETAL MUSCLES

M. Oe*, I. Nakajima, S. Muroya and K. Chikuni

National Institute of Livestock and Grassland Science, Tukuba, Ibaraki 305-0901, Japan. Email: mooe@affrc.go.jp

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Introduction

Tropomyosin (TPM) and Myosin heavy chain (MyHC) are myofibril proteins that work together on muscle contraction. In skeletal muscle, TPM has three isoforms: TPM1, TPM2 and TPM3 (Perry, 2001). TPM1 is expressed in fast skeletal muscle and TPM3 is expressed in slow skeletal muscles, forming a dimer with TPM2. MyHC expressed three isoforms in bovine skeletal muscles (Chikuni *et al.*, 2004). MyHC-slow is a slow-type isoform and MyHC-2a and -2x are fast-type isoforms.

Although there are one TPM and two MyHC fast-type isoforms, the relationships between the fast type TPM and MyHC isoforms in muscle fibres are not clear. The aim of this study was to analyse the presence of MyHC and TPM isoforms in ten different types of skeletal muscles using real time polymerase chain reaction (real time-PCR).

Materials and Methods

Skeletal muscle samples for real time-PCR were obtained from the tongue, *masseter*, *pectralis profundus*, diaphragm, *psoas majoir*, *longissimus thoracis*, *semispinalis*, *semitendinosus*, *semimembranosus* and *biceps femoris* of three Holstein cows. The muscle pieces were excised within 1 h after slaughter and immediately frozen in liquid nitrogen. The frozen samples were crushed into fine powder, and total RNA was extracted from the frozen muscle powder by an ISOGEN total RNA extraction kit (NipponGene, Tokyo, Japan). Single-stranded cDNA was synthesized by RNase H minus M-MLV reverse transcriptase (TOYOBO, Osaka, Japan). The cDNAs from the ten muscles were used as a template of multiplex PCR (Table 1). PCR products were electrophoresed on 4% NuSieve GTG agarose gel and then stained with ethidium bromide. The image of the PCR products was scanned by FluorChem™8900 with AlphaEase FC software (Alpha Innotech, San Leandro, CA, USA).

Results and Discussion

The TPM2 isoform comprised about 50% of the total TPM in all muscles examined, while the amounts of TPM1 and TPM3 isoforms differed among the muscles. Comparing the TPM isoform composition with the MyHC isoform composition, there was high positive correlation between MyHC-slow and TPM3 ($r = 0.92$) (Table 2). The sum of MyHC-2a and -2x (fast-type MyHC isoforms) had high positive correlation with TPM1 ($r = 0.85$), but the correlations between MyHC-2a or -2x alone and particular TPM isoforms were not as high.

In the tongue and *semispinalis* muscles, the MyHC-2a isoform was the only fast-type MyHC isoforms expressed, while both TPM1 and TPM3 isoforms were expressed in these muscles. Therefore, the IIA type fibre, which expresses MyHC-2a, would be regulated delicately by changing the TPM isoform composition.

Conclusions

TPM isoforms affect muscle contraction together with the MyHCs during post mortem rigor development.

References

Chikuni, K., Muroya, S. and Nakajima, I. (2004). Myosin heavy chain isoforms expressed in bovine skeletal muscles. Meat Science, 67(1); 87-94.

Perry, S. V. (2001). Vertebrate tropomyosin: distribution, properties and function. J Muscle Res Cell Motil, 22(1); 5-49.

Table 1: Nucleotide sequence of the PCR primers used for RT-PCR.

	Forward	Reverse
TPM 1	5'ATGGAGGCCATCAAGAAGAAGATGCAGATG 3'	5'GGAGTATTTGTCCAGTTCATCTTCGGTGG 3'
TPM 2	↑	5'TCCCTTTTAGCTTTTTCTGGAGGGCCTG 3'
TPM 3	↑	5'CTTACTTCTTTCTTCTGCCTGCTTCTGC 3'
MyHC-slow	5'TGCTGCTCTCAGGCCCCTGCCACCTT 3'	5'GTCATCATGGCCATGTCCTCGATCTTGTC 3'
MyHC-2a	5'CACTTGCTAACAAGGACCTCTGAGTTCA 3'	↑
MyHC-2x	5'CTTTCCTCATAAAGCTTCAAGTTCTGACC 3'	↑

Table 2: Correlation coefficients (r) between content of TPM isoforms and content of MyHC isoforms in skeletal muscle (n = 30).

	MyHC-slow	MyHC-2a	MyHC-2x	MyHC-2a + -2x
TPM 1	- 0,85	0.48	0.69	0.85
TPM 3	0.92	- 0.65	- 0.62	- 0.92