ABSENCE OF MYOSIN HEAVY CHAIN 2B mRNA IN BOVINE SKELETAL MUSCLES

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Background

Skeletal muscles contain heterogeneous myosin heavy chain (MyHC) isoforms, each of which are associated with the muscles' different contractile properties. Three fast- and one slow-type MyHC isoforms are expressed in the porcine *longissimus* muscle and would be partially responsible for the diversity of meat texture (Tanabe et al. 2001). To study the relationships between the MyHC isoforms and meat quality in beef, we determined the sequences of the bovine MyHC-slow, -2a, and -2x isoforms, but the MyHC-2b isoform has not yet been identified. The three fast types have been identified in some mammalian animals, but the bovine MyHC-2b remains unelucidated.

Objectives

In the present study, we try to clarify the presence or absence of the bovine MyHC-2b isoform on the mRNA level. If the MyHC-2b protein is present in bovine muscles, its mRNA should also be expressed in the bovine muscles.

Methods

Muscle samples were obtained from adult Holstein cows within one hour of slaughter. Total RNA was extracted from frozen muscle pieces by means of acid guanidium thiocyanate-phenol-chloroform extraction. First-stranded cDNA was synthesized by M-MLV reverse transcriptase RNase H minus and then used as a template for RT-PCR. The RT-PCR was conducted on the three different regions of MyHC cDNAs. The MyHC-2b-specific primer pairs P31/BP02, P33/BP02, and P35/BP02 were used for the amplification of the 5'UTR (un-translating region). The MyHC common primer pair B001/B002 was used for the amplification of the Loop 2 region on the MyHC amino acid coding region. The MyHC fast type specific primer pair B003/3ADP1 was used for the amplification of the 3'UTR. Sequences of the RT-PCR primers were follows; P31, 5'CATCTGGTAACATAAGAGGTACATCTAG3'; P33, 5'GCCTTGAGCCTGCCACCGTCTTC ATCTG3'; P35; 5'TTAAGTAGTTGTCTGCCTTGAGCCTGCCA3'; BP02, 5'GTCATCATGGCCATGTCCTCGATCTTGTC3'; B001, 5'GTGGACTACAACATTACTGGCTGGCTG3'; B002, 5'GGAGTCTTAGTTTCATTGGGGATGATGCA3'; B003, 5'CGGGAGGTTCAC ACAAAARTCATMAG3'; 3ADP1, 5'CTGCAGGAATTCGATATCGAAGCTTGC3'.

Results and Discussion

In a previous study, we were unable to obtain the 5'UTR fragments of the bovine MyHC-2b isoform from the *biceps femoris* muscle of 7 month-old cattle (Tanabe et al. 1998). To confirm the presence or absence of the bovine MyHC-2b isoform, we conducted three RT-PCR experiments on the MyHC isoform-specific regions. In the first experiment, new specific primers of the 5'UTR were designed for the RT-PCRs on seven muscles of each animal. The MyHC-2b specific primer pairs amplified the porcine fragments, but not the bovine fragments from the muscles of three adult cows (Fig.1). In the second experiment, the Loop 2 region of bovine *biceps femoris* and *semitendinosus* and porcine *longissimus thoracis* muscles was amplified by the MyHC common primer pair B001/B002 (Fig.2) and then sequenced following restriction enzyme digestion. The common primer pair amplified the bovine and porcine MyHC-2a, -2x, and the porcine -2b isoforms, but not the bovine -2b isoform. In the third experiments involving the 3'UTR, the results were the same as the second experiment (data not shown). Our results conflict with the electrophoresis of MyHC protein isoforms performed by Picard et al.(1999) who separated three fast isoforms from bovine muscles. There is no other report concerning the presence of bovine MyHC-2b isoform.

Conclusions

The MyHC-2b isoform was not detected in the bovine muscles in contrast to the other fast-type isoforms. These RT-PCR amplifications were conducted independently on the three different regions of the bovine MyHC mRNA, and the results were identical. These results suggest that bovine MyHC proteins are composed of the slow, 2a and 2x isofroms in the adult skeletal muscles, and that the 2b isoform does not contribute to the bovine muscle conformation.

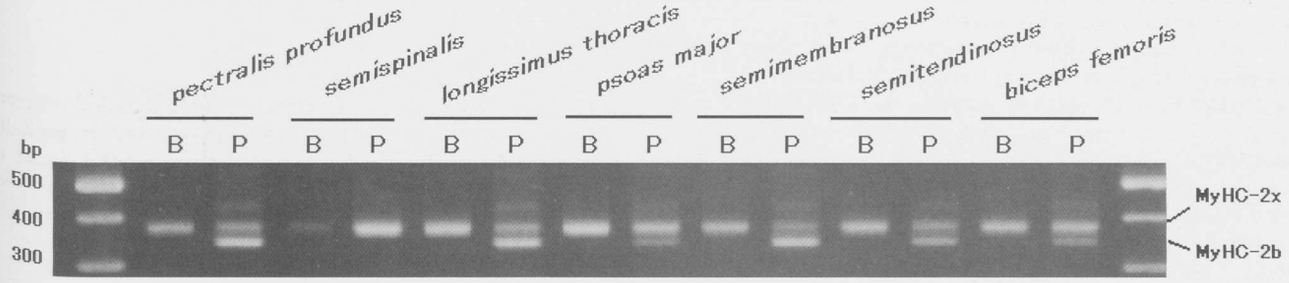
Pertinent literature

Picard, B., Barboiron, C., Duris, M.P., Gagniere, H., Jurie, C, & Geay, Y. (1999). Separation of bovine muscle myosin heavy chain isoforms. *Meat Science*, 53, 1-7.

Tanabe, R., Murakami T, Kawahara, T., Yamashiro, R., Mitsumoto, M., Muroya, S., Nakajima, I., & Chikuni, K. (2001). Composition of myosin heavy chain isoforms in relation to meat texture in Duroc, Landrace and Meishan pigs. *Animal Science Journal*, 72, 230-237.

Tanabe, R., Muroya, S, & Chikuni, K. (1998). Sequencing of the 2a, 2x, and slow isoforms of the bovine myosin heavy chain and the different expression among muscles. *Mammalian Genome,* 9, 1056-1058.

A) electrophoresis of the RT-PCR products from skeletal muscles



B) The 5'UTR of the porcine and human MyHC-2b isoforms

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                -90   (primer P35)          (primer P33)      (primer P31)    -30                    1
Pig     aacacttaagtagttgtctgccttgagcctgccaccgtcttcatctggtaacataagaggtacatctagtgccctgctgccatcaataacccgcagccATG
Human   ··tt···g·············t·············t·········a···t·c·····t·c·····c·agca·············t·········
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Fig.1. RT-PCR amplification of the bovine and porcine MyHC-2x and -2b isoforms on the 5'UTR.
Open boxes and an underline indicate the MyHC-2b specific forward primers. The primer P31, -57 to -30, was used as a forward primer on the RT-PCR shown in Fig.1-A).

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Bovine-2x       1 GTGGACTACAACATTACTGGCTGGCTGGACAAGAACAAGGACCCCCTGAATGAGACGGTGGTCGGGCTGTACCAGAAGTC
Bovine-2a       1 ..............................G.....................C..T..T.....T.................
Bovine-slow     1 ..............C.TA.........C.G..A.....A.....G..C..C...........G.ACT.....A.......
Pig-2b          1 ...............................................................................
Human-2b        1 ..............CG.C..............A.......................T.....G.................

Bovine-2x      81 TTCAGTGAAGACTCTGGCTTTACTGTTCTCTGGCCCAGCATCTGGTGAAGCAGAGGGCGGTC------CAAAGAAAGGTG
Bovine-2a      81 .G.GT....A.....A.....C..T........GA.TC..A.......CT......CAA..GGTGGAA.T..........
Bovine-slow    81 ...CC.C....TG..CAGCAGC..C..TG.CAA.TAT..TGGGTT...CA..CCTATT.AGAAGGGC------.....CA
Pig-2b         81 ...C.................C..C..TG...AGAG.CAGAG.TC.....AG.GT..TA---------..........
Human-2b       81 .G..A................C..C........GG..CA.A...C............T...GGTGGAA---.........

Bovine-2x     155 GCAAGAAGAAGGGTTCTTCTTTCCAGACCGTGTCTGCTCTTTTCAGGGAGAACCTGAATAAGCTGATGACCAACCTGAGG
Bovine-2a     161 .T...........C.............G........A...........A..T.....C..A.............C...
Bovine-slow   155 AGGCC.....A..C..A..C..T...........A.....GCA.........T.....C..............T..C.C
Pig-2b        152 ..................................A.................T.....C.....................
Human-2b      158 ....A.......................A.....A.................TT.....................T.....

Bovine-2x     235 AGCACTCACCCCCACTTTGTACGCTGCATCATCCCCAATGAAACTAAAACTCC
Bovine-2a     241 ..T..C..T..T........G..G.....................G..G..C..
Bovine-slow   235 TC...A...........C..G..G.................G..A..GT....
Pig-2b        232 .....C.....T........G.....................G..C........
Human-2b      238 ....................G..G..............................
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Fig.2. Nucleotide sequences of the MyHC Loop 2 region.
Underlines indicate the forward (B001) and reverse (B002) primers that are capable of amplifying all MyHC isoform types.