RT-PCR analysis of myosin heavy chain isoform expressions in porcine muscles

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Background

Myosin is a major structural protein of skeletal muscle. A myosin molecule comprises two identical heavy chains and two pairs of light chains. Many studies concerned with muscle fiber characteristics of farm animals have been related to the myosin heavy chain (MyHC) isoforms. In those studies, myosin ATPase activity and metabolic properties in each muscle fiber have been evaluated by histoenzymatic method, and muscle fibers have been divided into three or four types. The porcine muscle fiber characteristics are different among different breeds and muscles. It was suggested that muscle fiber characteristics might be an important source of variation in meat quality such as meat texture. The mean diameter of the porcine FOG fibers explained a significant amount of total variation in instrumental texture of meat. As the mean diameter increased, the required force, the total compression and the total work done increased, indicating tougher muscle (Maltin et al., 1997).

It is known that muscle fiber types result from expression of different MyHC gene isoforms. In mammals, there are four known isoforms of MyHCs: type 2a, 2b, 2x, and slow/β, which are encoded by a distinct gene, and with their own ATPase activity(Schiaffino and Reggiani, 1994). So, the expression patterns of MyHC isoforms, which differed among different breeds and muscles would thus be a determinant of meat texture.

Objectives

Our objective was to develop a procedure to detect the expression of all four MyHC isoforms in porcine muscles by reverse transcription-polymerase chain reaction (RT-PCR) method with the use of the multiplex PCR technique.

Methods

Oligonucleotide primers for Multiplex PCR

For the multiplex PCR analysis, four sense primers named MYO207, MYO305, MYO107 and MYO403, being specific against each MyHC cDNA were synthesized. Those primers were based on 2x, 2b, 2a and slow/β type MyHC isoforms cDNA, respectively. Those primers were designed to amplify the cDNA fragments whose size were 429 bp, 398 bp, 375 bp and 384 bp, for type 2x, 2b, 2a and slow/β, respectively, with the use of as a single common antisense primer(MYO2).

Multiplex PCR

In multiplex PCR, more than two pairs of primers were mixed in one PCR reaction tube to amplify more than two cDNA fragments simultaneously. In this study, the multiplex PCR was carried out to amplify the two MyHC isoform cDNA fragments simultaneously. MYO2 was always contained in the PCR reaction mixture as a single common antisense primer. MYO207 was also contained in every PCR reaction mixture as a sense primer to amplify the 429 bp of the cDNA fragment at any time, which correspond to type 2x MyHC isoform: Another sense primer was selected dependent on another target cDNA amplified simultaneously. PCR Amplifications were performed, and amplified DNA was electrophoresed on 4% agarose gel.

Standard reaction of multiplex PCR

The standard amplifications of multiplex PCR were carried out with the use of the mixture of the purified DNA fragment as templates. The mixture contained the purified DNAs corresponding to type 2x isoform and another MHC isoform. The mixture varied on the proportion (0, 10, 25, 50, 75, 90 and 100%) of each MHC isoform DNA in mole concentration level. The PCR amplifications were carried out on those mixtures.

Analysis of MyHC isoform gene expression by RT-PCR

M. longissimus thoracis (LT), M. semispinalis (SS), M. semitendinosus (ST), tongue(TON) and diaphragm(DIA) were excised from six-month-old Landrace pigs. Total RNA was extracted from the muscle sample. First-strand cDNA synthesis was performed on each RNA sample by using of M-MLV Reverse Transcriptase. The multiplex PCR was carried out with the use of the first-strand cDNA as the template.

Results and discussion

Figure 1 shows the results of the standard reactions of the multiplex PCR amplifications. In every case, with increasing the ratio of the cDNA corresponding to type 2x MHC isoform in the template, the band of 429 bp became stronger, which was produced from the primer MYO207 and MYO2. The strength of another band also changed dependent on the proportion of the DNA corresponding to each MHC isoform in the template. These results indicate clearly that the multiplex PCR used in this study could detect all four MHC isoform DNAs, and amplified the DNA according to the ratio of each MHC isoform DNA in the template. This procedure could also evaluate the rough proportion of MHC isoform DNA. These results of standard reaction revealed that this procedure could approximately evaluate the rough proportion of MHC isoforms between 0, 25, 50, 75 and 100%.

Figure 2 shows the results of RT-PCR analysis for porcine muscles with the use of the multiplex PCR technique. Each band indicates the gene expression of each MyHC isoform in the muscle. The electrophoresis patterns of the amplified MyHC isoform DNAs differed among muscles. The strong bands indicate the much expression of the MyHC isoform corresponding to that band. In LT and ST, all four types of MyHC isoforms were expressed. And in those muscles, higher expression of type 2b and 2x than that of type 2a and slow/ β (MyHC isoforms was observed (Fig. 2, lane 1-2, 5-6). In SS, higher expression of type 2a and slow/ β MyHC isoforms was observed than that in LT and ST (Fig 2, lane 3-4). The expression of type 2b MyHC isoform was not observed in SS, TON or DIA (Fig. 2A, lane 3-4, 7-10). In TON, proportion of type 2a MyHC isoform was higher than slow/ β (MyHC was observed (FIG. 2B-C, lane 7-8). These observations were reproducible between two pigs used in this study (Fig. 2). These results indicated clearly that we could detect the expression patterns of all four porcine MyHC isoforms by RT-PCR method with the use of the multiplex PCR technique.

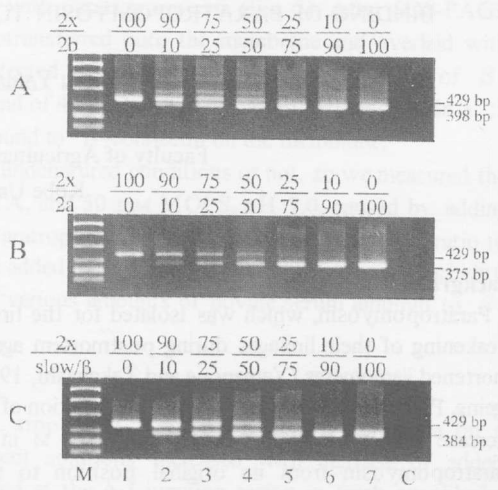

Fig. 1--Standard reactions of the multiplex PCR. C: control(no template), M: marker.

In this study, the expression of MyHC isoforms was evaluated in mRNA levels, and all four MyHC isoforms could be distinguished.

It is known that there are correlations between fiber type and MyHC isoform expressions. Our data revealed that fast type MHC isoforms are dominant in porcine LT and more slow type MHC isoforms exist in SS than in LT. Those findings were in good agreement with the histoenzymatic observation, which showed high proportion of fast fiber in LT and higher proportion of slow fiber in SS than in LT.

Skeletal muscle consists of a mixture of fiber types having different proportions of MyHC isoform expression. Many researchers have discussed the relationship between muscle fiber characteristics and meat texture. Despite the speculation as to the roles of muscle fiber characteristics in meat texture, the precise relationship is not yet cleared. Thus, it is necessary that meat texture be considered as to not only muscle fiber characteristics but also the MyHC isoform expression. The procedure developed in this study would be very useful in that regard.

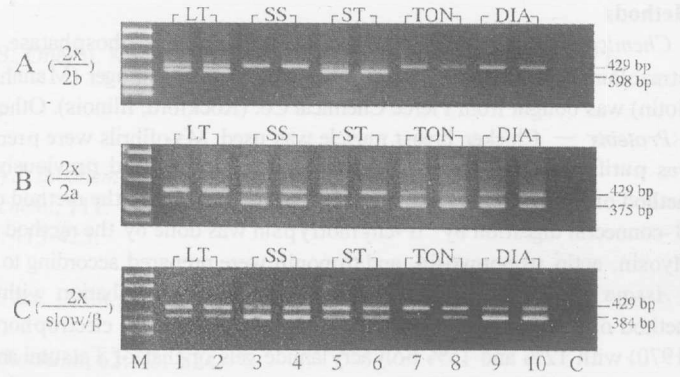

Fig. 2--Analysis of the expression of MyHC isoforms in the porcine muscles by RT-PCR. C: control(no template), M: marker.

Conclusion

The procedure of RT-PCR was developed to analyze the expression patterns of MyHC isoforms in porcine muscles. All four MyHC isoforms in postnatal porcine muscles could be analyzed by this method. The MyHC isoforms showed different expression patterns according to the porcine muscle types. The procedure developed in this study would be very useful to elucidate the relationships between meat quality and MyHC isoforms composition.

References

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