The difference in the ratio of each myosin heavy chain isoform content among porcine skeletal muscles.

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SUMMARY

A sodium dodecyl sulfate-polyacrylamide gel electrophoresis(SDS-PAGE) method was developed for analyzing the porcine myosin heavy chain(MHC) isoforms. On the gel containing 6% acrylamide and 25% glycerol, porcine MHC isoforms were separated to two bands, fast and slow type isoforms.

By this method, we analyzed the ratio of the slow type isoform to the whole MHC isoforms in porcine muscles. That ratio was 5, 32 and 51% in M. longissimus thoracis, M. rhomboideus and diaphragm, respectively. So, the difference in the ratio of each myosin isoform content among porcine skeletal muscles was clarified. This difference would affect the meat quality of each muscle.

INTRODUCTION

Myosin is present in different isoforms whose ATPase differs. The coexistence of the isoforms causes the difference in the contraction velocity among muscles. In a muscle, several types of myosin coexist in a certain ratio according to the physiological condition of that muscle. That ratio would affect the process of rigor mortis and conditioning of meats and would affect the meat quality such as tenderness, texture and water holding capacity.

Most studies of MHC isoforms were carried out in relation to the classification of muscle fibers using histochemical ATPase activity assay technique. In this study, we developed the SDS-PAGE technique for analyzing the porcine MHC isoforms. And we analyzed the ratio of each MHC isoform in porcine muscles.

MATERIALS AND METHODS

SDS-PAGE Muscles were homogenized with 5 mM Tris-HCl(pH 8.0), 5 mM EDTA. Samples for SDS-PAGE analysis were prepared from these homogenates according to Laemmli(1970). Laemmli's system was adapted to some types of polyacrylamide gel which differed concentration of acrylamide, bis-acrylamide and glycerol. We determined the best composition of the gel for porcine MHC isoform separation.

Immunoblot The proteins separated by the SDS-PAGE were transferred onto a nitrocellulose sheet. The bands corresponding to MHC were detected by the polyclonal anti-myosin antibody or the monoclonal anti-fast type myosin antibody(sigma, USA).

Densitometry The ratio of a MHC isoform content to the whole of MHC was analyzed by a densitometer(CS-9000, Shimadzu, Japan).

RESULTS AND DISCUSSION

Polyacrylamide gel containing 6% acrylamide(6%T, 5%c) and 25% glycerol was most suitable to separate porcine MHC isoforms. By using this type of gel, the porcine MHC isoforms were separated to two bands(Fig. 1, lane1, 4).

By immunoblot analysis, it was confirmed that these two bands were MHC. The fast type MHC isoform displayed a mobility slightly lower than the slow type one(Fig. 1, lane2, 5).

In other studies, the rat myosin isoforms were separated to three or four MHC isoforms by electrophoresis(LaFramboise et al.,1990; Schiaffino and Reggiani,1994). The electrophoresis used in this study also allowed consistent separation of three rat MHCs which were two fast and one slow type MHC isoforms(Fig. 2). So, the electrophoresis used in this study had enough ability to separate MHC isoforms. Figure 1 shows only one band corresponding to the fast type MHC isoform, however more than two fast type MHC isoforms may exist in porcine muscles. Fernandez et al.(1995) demonstrated

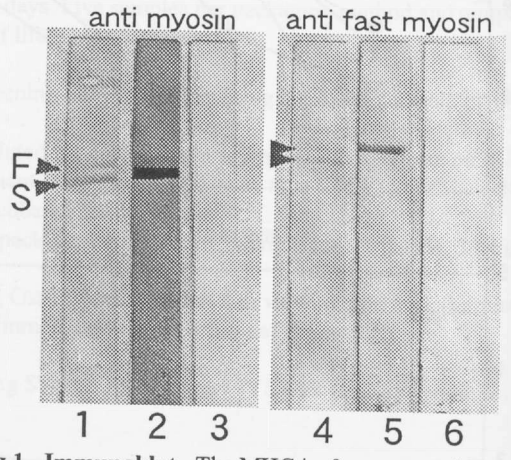

Fig.1. Immunoblot. The MHC isoforms were detected with anti-myosin(right panel) or anti-fast type myosin (left panel) antibodies. 1,4:amido black 10B stain; 2:treated with anti myosin antibody; 5:treated with anti fast type myosin antibody; 3,6:treated with second antibody alone. F:fast type MHC; S:slow type MHC.

that there were two types of fast-twitch muscle fiber in porcine muscles. Their results suggest the existence of more than two types of fast type MHC isoforms in porcine muscles. Fast type MHC isoforms in porcine muscles would be almost equal in that molecular weight.

Manabe et al.(1995) developed a immunohistochemical micro method for measuring fast myosin isoform contents in histological sections. Their method could preserve the muscle structure. though required the antibody which reacted specifically with the antigen. So, their method and our method using the SDS-PAGE in this study would be complementary each other.

The ratio of each MHC isoform content to the whole MHC isoforms was different among porcine muscles(Fig. 3). Table 1 indicates the ratio of the slow type MHC isoform in several porcine muscles obtained from SDS-PAGE patterns with a densitometer. That ratio was different among muscles. Especially, the ratio was high in M. rhomboideus and diaphragm. These results were consistent with other studies by histochemical enzyme activity assay technique.

This difference among porcine muscles would affect the process of the rigor mortis and conditioning of meats through the binding between myosin and actin. So, the ratio of each MHC isoform content would affect the meat quality of each muscle.

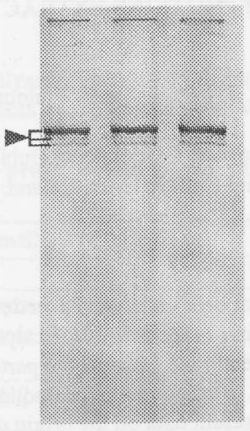

Fig.2. Electrophoretic separation of rat MHC isoforms. Arrow heads indicate MHC isoforms.

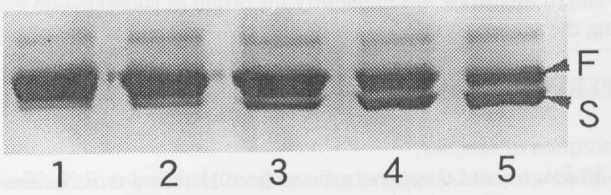

Fig.3. The difference in the ratio of MHC isoform content among porcine skeletal muscles.
1:M. longissimus thoracis; 2:M. sartorius; 3:M.gastrocnemius; 4:M.rhomboideus; 5:diaphragm; F:fast type MHC; S:slow type MHC.

Table 1. The ratio of the slow type MHC isoform in porcine muscles.

M. longissimus thoracis	4.8%[a]
M. biceps femoris	6.2
tongue	7.7
M. gastrocnemius	7.8
M. semimembranosus	8.0
M. rhomboideus	32.3
diaphragm	50.5

[a]The ratio(percent) of the slow type MHC isoform content to the whole of the MHC isoforms was calculated from SDS-PAGE patterns with a densitometer. MHC:myosin heavy chain.

REFERENCES

Fernandez, X., Lefaucheur, L. and Candek, M., 1995. Comparative study of two classification of muscle fibers: Consequence for the photometric determination of glycogen according to fibre type in red and white muscle of the pig. Meat Sci., 41, 225-235.

Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680-685.

LaFramboise, W. A., Daood, M. J., Guthrie, R. D., Moretti, P., Schiaffino, S. and Ontell, M. 1990. Electrophoretic separation and immunological identification of type 2X myosin heavy chain in rat skeletal muscle. Biocem. Biophysica Acta, 1035, 109-112.

Manabe, N., Azuma, Y., Furuya, Y., Kuramitsu, K., Nagano, N. and Miyamoto, H. 1995. Immunohistochemical microquantification of fast-myosin in frozen histological sections of mammalian skeletal muscles. J. Anim. Sci.,73, 88-95.

Schiaffino, S and Reggiani, C. 1994. Myosin isoforms in mammalian skeletal muscle. J. Appl. Physiol., 77, 493-501.