CONFIRMATION OF MYOSIN HEAVY CHAIN ISOFORMS IN PORCINE *LONGISSIMUS* MUSCLE BY GEL ELECTROPHORESIS AND MALDI-TOF/TOF MASS SPECTROMETRY

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Abstract – The purpose of this study was to find the identification method for myosin heavy chain (MyHC) isoforms separated by 1-dimensional gel electrophoresis. Four bands were separated and identified to four isoforms (MyHC-2a, -2x, -2b and -slow) after in-gel digestion and peptide mass fingerprint by MALDI-TOF/TOF analysis. Two bands of MyHC-slow and co-expressed bands (MyHC-2a and -2b; MyHC-2x and -b) were found and their specificities were confirmed by immunoblot analysis. We also selected three major peptides and compared their amino acid sequences among MyHC isoforms. Results suggest that mass spectrometry is useful for the accurate identification of MyHC isoforms separated by gel electrophoresis.

Key Words – Myosin heavy chain, Mass spectrometry, Porcine muscle.

I. INTRODUCTION

One of the most popular means of typing skeletal muscles is identification of myosin heavy chain (MyHC) distribution. In the *longissimus* muscles of meat animals, there are three or four MyHC isoforms (MyHC-slow, MyHC-2a, MyHC-2x or MyHC-2b), and their distribution is a major determinant of muscle fiber characteristics which are closely related to meat quality and carcass traits [1, 2].

It is widely recognized that gel electrophoresis is the most convenient method for separation of MyHC isoforms, but it is difficult to clearly separate them. The migration rate of MyHC isoforms can be identified according to their apparent molecular masses and confirmed by immunoblot analysis [3]. However, there are divergent views on the migration rates of MyHC isoforms among researchers. For example, Bamman *et al*. [3] reported that the migration rate of MyHC isoforms was I>IIa>IIx in human muscle, while Picard *et al*. [4] separated three MyHC isoforms of bovine muscles in the order I>IIa>IIb. Four MyHC isoforms in the order I>2b>2x>2a were separated from marsupial mammals by Zhong *et al*. [5]. Immunoblot analysis allows the confirmation of the migration rate and distribution of specific MyHC isoforms. The specificity of some monoclonal antibodies (MAbs) for the identification of MyHC isoforms has been reported in a number of mammals [6], but the specificity of the antibody for a particular MyHC differs among the animal species.

Therefore, the purpose of this study was to find a useful and clear method for the identification of skeletal muscle MyHC isoforms separated by gel electrophoresis. In the present study, we separated four MyHC isoforms from porcine *longissimus* muscle by 1-dimensional gel electrophoresis and analyzed their peptide mass fingerprint by in-gel digestion and MALDI-TOF/TOF mass spectrometry, confirming their migration rate by immunoblot analysis.

II. MATERIALS AND METHODS

Muscle sample was taken from three adult porcine (Yorkshire × Landrace × Duroc, 185 days old and 110±1.5 kg in body weight) *longissimus* muscles immediately after slaughter, frozen in liquid nitrogen and kept at -78°C until protein extraction. Protein extraction, sodium dodecyl sulfate-polyacrylamide gel preparation and running condition were performed according to Talmadge & Roy [7] method, and protein concentration was determined by Bradford method [8] using bovine serum albumin as a standard. Spectrometric analysis using the 4800 MALDI-TOF/TOF mass spectrometer (Applied

Biosystems, CA, USA) was analyzed the mass spectra of tryptic peptides after in-gel digestion with trypsin solution. Mass peaks were submitted to MASCOT (Matrix Science, MA, USA) under the following searching conditions: NCBInr database, mammalian species, trypsin digest, no fixed modifications, one maximum missed cleavage and mass tolerance of 0.2 Da. At least two unique peptides were identified for one protein. A battery of four MAbs was used whose specificities (BA-D5, anti-MyHC slow; BF-F3, anti-MyHC 2b; BF-35, anti-MyHC slow and 2a; SC-71, anti-MyHC2a and 2x) for MyHC isoforms have been demonstrated in previous reports [6]. Immunoblot analysis was carried out according to Rossini *et al*. [9].

III. RESULTS AND DISCUSSION

Four resolvable bands were separated in porcine *longissimus* muscle by gel electrophoresis (Figure 1-a). Identification of these MyHC bands was determined by mass spectrometry and immunoblotting. The fastest migrating MyHC band (Line 4) was identified as MyHC-slow (Myosin, heavy chain 7), and Line 3 was identified as MyHC-slow (Table 1).

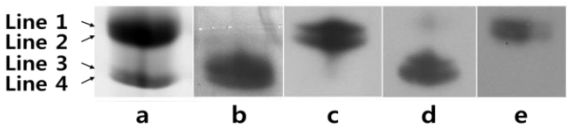

Figure 1. 1-dimensional gel electrophoresis (a) and immunoblot analysis (b-e) of myosin heavy chain (MyHC) isoforms in porcine *longissimus* muscle. Monoclonal antibodies (MAbs) BA-D5 (b, anti-MyHC slow), BF-F3 (c, anti-MyHC 2b), BF-35 (d, anti-MyHC slow and 2a) and SC-71 (e, anti-MyHC2a and 2x) were used.

Also, the slowest migrating band (Line 1) was identified as MyHC-2a and MyHC-2b, and the Line 2 band was found to be MyHC-2x and MyHC-2b. In this study, the migration rate of MyHC isoforms was slow (Line 1)>slow (Line 2)>2b and 2x>2b and 2a; that is, the more slowly migrating bands (Line 3 and Line 4) were identified as the same MyHC isoform (slow), while both Line 1 and Line 2 contained MyHC-2b.

Table 1. Identification of myosin heavy chain isoforms by mass spectrometry and NCBInr search

Label	Accession number[a)]	Protein name[b)]	Size (aa)[c)]	Theoretical MW (Da)[d)]	Score[e)] / Matched peptides[f)]	Sequences
Line 1	gi|55741490	Myosin, heavy chain 2 (2a)	1939	223924	348 / 44	RQLDEKEALVSQLSRG RDLEEATLQHEATAAALRK KHADSVAELGEQIDNLQRV
	gi|178056718	Myosin, heavy chain 4 (2b)	1937	224010	227 / 26	KTLAFLFAERQ RDLEEATLQHEATAAALRK KLAQRLQDAEEHVEAVNAKC
Line 2	gi|178056718	Myosin, heavy chain 4 (2b)	1937	224010	250 / 29	RILYADFKQRY RQLDEKEALVSQLSRG RDLEEATLQHEATAAALRK
	gi|157279731	Myosin, heavy chain 1 (2x)	1939	223947	185 / 27	RQVEEKDALISQLSRG RDLEEATLQHEATAAALRK
Line 3	gi|55741486	Myosin, heavy chain 7 (slow)	1935	223743	517 / 48	RVVDSLQTSLDAETRS RQLDEKEALISQLTRG TILNPAAIPEGQFIDSRK RDLEEATLQHEATAAALRK
Line 4	gi|55741486	Myosin, heavy chain 7 (slow)	1935	223743	409 / 43	RILYGDFRQ RVVDSLQTSLDAETRS RQLDEKEALISQLTRG RILNPAAIPEGQFIDSRK RDLEEATLQHEATAAALRK

[a,b)] Accession number and protein names were taken from the NCBI database.
[c)] Theoretical molecular weight; [d)] Peptide size (amino acids) (recorded in NCBI database).
[e)] The MASCOT baseline significant score is 70.
[f)] Number of matched peptides.

Table 2. Amino acid sequences of three major peptides obtained from each MyHC isoforms

MyHC[a)]	Observed mass (Da)[b)]	Calculated mass (Da)[c)]	Residues	Peptide
2a	1153.6647	1152.6291	717 - 725	ILYADFKQR
2x	1153.6680	1152.6291	715 - 723	ILYADFKQR
2b	1153.6377	1152.6291	716 - 724	ILYADFKQR
Slow	1167.6488	1166.6196	713 - 721	ILY**G**DF**R**QR
2a	1702.8822	1701.8243	1425 - 1438	LQNEVEDLMLDVER
2x	1702.8676	1701.8243	1424 - 1437	LQNEVEDLM**I**DVER
2b	1702.8372	1701.8243	1423 - 1436	LQNEVEDLMLDVER
Slow	1702.8940	1701.8243	1421 - 1434	LQNE**I**EDLM**V**DVER
2a	1607.8186	1606.7694	1597 - 1610	VVESMQS**M**LDAEIR
2x	1607.8240	1606.8167	1596 - 1609	**I**VESMQSTLDAEIR
2b	1593.7721	1592.7715	1595 - 1608	VVESMQSTLDAEIR
Slow	1533.7830	1532.7682	1593 - 1606	VV**D**S**L**QSTLDAE**T**R

[a)] Myosin heavy chain.
[b)] Molecular ion mass observed in the MALDI-TOF/TOF system.
[c)] Calculated relative molecular mass of the matched peptide.

Previous other studies documented that one band showed only one MyHC [4-7]. However, our results showed different migration pattern of the MyHC, although the MyHC separation method using gel electrophoresis was similar to other previous studies. As shown in Table 1, migrated bands had many matched peptides (26-48 peptides) and considerably high MASCOT scores (the baseline significant score was 70). Moreover, the sequences of two peptides were identified.

Three major peptides in same residues were selected to identify sequences of the peptide peaks from individual MyHC bands, and their amino acid sequences were compared to each other (Table 2). Residue from 717 to 725 in the MyHC-2a peptide corresponded to the 715-723 residue of MyHC-2x, the 716-724 residue of MyHC-2b and the 713-721 residue of MyHC-slow. However, these amino acid sequences were not corresponded to MyHC-slow sequence that was 'ILYADFKQR'. MyHC-slow showed different amino acids 'G' and 'R' in residues 716 and 719, respectively. Moreover, residues 1433 (I) and 1596 (I) of MyHC-2x, and 1604 (M) of MyHC-slow were not same as those of the others.

Four MyHC bands were detected by MAbs such as BA-D5, BF-F3, BF-35 and SC-71. BA-D5 reacted with two MyHC-slow bands which were Line 3 and Line 4 as presented in Figure 1-b. BF-F3 also reacted with two bands (Line 1 and Line 2) which were identified as MyHC-2b (Figure 1-c). BF-35 reacted strongly with two bands of MyHC-slow (Line 3 and Line 4) and slightly reacted with MyHC-2a (Line 1) (Figure. 1-d). Line 1 and Line 2 were stained with SC-71 which reacted with MyHC-2a and -2x (Figure. 1-e). We confirmed that two MyHCs were co-expressed in one band as identified by MALDI-TOF/TOF mass spectrometry (Table 1).

IV. CONCLUSION

Two MyHCs were co-expressed in one band and one MyHC was separated into two bands in this study. Mass spectrometry is very useful for the accurate identification of MyHC isoforms separated by gel electrophoresis, because the migration rate and pattern of MyHC isoforms are varied by separating method and animal species,

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