INTERSPECIES DIFFERENCES IN SKELETAL MUSCLE MYOSIN LIGHT CHAINS ISOFORMS BETWEEN CATTLE, PIG AND SELECTED POULTRY SPECIES

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***Abstract*—The aim of the study was to compare the expression of selected skeletal muscle proteins of six animal species (cattle, pig, chicken, turkey, duck and goose), the meat of which is most often consumed in Europe, and to reveal differences between them. This paper presents the interspecies differences detected in range of the myosin light chains (MLC1f, MLC2f, MLC3f). Proteins extracted from the muscle tissue were separated using two-dimensional electrophoresis. The separations revealed the differences between examined species in molecular weight (MW) and isoelectric point (pI) values of the fast essential as well as the fast regulatory myosin light chains. In the case of cattle and pig MLC1f isoforms had a similar pI values, but differed in MW up to 2.4 kDa. All poultry species (chicken, turkey, duck, goose) also differed in MW of the MLC1f. Only the MLC1f extracted from goose had the same MW like pig but its pI value was more alkaline (4.91) in relation to pig (4.79). Apparent differences were observed for proteins matching to MLC2f isoform. These proteins had similar pI values for all examined species but differed in MW. MLC3f isoforms also differed in their MW. The MLC3f extracted from chicken had the highest MW (17.4 kDa). The lowest one was found in duck muscles (16.1 kDa). The cause of that phenomenon might be slightly different their amino acid composition.**

***Index Terms*— MLC isoforms, species identification, two-dimensional electrophoresis**

I. INTRODUCTION

Myosin plays a key role in shape-maintaining and movement of eucariotic cells. It is the best-known motoric protein (molecular motor). Myosin has the ability to convert chemical energy into mechanical one through a conformational change – a contraction, which is possible due to the release of energy from ATP hydrolysis. At present, as many as 15 distinct classes of myosin were indentified, of which the role of molecular motor in muscle contraction perform class II myosins which are presented in muscle fibres, platelets, epithelial gastroenteric cells or neurons (Reggiani, Bottinelli and Stienen, 2000).

Skeletal muscles differ in their contractile properties. These differences occur not only between species but also between muscles within a single organism. The reason of that is slightly different muscle fibres structure due to the presence of different myofibrillar proteins isoforms, including myosin. In adult mammals fast fibres of skeletal muscles include three MHC isoforms (MHC-2A, 2X and 2B) and these in turn the fast alkali/essential two isoforms (MLC1f and MLC3f) and the fast regulatory isoform (MLC2f). Skeletal muscles contain also few slow fibres (type I) with the MHC-I isoform, which is associated with the slow alkali isoform (MLC1s) and the slow regulatory isoform (MLC2s) (Bottinelli, 2001; Andruchov, Andruchova, Wang and Galler, 2006).

Currently detailed information about the characterization of biochemical, energetic and mechanical parameters or sequences of sarcomeric myosins are available for only several species, among mammals for rat, rabbit and human, among birds for chicken. The amino acid sequences of rat skeletal muscles indicate that the myosin fast regulatory light chains (RLC) type I and II are identical in 98.8% (Szczesna-Cordary, 2003). The role of MLC isoforms is much less known in comparison with the MHC (Andruchov et al., 2006). At present it is believed that the RLC plays a physiological role in the regulation of contraction, probably through its phosphorylation but the exact mechanism of that process is still not known. An influence may have a functional coupling between phosphorylation and Ca^{2+} binding to RLC (Szczesna-Cordary, 2003).

In recent years a significant progress has been made in the methodology used in proteomic research. A considerable number of papers based on 2-DE and MS techniques in mapping of skeletal muscle proteins and examining connection between the content of protein and meat quality has been published. In case of farmed animals most of the papers concerned pigs and cattle, some - chicken, whereas a few of them concerned other poultry. Thus, the aim of the study was to compare the expression of skeletal muscle proteins, especially in the range of the myosin light chains of six animal species (cattle, pig, chicken, turkey, duck and goose), the meat of which is most often consumed in Europe, and to distinguish differences between them. Displaying the interspecies differences may also contribute to better

understanding of the structure and function of skeletal muscle proteins, as well as the mechanisms affecting the processes of meat aging.

II. MATERIALS AND METHODS

Five animals of six species: cattle (*Bos taurus*), pig (*Sus scrofa*), chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*), duck (*Anas platyrhynchos*) and goose (*Anser anser*) were used in this study. Both feeding and rearing conditions of the animals were controlled. Samples of *longissimus* muscle (LM) or *pectoralis* muscle (PM) were harvested within 45 min *post mortem*, immediately frozen in liquid nitrogen and then stored at -80°C until subsequent analysis.

Muscle sample of 0.1 g was homogenized in 1 ml extraction buffer (7 M urea, 2 M thiourea, 4% CHAPS, 2 % carrier ampholytes, 40 mM DTT, protease inhibitor mixture) using Ultra-Turrax T25 at 9500 rpm 2 x 20s. The homogenate was centrifuged (1h at 11000 rpm) at 10^{o}C. Protein concentration was determined using the 2-D Quant Kit (GE Healthcare). The samples containing 90 µg of protein were loaded onto the strips.

The separation in the first dimension (IEF) was carried out on an Immobiline DryStrip pH 3-10 and 4-7, 24 cm long (GE Healthcare) using the Ettan IPGphor 3 (GE Healthcare). Proteins were diluted to 450 µl with DeStreak Solution supplemented with 0.5% IPG Buffer pH 3-10 (GE Healthcare) and used for overnight rehydration of the IPG DryStrip. For the subsequent IEF, voltage was increased gradually up to 8000 V until reaching a total of 70000 Vh.

The two-dimensional electrophoresis (2-DE) was carried out on 15% SDS-polyacrylamide gels (200 x 260 x 1 mm) using Ettan Daltsix Large Vertical System (GE Healthcare). Strips were equilibrated in 6 M urea, 50 mM Tris-HCl pH 8.8, 80% glycerol, 2% SDS, 0.002% bromophenol blue and 1% DTT for 15 min, followed by another 15 min in the same buffer, but with 2.5% iodoacetamide instead of DTT, and then applied to the top of SDS-PAGE gels and sealed with 0.5% agarose. Electrophoresis was carried out at 1 W/gel for 45 min and 7 W/gel until the front reached the bottom of the gel. Gels were stained with silver nitrate and scanned using ImageMaster Scanner and analyzed using ImageMaster 2D Platinum 7.0 software.

III. RESULTS AND DISCUSSION

Two-dimensional electrophoretic separations of skeletal muscle proteins extracted from six species: cattle, pig, chicken, turkey, duck and goose, revealed the differences between examined species in molecular weight (MW) and isoelectric point (pI) values of the myosin light chains (Figure 1, Table 1). In the case of cattle and pig the MLC1f isoform (spot 1519) had a similar pI values, but differed in MW up to 2.4 kDa. All poultry species (chicken, turkey, duck, goose) also differed in MW of the MLC1f. Only the MLC1f extracted from goose had the same MW like pig but its pI value was more alkaline (4.91) in relation to pig (4.79). Apparent differences were observed for proteins described as spots 1595 and 1590, matching to MLC2f isoform. The amount of protein 1595 was especially lower in poultry than in cattle and pig meat. These proteins had similar pI values for all examined species but differed in MW. MLC3f isoforms (spot 1626) also differed in their MW. The MLC3f extracted from chicken had the highest MW (17.4 kDa). The lowest one was found in duck muscles (16.1 kDa). It is worth to mention that the spot 1618 was plainly visible in mammals, whereas only a trace amount of this protein was found in poultry. The correctness identification of the above discussed spots was confirmed by comparing our results with the data from literature, for beef (Bouley, Chambon and Piccard, 2004; Muroya, Ohnishi-Kameyama, Oe, Nakajima, Shibata and Chikuni, 2007) and for pork (Hwang, Park, Kim, Cho and Lee, 2005).

A few slow-twitch fibers exist in the *longissimus* and *pectoralis* muscles. Spots 1455 and 1580 correspond to the slow-twitch MLC1s and MLC2s isoforms, respectively. The MLC1s was found in pigs in the *diaphragm* and *soleus* muscles (Bicer and Reiser, 2004), while the MLC2s in the *longissimus* muscle (Muroya et al., 2007). The spot 1455 may be the same as MLC1sb in cat skeletal muscle reported by Bicer and Reiser (2004). Our results confirm that there are different isoforms of MLC even between closely related animal species, such as goose and duck. A slightly different amino acid composition is likely to be the cause of that phenomenon. Dalla Libera and Carpené (1997) comparing the content of myosin light chains isoforms in chicken and wild birds PM separated by SDS-PAGE technique found no apparent differences in electrophoretic mobility of MLC2f and MLC3f. The differences occurred only in MLC1f of jay and woodcock. However, the percentage contents of analyzed isoforms were various. The share of MLC3f was higher and of MLC2f was lower in chicken in comparison to wild birds.

LM in cattle is composed mainly of the slow-twitch-oxidative-red fibres, and in pig's case the fast-white fibres dominate in approx. 90%. Pectoralis muscle of chicken is composed almost entirely of the fast-white fibres, while in wild avian species the fast-red fibres dominate. Former studies inform that the light chains of fast-white and fast-red myosins in guinea-pig muscles contain the same type of LC1f, LC2f and LC3f with the same molecular weight and pI values (Dalla Libera, Sartore, Pierobon-Bormioli and Schiaffino, 1980). Our 2-DE separations showed there is a diversification in MW and pI values within the MLC extracted from LM of cattle and pig, which may indicate that these isoforms, derived from red and white fibres may have various structures, what can be the base to differentiate species.

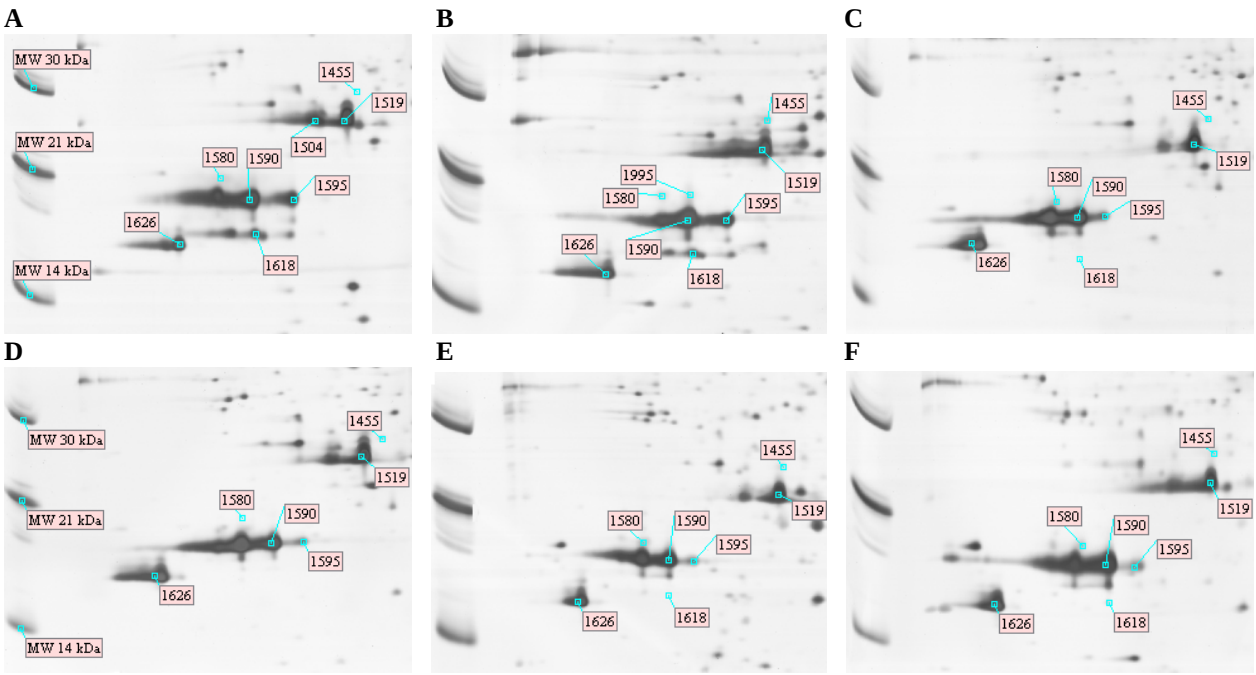

Figure 1. Pictures of representative 2-DE gels, protein spots found 45 min *post mortem*, pH range of 4-7. A – cattle, B – pig, C – chicken, D – turkey, E – duck, F – goose. Spots 1519, 1626 – the fast essential myosin isoforms (MLC1f and MLC3f, respectively); 1595, 1590 – the fast regulatory myosin isoforms (MLC2f); 1455, 1580 – the slow alkali isoform (MLC1s) and the slow regulatory isoform (MLC2s).

Table 1. Experimental pI and MW (kDa) of chosen spots

Spot	Cattle	Pig	Chicken	Turkey	Duck	Goose
1519	4.86/26.8	4.79/24.4	4.89/24.0	4.89/25.4	4.88/23.3	4.91/24.4
1595	4.71/19.8	4.71/19.0	4.65/19.0	4.72/18.7	4.65/18.5	4.73/18.6
1590	4.62/19.9	4.61/19.0	4.59/19.1	4.66/18.7	4.58/18.5	4.66/18.7
1626	4.41/17.2	4.39/16.2	4.34/17.4	4.37/16.9	4.34/16.1	4.37/16.3

IV. CONCLUSION

The obtained results showed differences in pI values and MW of the fast essential as well as the fast regulatory myosin light chains (MLC1f, MLC2f and MLC3f). The existence of different isoforms of MLC even between closely related animal species, such as goose and duck, was revealed. A slightly different amino acid composition is likely the cause of that phenomenon.

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