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MYOSIN LIGHT CHAINS (MLCs) HETEROGENEITY IN MAMMALIAN SMOOTH AND CARDIAC MUSCLE

R. K. MALHOTRA, SURENDER S. KATOCH*, RAMESH KUMAR AND AMBRISH SOOD

Department of Biosciences,
Himachal Pradesh University,
Summer Hill, Shimla -171 005

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Abstract: A comparative study of myosin light chains (MLCs) has been made in the aorta, uterine and cardiac muscles (auricle, ventricle) of mice, pig, sheep and goat. Analysis of myosin light chains by sodium dodecyl polyacrylamide gel elctrophoresis (SDS-PAGE) has revealed that (a) aorta myosin from mice, goat and pig has identical myosin light chains profile but pig aorta myosin lacks LC-2_f; (b) uterine smooth muscle myosin depicts absence of LC-1_f in sheep. Whereas satellite bands of LC-1_f and LC-2_f fractions are absent in pig uterine myosin, mice shows duplets for both the myosin light chains; (c) the auricular myosin in pig and goat is identical to chicken gizzard myosin used as reference and exhibits ALC-2_s and ALC-1_s fractions only while sheep auricular myosin lacks in ALC-1_f; (d) the mice ventricular myosin depicts two satellite MLCs associated with fast migrating VLC-1_s.

Key words: myosin light chains, uterus, isoforms, cardiac, slow-fast, aorta

INTRODUCTION

Myosin, one of the several myofibrillar proteins, is a major structural and functional component of contractile apparatus. The myosin macromolecule comprising two myosin heavy chain polypeptides (MHC; Mr. 220,000) and two pairs of light chains (MLC_s; Mr. 17,000 & 20,000) (1), expresses isoform variants in smooth as well as cardiac muscles (2, 3). Whereas the MHC isoforms have been studied in considerable details in a number of vascular and non-vascular smooth muscles (4-9) and cardiac muscles (10-12), the pattern of expression of MLC isoforms in these tissues have not been investigated in depth. The available data establishes the existence of 'light chain heterogeneity' in smooth and cardiac muscles (8, 9, 13-16) which is confirmed through studies employing atlernate splicing mechanism (17) and cDNA sequencing (18).

Myosin light chains (MLCs) form an all important functional component of the macromolecule. Both 17 kDa an 20 kDa myosin light chains exhibit isoformic variants. In mammalian vascular smooth muscle 17 kDa myosin light chain demonstrates two electrophoretically different components called as LC_{17}a and LC_{17}b (19, 20). Enzymatic and functional properties of actomyosin complex are modulated by a specific ratio between these two isoforms (9, 21). The regulatory myosin light chain (LC_{20} kDa) that plays a crucial role in the initiation of contraction (22, 23) and control the actinactivated ATPase activity (for review see 8, 9) has been reported to reveal two different isoforms in arterial smooth muscle (24) and three in chicken gizzard (25, 26).

This communication presents observations on myosin light chain heterogeneity in aortic and uterine smooth muscles and the cardiac (auricle, ventricle) muscles of mice, pig, sheep and goat. These functionally different muscles have been studied to understant the relationship

*Corresponding Author

if any, between the heterogenetic pattern of MLCs and the physiological differences among different muscle types.

METHODS

The study was conducted on aortic smooth muscle, uterine smooth muscle and cardiac muscles (auricle and ventricle separately) from goat, sheep, pig and mice. Fresh muscles from animals listed above were collected at the local slaughter house within minutes of their killing. Swiss albino mice of *lacca* strain were procured from Central Research Institute, Kasauli (H.P.) The muscles samples were washed extensively and brought to the laboratory in ice cold physiological salt solution (27) maintained at a pH of 7.45. The physiological salt solution comprised (in mM): NaCl (118.00), KCl (5.00), $MgCl_2$ (1.2), $CaCl_2$ (1.6), Na_2HPO_4 (1.2), glucose (10.00) and $NaHCO_3$ (23.00). The intimal surfaces of dorsal aorta and uterus were thoroughly rubbed with cotton gauge to remove adventitia and myometrium. The tissue were finally stored at —20°C and processed further, the same day.

All preprations were made on ice. Muscle tissues were homogenized in sorrenson buffer containing 50 mM Na_2HPO_4 containing 1% SDS and 50 mM KH_2PO_4 with 1% SDS in the ratio of 7:1. The homogenate was centrifuged at 6000 rpm, for 10 min and the supernatant employed in electrophoretic studies. Protein concentration was estimated following Lowry's method (28). Equal amount of proteins were loaded into the gel. The different myofibrillar proteins were resolved on 7.5-20% gradient polyacrylamide slab gel as per Laemmli (29). Chicken gizzard actomyosin prepared as per Sobieszek and Bremel (30) was employed as reference marker to the myosin light chains. Twin system Biometra (Germany) slab gel electrophoresis apparatus was used to separate the myofibrillar proteins. After electrophoresis, the gels were stained in Coomassie brilliant blue R 250 stain (Bio Rad, USA). Reproducible myosin light chain pattern was photographed.

RESULTS

The distribution pattern of MLCs in different muscles are presented in Figures 1-4.

Aortic myosin (Fig. 1) from mice exhibited four myosin light chains ($LC\text{-}2_s$, $LC\text{-}2_f$, $LC\text{-}1_s$ and $LC\text{-}1_f$) in order of increasing electrophoretic mobilities. Pig aorta myosin revealed only three bands corresponding to $LC\text{-}2_s$, $LC\text{-}1_s$ and $LC\text{-}1_f$. This myosin is characterized by an absence of $LC\text{-}2_f$. Aortic myosin from goat mimicked the MLC pattern of mice but differed in demonstrating conspicuously low contents of $LC\text{-}2_f$ and $LC\text{-}1_s$. Myosin from sheep aorta exhibited all four MLCs. The $LC\text{-}2_s$ and LC-1 were recorded as prominent bands whereas $LC\text{-}2_f$ and $LC\text{-}1_s$ were noticed as minor fractions.

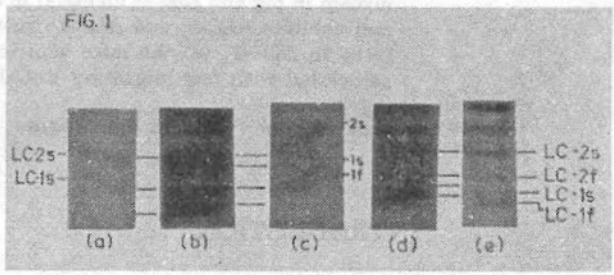

Fig. 1 : Low molecular weight regions of coomassie blue stained 7.5-20% SDS-PAGE gels loaded with aortic myofibrillar preparations from (a) chicken gizzard (as reference maker), (b) mice, (c) pig, (d) goat and (e) sheep.

Uterine smooth muscle myosin (Fig. 2) from pig and goat (Fig. 2 c, d) exhibited a typical gizzard myosin like MLC profile. As such only $LC\text{-}2_s$ and $LC\text{-}1_s$ were distinctly recorded. Uterine myosin from mice (Fig. 2 b) displayed one additional band each, associated with two main myosin light chains as is characteristic to gizzard myosin. Thus overall four MLCs i.e., $LC\text{-}2_s$, $LC\text{-}2_f$, $LC\text{-}1_s$ and $LC\text{-}1_f$ were invariably observed in these muscles ($LC\text{-}2_f$ and $LC\text{-}1_f$ were minor fractions). The myosin from sheep uterus (Fig. 2 e) demonstrated a different picture. It resembled pig and goat myosin in the absence of $LC\text{-}1_f$ but exhibited $LC\text{-}2_f$ fraction.

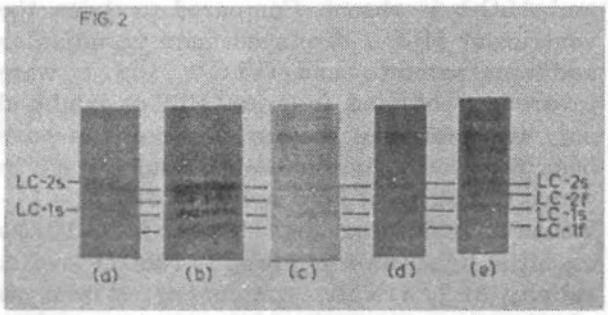

Fig. 2 : Stained gels with uterine myofibrillar preparations showing myosin light chains (a) gizzard smooth muscle, (b) mice, (c) pig, (d) goat and (e) sheep.

The cardiac myosins (Fig. 3) from pig and goat auricles (Fig. 3 b, c) were identical. No differences in MLCs of left and right auricles could be discerned. Such myosins displayed two MLCs i.e., ALC-2_s and ALC-1_s. Auricular myosin obtained from mice (Fig. 3a), however, depicted four MLCs (ALC-2_s, ALC-2_f, ALC-1_s and ALC-1_f in order of increasing electrophoretic mobilities). ALC-2_f and ALC-1_f were however found to be present in minor amounts. Myosin obtained from left and right auricles of sheep (Fig. 3d) were similar in their MLCs pattern but were typically characterized by an absence of ALC-1_f. Thus sheep auricular myosin differed from that of pig and goat by exhibiting an additional ALC-2_f and also from mice auricles by the absence of ALC-1_f.

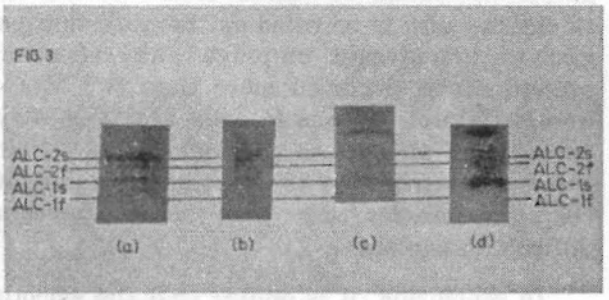

Fig. 3 : SDS-PAGE gradient gels (7.5-20%) showing myofibrillar preparations from auricles of (a) mice, (b) pig, (c) goat and (d) sheep.

ALC-1_s and ALC-1_f : LC_{17} slow and fast.

ALC-2_s and ALC-2_f : LC_{20} slow and fast.

Ventricular myosin (Fig. 4) exhibited maximum variation in MLC distribution pattern among different mammalian species under investigation. Compared to the standard actomyosin preparation from chicken gizzard which exhibited only two MLCs, the ventricular myosin from mice (Fig. 4a) displayed as many as five light chains. These corresponded to VLC-2_S, VLC-2_f, VLC-1_S, VLC-1_{si} and VLC-1_{sii}. The pig ventricular myosin (Fig. 4b) revealed three MLCs in equal proportions designated as VLC-2_S, VLC-2_f and VLC-1_S and thus differed from that of mice by the absence of VLC-1_f. Goat ventricular myosin (Fig. 4c) displayed only two prominent MLCs viz., VLC-2_S and VLC-1_S. A third band in minor proportions (less than 5%) could also be distinguished between these two MLCs. Sheep ventricular myosin (Fig. 4d) also exhibited three bands which corresponded to VLC-2_S, VLC-2_f and VLC-1_S.

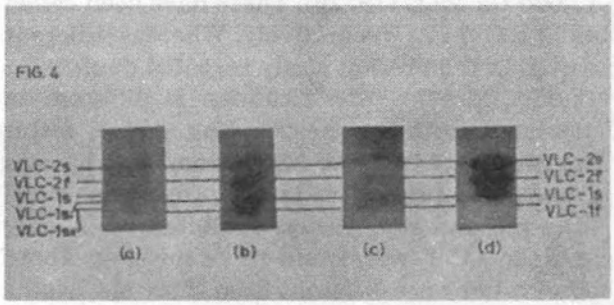

Fig. 4: SDS-PAGE gradient gels (7.5-20%) of myofibrillar preparations from ventricles showing light regions (a) mice, (b) pig, (c) goat and (d) sheep.

VLC-1_s and VLC-1_f : LC_{17} slow and fast.

VLC-2_s and VLC-2_f : LC_{20} slow and fast.

DISCUSSION

The present observation confirm the existence of the phenomenon of polymorphism in myosin light chain subunits in different muscles of four mammalian species investigated. The expression of this kind of structural dissimilarity among muscles belonging to different categories (dorsal aorta, uterus, auricular and ventricular muscles) is deduced

from varying number of bands obtained in myosin light chain region. The number of such bands may vary from three to four in auricles, four in aorta and uterine smooth muscle to as many as five in ventricles.

Two variants of 17,000 Da MLCs have been reported in mammalian vascular and non-vascular smooth muscles (19, 20, 30, 31) which has subsequently been confirmed from alternate splicing (17) and nucleotide sequencing (18). By and large, the muscles studied during the present investigation also demonstrate more than one myosin light chain corresponding to 17,000 Da region. These have been designated as LC-1_s and LC-1_f or ALC-1_s, ALC-1_f, VLC-1_s and VLC-1_f. The differences observed in MLC profiles in differnt animals indicate that the presence of 17,000 Da MLC duplets as seen in smooth muscle of aorta, may not be a universal phenomenon. Myosin prepared from bovine arterial smooth muscle display two isoforms of 17,000 Da MLC (19, 20). These have been called as 17 a and 17 b respectively. Whereas different aortae in the present study revealed duplets for 17,000 Da MLC, the situation is different in uterine smooth muscles. Among aortae, faster migrating 17,000 Da MLC from (LC-1_f) was found to be present in larger proportion. Uteri from pig and goat always exhibited LC-1_s only and the LC-1_f was found to be missing. These observations are different from those of Cavellie et al (30) who have reported two isoforns of 17,000 Da MLC in rat, monkey and human uterine smooth muscles. Myosin light chain lying in 20,000 Da region also exhibited animal to animal differences but duplets were invariably recorded suggesting different forms of this MLC. Such differences extend from an absence of LC-2_f in pig aorta to presence of additional LC-2_f in uterine MLCs. Whether or not these multiple forms of 20,000 Da MLC reflect physiological states of muscle is difficult to interpret.

The study on the MLCs in myocardial tissue of the four animals shows that the auricles of pig and goat have only two myosin light chains (ALC-2_s and ALC-1_s). On the other hand, sheep auricular myosin exhibits three light chains and ALC-1_f is absent. Compared to these, the ventricular MLC-1 displayed more variation as additional satellite bands (VLC-1_{si}, VLC-1_{sii} were invariably obtained in mice. VLC-2 exhibited only two isoforms. Similar observations have been recorded in human ventricular myosin in which two forms of LC-1 and single form of LC-2 exist in 18,000 Da range (13, 14). MLC-1 is an essential light chain required to control myosin activity (8). The relative contents of two isoforms of MLC17 as reported earlier (19, 20) regulate actin activated myosin ATPase activity of tissue. The actin activated Mg^{+2} ATPase activity of bovine smooth muscle are proportional to LC_{17a} content. The activity declines with increasing LC_{17b} (21). This seems to be more relevant in ventricular muscle which plays a prominent role in the propulsion of blood. Interestingly the MLCs in this muscle display larger variation. The heterogeneity in MLC-1 as well as MLC-2 components of ventrucular myosin as observed in the present study may thus reflect probably the functional status of the tissue. Variation in the contents of two different isoforms would be responsible for differential and regulated but forceful propulsion of blood.

The possibility of proteolyzed fragments of myosin light chains appearing as multiple forms has to be ruled out because the separation of myofibrillar proteins was conducted immediately on ice following the method of sobieszek and Bremel (32). Similarly a contribution towards additional bands of MLCs from non-muscle source has also to be ruled out because chicken gizzard actomyosin employed as reference protein never displayed more than two MLCs (n=5). While non-muscle cells are invariably present in all muscles under study, their selective contribution towards additional light chains in muscles other than gizzard, appears difficult to explain.

In conclusion, it is opined that the smooth muscles and cardiac muscles express different isoforms of myosin light chains and these variations probably accrue from the differences in kind of functions performed by muscles. Unlike sarcomeric skeletal muscles, smooth

muscle lacks structural differentiation into differing cell types and hence expression of isoform variants in latter category can be explained to denote the functional differences among different smooth muscles.

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