Volume 219, number 2, 469–471 FEB 04950 July 1987

Myosin light chain phosphorylation in intact human muscle

M.E. Houston, M.D. Lingley, D.S. Stuart and R.W. Grange

Department of Kinesiology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

Received 22 April 1987

The phosphate content of the fast (LC2F) and two slow (LC2S and LC2S[1]) phosphorylatable light chains (P-light chains) in myosin isolated from biopsy samples of rested human vastus lateralis muscle averaged 0.21, 0.28 and 0.25 mol of phosphate per mol of P-light chain, respectively. Following a 10 s maximal contraction, phosphate content was increased by almost 2-fold in the fast and two slow P-light chains. After prolonged, moderate cycling activity phosphate content was only slightly increased in the three P-light chains. These data suggest that, unlike animal skeletal muscle, myosin light chain kinase and phosphatase activities are similar in human fast and slow muscle fibres.

Myosin light chain; Contractile activity; Myosin light chain phosphorylation; (Human skeletal muscle)

1. INTRODUCTION

Skeletal muscle myosin contains a class of light chains (18–20 kDa) that can be phosphorylated by myosin light chain kinase (MLCK) in a Ca^{2+}-dependent manner. The MLCK is activated by Ca^{2+}-bound calmodulin, and activation occurs when the Ca^{2+} concentration is increased to the level found in contracting muscle [1]. Dephosphorylation of the phosphorylatable light chain (P-light chain) is catalyzed by a myosin phosphatase. Phosphorylation of P-light chains has been correlated with potentiation of the isometric twitch tension in fast skeletal muscle of the rat [2] and rabbit [3]. Twitch potentiation accompanied by P-light chain phosphorylation has not been observed in slow skeletal muscles, the reason for which is attributed mainly to a reduced MLCK activity and increased phosphatase activity [2,3].

In the human vastus lateralis muscle, composed of an approximately equal mix of fast and slow twitch fibres, P-light chain phosphorylation was markedly increased in isolated fast myosin following a brief maximal, voluntary contraction [4]. However, there was no clear evidence of phosphorylated P-light chains isolated from the slow myosin. The purpose of this study was to re-investigate the phosphorylation of fast and slow P-light chains following two forms of contractile activity utilizing the in vivo human model.

Correspondence address: M.E. Houston, Department of Kinesiology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

2. EXPERIMENTAL

Muscle samples were obtained from the vastus lateralis portion of the quadriceps muscle group of 15 male and female volunteers using the needle biopsy technique [5]. Muscle samples, taken at rest or immediately following a 10 s maximal voluntary isometric contraction or after 60 min of cycling on a stationary ergometer at a moderate intensity, were rapidly frozen in liquid nitrogen. Two muscle samples from rested muscle were immediately placed in an oxygenated Ringer's solution [6] maintained at 30°C for 20 min, then rapidly frozen with aluminum tongs cooled in liquid nitrogen.

Muscle samples (6–10 mg) were homogenized at 0°C in a glycerol-pyrophosphate solution [7]. Gel

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

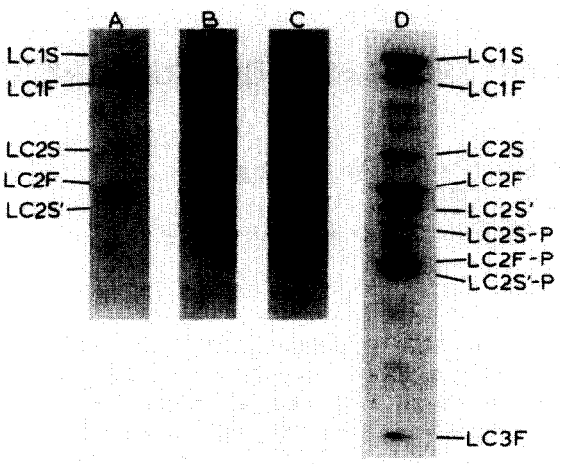

Fig.1. Isoelectric focusing gel showing nonphosphorylated and phosphorylated light chains of myosin from human vastus lateralis muscle. Biopsy samples from rested muscle were immediately frozen or incubated in oxygenated Ringer's solution, then frozen. Native myosins from homogenates were isolated from pyrophosphate-polyacrylamide gels and the light chains were separated by isoelectric focusing under denaturing conditions. Lanes A, B and C: light chains from fast, whole and slow myosin, respectively, prepared from rested, incubated muscle samples. Lane D: light chains in whole myosin from rapidly frozen, rested biopsy sample.

slices representing whole myosin or fast and slow myosin isozymes were isolated from pyrophosphate-polyacrylamide gels following electrophoresis for 3 or 20 h, respectively [4]. Whole myosin or fast and slow isomyosins were subjected to isoelectric focusing [4] using a pH range of 4.5–6.0. After silver staining, individual P-light chains and their phosphorylated derivatives were quantified using a laser densitometer.

3. RESULTS AND DISCUSSION

The isoelectric focusing pattern of light chains from whole human myosin or isolated fast and slow isomyosins are shown in fig.1. We found that whole myosin obtained from rapidly frozen biopsy samples contains a mix of fast and slow P-light chains (LC2F, LC2S and LC2S[1]) and their phosphorylated derivatives (LC2F-P, LC2S-P and LC2S[1]-P). Fast and slow myosin isolated from muscle samples frozen after incubation in Ringer's solution do not show evidence of the phosphorylated forms of the fast and two slow P-light chains. Presumably, the phosphatase has adequate time to catalyze dephosphorylation during the 20 min incubation period for the relaxed tissue samples.

In an earlier study [4] in which slow and fast isomyosins were isolated before isoelectric focusing, we were unable to clearly determine phosphorylated forms of the two slow P-light chains. This was partly due to the difficulty in isolating slow isomyosins without contamination by fast light chains, the poor recovery of slow

Table 1

Effect of muscle activity on additional phosphate incorporation into human skeletal muscle myosin P-light chains

Contractile activity	*n*	mol phosphate/mol P-light chain		
		LC2F	LC2S	LC2S[1]
Maximal contraction	8	0.40 ± 0.24	0.41 ± 0.21	0.35 ± 0.20
60 min cycling	5	0.06 ± 0.03	0.10 ± 0.03	0.08 ± 0.02

Muscle biopsy samples were obtained from the vastus lateralis muscle after a 10 s maximal isometric contraction or after 60 min of cycling exercise at a moderate intensity. The phosphate content of the fast (LC2F) and two slow (LC2S and LC2S[1]) P-light chains was determined in isolated myosin as described in section 2. Phosphate incorporation values represent the differences between the extent of phosphate content in P-light chains in rested muscles. Data are reported as means ± SD, and *n* represents the number of human subjects for each condition

myosin from the pyrophosphate gels and also because of the technique used for isoelectric focusing. Using a narrower pH range and a wider resolving width, clear separation is possible. Indeed, as fig.1 illustrates, we can now resolve all forms of the P-light chains from whole human myosin in one lane of an isoelectric focusing apparatus. However, it should be noted that LC3F is not always apparent in our human muscle samples; this point has been acknowledged [8]. Moreover, a minor form of LC1S, identified as LCS1a, is not apparent in our system since it has a p*I* value >6 [8].

The effects of two forms of muscle activity on phosphate incorporation into the fast and two slow P-light chains is illustrated in table 1. In rested vastus lateralis muscle from 13 subjects, phosphate content of LC2F, LC2S and LC2S[1] averaged 0.21 ± 0.07, 0.28 ± 0.09 and 0.25 ± 0.09 (mean ± SD) mol of phosphate per mol of P-light chain. Immediately after the 10 s maximal voluntary isometric contraction, phosphate content of the individual P-light chains was more than twice that observed for rested muscle in these subjects. Following 60 min of moderate level cycling activity, P-light chain phosphate content was only slightly above that found in rested human muscle. This slight increase is probably due to the contraction force of less than 25% maximal necessary to sustain the cycling exercise we employed [9].

The results of this study reveal that there are significant differences between human and animal skeletal muscle in terms of P-light chain phosphorylation. The slow P-light chains in the relaxed slow soleus muscle of rat and rabbit show virtually no phosphate content, whereas the P-light chains of fast rat and rabbit muscles contain 0.1–0.2 mol of phosphate per mol of P-light chain [2,3]. Moreover, while a brief tetanic contraction causes a marked phosphate incorporation into fast P-light chains, slow myosin from the rat and rabbit is phosphorylated only to a modest extent following a prolonged tetanus [2,3]. These observations are consistent with the greatly reduced MLCK activity and the larger myosin phosphatase activity in slow compared to fast skeletal muscles [2]. On the other hand, the data we report for rested and active human skeletal muscle suggest that MLCK and myosin phosphatase activity may be similar in fast and slow twitch fibres of man.

ACKNOWLEDGEMENTS

This study was supported by grants provided by the Medical Research Council and the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

[1] Blumenthal, D.K. and Stull, J.T. (1980) Biochemistry 19, 2386–2391.
[2] Moore, R.L. and Stull, J.T. (1984) Am. J. Physiol. 247, C462–C471.
[3] Moore, R.L., Houston, M.E., Iwamoto, G.A. and Stull, J.T. (1985) J. Cell. Physiol. 125, 301–305.
[4] Houston, M.E., Green, H.J. and Stull, J.T. (1985) Pflügers Arch. 403, 348–352.
[5] Bergstrom, J. (1962) Scand. J. Clin. Lab. Invest. 14 (suppl.68), 10–13.
[6] Stevens, J.C., Dickinson, V. and Jones, N.B. (1980) Med. Biol. Eng. Comput. 18, 1–9.
[7] Silver, P.J. and Stull, J.T. (1982) J. Biol. Chem. 257, 6137–6144.
[8] Salviati, G., Betto, R., Danieli-Betto, D. and Zeviani, M. (1983) Biochem. J. 224, 215–225.
[9] Citterio, G. and Agostoni, E. (1984) J. Appl. Physiol. 57, 371–379.