Phosphorylation of the 18,000-Dalton Light Chain of Myosin during a Single Tetanus of Frog Muscle*

(Received for publication, April 15, 1977, and in revised form, May 19, 1977)

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SUMMARY

Changes in the 32P content of proteins due to muscle contraction were investigated, using muscles dissected from liver frogs injected with [32P]orthophosphate. The only significant change found was in the radioactivity of the 18,000-dalton light chain of myosin; during a single tetanus, an increase of 85 to 90% occurred as compared to the resting muscle. This increase corresponded to about 0.4 mol of 32P per mol of light chain. The same increase in radioactivity of this light chain was also found upon caffeine-induced contraction of the intact muscle. It is postulated that the increased Ca2+ concentration in the sarcoplasm resulting from 5.0 mCi of carrier-free [32P]orthophosphate and were left at room temperature for periods of 2 to 3 days. The frogs were then pithed, and the paired sartorius and semitendinosus muscles were dissected. The muscles were clamped at rest length in the electrode assembly which was adapted to allow freezing during isotonic contraction as previously described (11). One muscle, lifting a 5.0-g weight, was given tetanic stimulation at 25°C for 30 s (frequency 30 pulses/s), and then immersed into isopentane chilled by liquid nitrogen. The paired muscle was treated and frozen under identical conditions. Without stimulation. The pooled frozen muscles from three such runs were pulverized in a special all-stainless steel apparatus designed to allow all manipulations to be carried out under liquid nitrogen cooling. First, 6 ml of the appropriate homogenizing solution (see below) was frozen over a smooth surface in a cold mortar. The muscles were placed over the frozen solution and brought in contact with a chilled pestle. Pulverization was achieved by striking the pestle several times with a mallet. The powdered homogenate was transferred from the mortar into a small Waring Blender, where it was immediately homogenized in an additional 30 ml of the same solution, at 0°C for 40 s. This procedure prevented a change in the 32P content of the muscle proteins since the muscle was thoroughly mixed with the homogenizing solution before it could be thawed.

For the determination of 32P incorporation into total muscle proteins, homogenization was carried out in 5% trichloroacetic acid containing 5 mM Na2HPO4. The homogenate was centrifuged at 40,000 × g for 5 min, and the residue was washed with this solution six more times. The final residue was dissolved in 3 ml of 2% SDS and 0.25 M sodium phosphate, pH 8.0, using a Polytron homogenizer (Brinkmann) at 0°C and dialyzed against 2 liters of 0.1% SDS and 0.1 M sodium phosphate buffer, pH 7.0, 25°C overnight. After clarification at 80,000 × g, 25°C, for 30 min, the samples from contracting and resting muscles were electrophoresed on 10% polyacrylamide gels at room temperature for periods of 2 to 3 days. The frogs were then given tetanic stimulation at 25°C for 30 s (frequency 30 pulses/s), and then immersed into isopentane chilled by liquid nitrogen. The paired muscle was treated and frozen under identical conditions. Without stimulation. The pooled frozen muscles from three such runs were pulverized in a special all-stainless steel apparatus designed to allow all manipulations to be carried out under liquid nitrogen cooling. First, 6 ml of the appropriate homogenizing solution (see below) was frozen over a smooth surface in a cold mortar. The muscles were placed over the frozen solution and brought in contact with a chilled pestle. Pulverization was achieved by striking the pestle several times with a mallet. The powdered homogenate was transferred from the mortar into a small Waring Blender, where it was immediately homogenized in an additional 30 ml of the same solution, at 0°C for 40 s. This procedure prevented a change in the 32P content of the muscle proteins since the muscle was thoroughly mixed with the homogenizing solution before it could be thawed.

For the determination of 32P incorporation into myofibrils, homogenization was carried out in a solution containing 20 mM sodium phosphate, 1 mM EDTA, and 1 mM iodoacetamide, pH 7.0, and the fibrils were sedimented at 600 × g. The pellet was washed with the same medium six times and prepared for gel electrophoresis as described before.

Caffeine-induced contraction was produced by incubating the dissected sartorius and semitendinosus muscles in Ringer’s solution containing 10 mM caffeine at 25°C for 20 min; the paired muscles were kept in normal Ringer’s solution. The muscles were homogenized, without prior freezing, at 0°C in one of the described solutions, and the 32P-labeled phosphoproteins were analyzed on SDS gels.

The specific radioactivity of the phosphate groups of ATP was determined upon a 5% perchloric acid extract of the 32P-labeled frog leg muscles remaining after dissection of the sartorius and semitendinosus muscles. Control experiments showed that the measured specific radioactivity of ATP was independent of the source of the muscle (mixed leg muscles compared with either sartorius or semitendinosus). The specific radioactivity of the γ-phosphate group of ATP was determined in two separate ways as previously described by us (11, 12). The procedures of Ullbrecht and Uhbrecht (13) were used to determine the specific radioactivities of each of the α-, β-, and γ-phosphates.

Phosphorylation of the DTNB-light chain of rabbit skeletal myosin was first reported by Perrie and collaborators (1). The British workers also isolated and characterized myosin light chain kinase and phosphatase, the enzymes specific for this phosphate turnover (2, 3). A possible physiological significance for the phosphorylation and dephosphorylation of the DTNB-light chain was suggested by a report that in rabbit heart perfused with adrenaline dephosphorylation of this light chain occurred when the force developed by the myocardium was raised. Furthermore, a similar dephosphorylation of the light chain was found when the contractile force of the heart was raised by decreasing the Na+ and increasing the Ca2+ concentrations in the perfusion medium (4).

In this light chain kinase which phosphorylates the 18,000-dalton light chain.

Phosphorylation of the DTNB-light chain of myosin enhanced its actin-activated ATPase activity (8). Adelstein and co-workers described phosphorylation of the 20,000 dalton light chain of human platelet myosin (5) and prepared the corresponding kinase (6). Phosphorylation of platelet myosin increased its actin-activated ATPase activity. Upon removal of the phosphoryl group, the ATPase decreased (7). This finding indicated for the first time that phosphorylation plays a role in the control of the enzymatic interaction between actin and myosin. Subsequently, it was shown that phosphorylation of the 20,000-dalton light chain of gizzard myosin enhanced its actin-activated ATPase activity (8).

Techniques have been developed in our laboratory for measuring protein phosphorylation in live muscle (9, 10). Skeletal muscle taken from frog injected with inorganic 32Porthophosphate.
The molar content of $^{32}$P in the 18,000-dalton light chain of frog myosin was determined on the basis of counts eluted from the appropriate zone of the gel. The counts were standardized using the specific radioactivity of the terminal phosphate of ATP as a reference. Values were expressed in terms of mole of $^{32}$P per mole of myosin light chain from the known amount of protein applied to the gel and from the following conversions: 50% of the total frog muscle proteins or 70% of the myofibrils are myosin (14), and there are two 18,000-dalton light chains per 500,000-dalton myosin (4).

RESULTS AND DISCUSSION

Live frogs injected with [$^{32}$P]orthophosphate and left at room temperature rapidly incorporate $^{32}$P into ATP. Within a day after the injection, the $\alpha$, $\beta$, and $\gamma$-phosphate groups of ATP were found to be equally labeled; this finding indicates that the total $^{32}$P pool in the muscle had reached equilibrium. In our experiments, the frogs were kept alive for at least 1 day in order to ensure that any changes in the radioactivity of muscle proteins caused by the stimulus would represent differences in phosphoprotein concentration rather than in phosphoprotein turnover. A further requirement for the validity of these studies is the complete inhibition of protein kinase and phosphorylase activities. We used trichloroacetic acid for denaturation of these enzymes. Recently, we reported that trichloroacetic acid-insoluble muscle proteins isolated from $^{32}$P-injected frogs contained considerable amounts of label (15). However, only a few of these phosphoproteins show changes in $^{32}$P content during a single tetanus. Fig. 1 compares the radioactivities of the total muscle proteins from the contracting muscle with those from the resting one. The only major change taking place, as a result of tetanus, was an 85% increase in radioactivity of the 18,000-dalton protein. An increase amounting to 36% was also seen in the 34,000-dalton protein zone. The amount of protein isolated from contracting or resting muscle for these analyses was the same: on average 170 mg of total protein per g of muscle.

To identify the 18,000-dalton protein that became labeled during contraction, we compared the radioactivities of myofibrillar proteins from contracting and resting muscles. Fig. 2 shows a 90% increase in $^{32}$P label of the 18,000-dalton protein in fibrils of contracting muscle under conditions when essentially no other change was found. A radioactivity profile similar to that shown in Fig. 2 was also obtained on actomyosins derived by further fractionation of these myofibrils. The mobility of the 18,000-dalton protein zone was found to be identical with that of the DTNB light chain from rabbit skeletal myosin (16, 17). The myofibrillar proteins were also analyzed by electrophoresis on SDS-urea gels (15) under conditions which separated the Ca$^{2+}$-binding subunit of troponin (troponin C) from the DTNB light chain. This experiment showed clearly that the label is associated with the light chain.

The molar content of $^{32}$P in the 18,000-dalton light chain of frog myosin was calculated using the specific radioactivity of the terminal phosphate of ATP as a reference, as further detailed under "Experimental Procedures." These values were 0.45 and 0.85 mol of $^{32}$P per mol of light chain before and after tetanus, respectively, when the frozen muscles and myofibrils were treated with trichloroacetic acid, and 0.30 and 0.55 mol of $^{32}$P per mol of light chain in the myofibrillar fractions of resting and tetanized muscles, respectively. The fact the $^{32}$P content of light chains in myofibrils was lower than that in the acid-treated muscles indicates that inhibition of the light chain phosphorylase was not complete under our conditions. However, the data from trichloroacetic acid-treated muscles show that about 0.4 mol of phosphate is transferred from ATP to the 18,000-dalton light chain during a single tetanus.

Pires et al. (2) established that rabbit myosin light chain kinase requires Ca$^{2+}$ for its activity. Based on the assumption that the frog enzyme behaves similarly, we postulated that the phosphorylation of light chain in the stimulated muscle is caused by an elevated Ca$^{2+}$ concentration demonstrated by Ashley and Ridgway (18). In order to test our hypothesis, we treated the frog muscles with caffeine, an agent known to release Ca$^{2+}$ from the sarcoplasmic reticulum (19). Total proteins and myofibrils isolated from caffeine-treated muscle showed a specific increase in the radioactivity of myosin light chain, as compared to the untreated muscles. This increase
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occurred to the same extent as was found for electrically stimulated muscles (see Figs. 1 and 2). Thus, it appears that the increased Ca^{2+} concentration in the sarcoplasm initiates the phosphorylation of the 18,000-dalton myosin light chain.

These results demonstrate for the first time phosphorylation of a muscle protein during a single contraction. It is of interest that the myosin light chain is phosphorylated in the working skeletal muscle, whereas in the beating heart, dephosphorylation of this light chain occurs. A major difference in the phosphorylation of the inhibitory component of troponin from skeletal as compared to heart muscle has also been recently described.

The apparent relationship between the Ca^{2+} level in the myoplasm and skeletal myosin light chain phosphorylation suggests a regulatory role for the DTNB light chain. Myosin-linked regulation of contraction through the 18,000-dalton light chain is well known in molluscan muscles. Based on x-ray diffraction patterns, Haselgrove raised the possibility that an activation control mechanism exists for frog myosin filaments. Native vertebrate myosin filaments were shown to possess Ca^{2+}-binding sites which were localized in the DTNB light chains, hence implying a Ca^{2+}-sensitive regulatory mechanism at the level of myosin. The ATPase activity of myosin in the intact muscle, determined by 31P nmr, is extremely low, suggesting a special inhibitory mechanism in vivo. Cyclic phosphorylation and dephosphorylation of the 18,000-dalton light chain could be the molecular switch regulating the myosin ATPase activity in living muscle.

Acknowledgments — We thank Mr. Scott T. Sayers and John D. Glowicki for their expert and enthusiastic assistance.

REFERENCES

1. Perrie, W. T., Smillie, L. B., and Perry, S. V. (1973) Cold Spring Harbor Symp. Quant. Biol. 37, 17-18
2. Pires, E., Perry, S. V., and Thomas, M. A. W. (1974) FEBS Lett. 41, 292-296
3. Morgan, M., Perry, S. V., and Ottaway, J. (1976) Biochem. J. 157, 687-697
4. Fearon, N., Solaro, R. J., and Perry, S. V. (1976) Nature 264, 801-802
5. Adelstein, R. S., Conti, M. A., and Anderson, W., Jr. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 3115-3119
6. Daniel, J. L., and Adelstein, R. S. (1976) Biochemistry 15, 2370-2377
7. Adelstein, R. S., and Conti, M. A. (1975) Nature 256, 597-598
8. Gorecka, A., Aksouy, M. O., and Hartshorne, D. J. (1976) Biochem. Biophys. Res. Commun. 71, 325-331
9. Ribolow, H., Báránya, K., Stein Schneider, A., and Báránya, M. (1977) Arch. Biochem. Biophys. 179, 81-85
10. Ribolow, H., and Báránya, M. (1977) Arch. Biochem. Biophys. 179, 718-720
11. Báránya, M., Báránya, K., Gaetjens, E., and Horvath, B.Z. (1974) in Exploratory Concepts in Muscular Dystrophy (Milhorat, A. T., ed) Vol. 2, pp. 451-462, Excerpta Medica, Amsterdam
12. Báránya, M., Báránya, K., and Trautwein, W. (1960) Biochim. Biophys. Acta 45, 317-335
13. Ulbrecht, G., and Ulbrecht, M. (1957) Biochim. Biophys. Acta 25, 100-109
14. Báránya, M., and Báránya, K. (1973) Cold Spring Harbor Symp. Quant. Biol. 37, 157-167
15. Báránya, M., Báránya, K., Gaetjens, E., and Stein Schneider, A. (1977) Biochim. Biophys. Acta 481, 387-397
16. Woods, A. G. (1960) Nature 223, 1362-1364
17. Gazith, J., Himmelfarb, S., and Harrington, W. F. (1970) J. Biol. Chem. 245, 15-22
18. Ashley, C. C., and Ridgway, E. B. (1970) J. Physiol. 209, 166-180
19. Endo, M. (1977) Physiol. Rev. 57, 71-108
20. Szent-Györgyi, A. G., Szentkirályi, E. M., and Kendrick-Jones, J. (1973) J. Mol. Biol. 74, 179-203
21. Szent-Györgyi, A. G. (1975) Biophys. J. 15, 707-723
22. Haselgrove, J. C. (1975) J. Mol. Biol. 92, 113-143
23. Motomura, K., and Harrington, W. F. (1974) J. Mol. Biol. 88, 695-709
24. Burt, C. T., Labotka, R., Flaherty, J., Danon, M., Glonek, T., and Báránya, M. (1977) Biophys. J. 17, 203a
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J. Biol. Chem. 1977, 252:4752-4754.

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