Hardwicke, P. M. D., T. Wallimann, and A. G. Szent-Györgyi. 1983. Light chain movement and regulation in scallop myosin. *Nature (Lond.)*. 301:478–482.

Huxley, H. E., and M. Kress. 1985. Crossbridge behavior during muscle contraction. *J. Musc. Res. Cell Motil.* In press.

Kendrick-Jones, J., and R. Jakes. 1977. Myosin linked regulation: a chemical approach. *In* International Symposium on Myocardial Failure. G. Riecker, A. Weber, and J. Goodwin, editors. Springer-Verlag, Amsterdam. 28–40.

Mornet, D., R. Bertrand, P. Pantel, E. Audemard, and R. Kassab. 1981. Proteolytic approach to structure and function of actin recognition sites in myosin heads. *Biochemistry*. 20:2110–2120.

Persechini, A., and D. J. Hartshorne. 1981. Phosphorylation of smooth muscle myosin: evidence for cooperativity between the myosin heads. *Science (Wash. DC)*. 213:1383–1385.

Sellers, J. R., and E. V. Harvey. 1984. Localization of a light-chain binding site on smooth muscle myosin revealed by light-chain overlay of SDS-polyacrylamide electrophoretic gels. *J. Biol. Chem.* 259:14203–14207.

Stafford, W. F., and A. G. Szent-Györgyi. 1978. Physical characterization of myosin light chains. *Biochemistry*. 17:607–614.

Stafford, W. F., E. M. Szentkiralyi, and A. G. Szent-Györgyi. 1979. Regulatory properties of single-headed fragments of scallop myosin. *Biochemistry*. 24:5273–5280.

Sutoh, K. 1983. Mapping of actin-binding sites on the heavy chain of myosin subfragment 1. *Biochemistry*. 22:1579–1585.

Szent-Györgyi, A. G., and P. D. Chantler. 1985. Control of contraction by myosins. *In* Myology. A. G. Engel, and B. Q. Banker, editors. McGraw-Hill, Inc., New York.

Szent-Györgyi, A. G., E. M. Szentkiralyi, and J. Kendrick-Jones. 1973. The light chains of scallop myosin as regulatory subunits. *J. Mol. Biol.* 74:179–203.

Szentkiralyi, E. M. 1982. Scallop regulatory and essential light chains complex with the same heavy chain peptide fragment. *Biophys. J.* 37(2, Pt. 2):399*a*. (Abstr.)

Szentkiralyi, E. M. 1984. Tryptic digestion of scallop S1: evidence for a complex between the two light chains and a heavy-chain peptide. *J. Musc. Res. Cell Motility*. 5:147–164.

Vibert, P., and R. Craig. 1982. Three-dimensional reconstruction of thin filaments decorated with a Ca^{2+}-regulated myosin. *J. Mol. Biol.* 157:299–319.

Vibert, P. J., and R. W. Craig. 1983. Electron microscopy and image analysis of myosin filaments from scallop striated muscle. *J. Mol. Biol.* 165:303–320.

Vibert, P., and R. Craig. 1985. Structural changes occurring in scallop myosin filaments on activation. *J. Cell Biol.* In press.

Vibert, P., C. Cohen, P. M. D. Hardwicke, and A. G. Szent-Györgyi. 1985. Electron microscopy of cross-linked scallop myosin. *J. Mol. Biol.* 183:283–286.

Wells, C., and C. R. Bagshaw. 1985. Calcium regulation of molluscan myosin ATPase in the absence of actin. *Nature (Lond.)*. 313:696–697.

Winkelmann, D. A., S. Lowey, and J. L. Press. 1983. Monoclonal antibodies localize changes in myosin heavy chain isozymes during avian myogenesis. *Cell*. 34:295–306.

Winkelmann, D. A., S. Almeda, P. Vibert, and C. Cohen. 1984. A new myosin fragment: visualization of the regulatory domain. *Nature (Lond.)*. 307:758–760.

HIGH-RESOLUTION EQUATORIAL X-RAY DIFFRACTION FROM SINGLE SKINNED RABBIT PSOAS FIBERS

LEEPO C. YU AND BERNHARD BRENNER
National Institutes of Health, Bethesda, Maryland; and University of Tübingen, Tübingen, Federal Republic of Germany

Recently it was shown by mechanical measurements (Brenner et al., 1982) and low-angle equatorial x-ray diffraction (Brenner et al., 1984) that in a relaxed skeletal muscle fiber, a substantial fraction of myosin heads (cross-bridges) is attached to the thin filaments at low temperature (5°C) and low ionic strength (μ = 20 mM), and that the fraction of attached cross-bridges decreases with increasing μ. These findings raised the question whether cross-bridges attached in the relaxed state assume the same structure as those found in other physiological states, such as in rigor. Results from the equatorial x-ray diffraction work (Brenner et al., 1984) and two-dimensional x-ray diffraction study by Matsuda and Podolsky (1984) suggested that the respective configurations of the attached cross-bridges in the relaxed and rigor states are probably different. To obtain additional evidence, in the present study we obtained improved electron density maps in axial projection of muscle fibers under relaxed and rigor conditions.

The unit cells of skeletal muscle consist of hexagonal arrays of interdigitating thick and thin filaments. Changes in the intensities of the two strongest x-ray equatorial reflections *10* and *11* have been interpreted to reflect mass movement associated with cross-bridge formation. However, with two reflections, the spatial resolution is limited to ~22 nm. The electron density maps of the projected thick and thin filaments are shown to be featureless cylinders (Yu et al., 1985). To improve the spatial resolution, higher orders beyond *10* and *11* are necessary.

In the past, most of the x-ray diffraction work was performed on intact whole muscle or bundles of skinned fibers. The present series of studies uses single, chemically skinned fibers where the sarcolemma is made permeable to large particles, whereby the medium surrounding the contractile proteins is directly controlled. Furthermore, with a single fiber preparation, the myofilament lattice is apparently better ordered for both relaxed and rigor fibers. This enables us to record several equatorial reflections not reported previously.

In the present study, fiber preparation and procedures of x-ray diffraction experiments mostly follow those reported previously (Brenner et al., 1984). Solutions were also

identical to those used in Brenner et al. (1984) except that K propionate was used instead of KCl to vary the ionic strength.

Diffraction patterns from relaxed and rigor fibers were obtained at ionic strengths μ = 20 mM and 100 mM. Twelve reflections up to the order *50* were recorded, although at μ = 100 mM (relaxed state) intensity were measured reliably only up to the reflection [3, 2], probably due to disorder within the lattice. All of the x-ray diagrams were dominated by the first two reflections, *10* and *11*, which contributed ~90% of the total intensities on the equator or ~75% of the total combined amplitutes after the Lorentzian correction.

To obtain electron density maps from these data, it is necessary to assign phase to each reflection. If a projected unit cell is centrosymmetric, the phases are either 0° or 180°. Even with such an assumption, which is reasonable at relatively low resolution, the twelve reflections would have 2^{12} possible phase combinations. Hitherto, only the first five reflections *10, 11, 20, 21, 30* have been studied in great detail and the combination [0°, 0°, 180°, 0°, 0°] was found to be the most likely (Yu et al., 1985) for the relaxed muscle. In the present study, we used only these five reflections and applied the same phase combination to the relaxed and rigor data, assuming that phases are the same in these two states. With these five reflections, the spatial resolution is improved from 22 nm to 12 nm.

A pronounced feature in the density maps thus obtained (Fig. 1 *a*) is that the thick filament is resolved into a backbone structure with an annular shell of mass of lower density distributed around it, a feature similar to that found in the relaxed intact frog sartorius muscle (Yu et al., 1985). This surrounding ring of mass has been suggested to be most likely that of the myosin heads (Yu et al., 1985), although the myosin heads need not be restricted only to this region. Qualitatively, these features of mass distribution are shared by all two-dimensional electron density maps obtained under various conditions, but the relative amounts of mass in each region depend on the physiological state.

At the present resolution (12 nm), objects with dimensions ≦12 nm would appear larger than their actual sizes. Consequently, the diameter of the thick filament backbone appears to be ~19 nm (apparent diameter) even though the actual diameter is ~15 nm. Similarly, the apparent diameter of the thin filament is ~15 nm (Yu et al., 1985).

Changes in reflection intensities indicate redistribution of mass in the muscle cell. Mass movement is best visualized in difference maps (Fig. 1 *b* and *c*), where mass transfer from one region to another is expressed in terms of gain and loss of mass. Fig. 1 *b* illustrates the mass movement occurring in transition from the relaxed to rigor state at μ = 100 mM. Fig. 1 *c* is the case for transition between two relaxed states, from μ = 100 mM to μ = 20 mM. In both cases, the final states have more cross-bridges attached as indicated by the gain of mass at the thin filament lattice points. However, the two difference maps show several features distinct from each other. Fig. 1 *b*

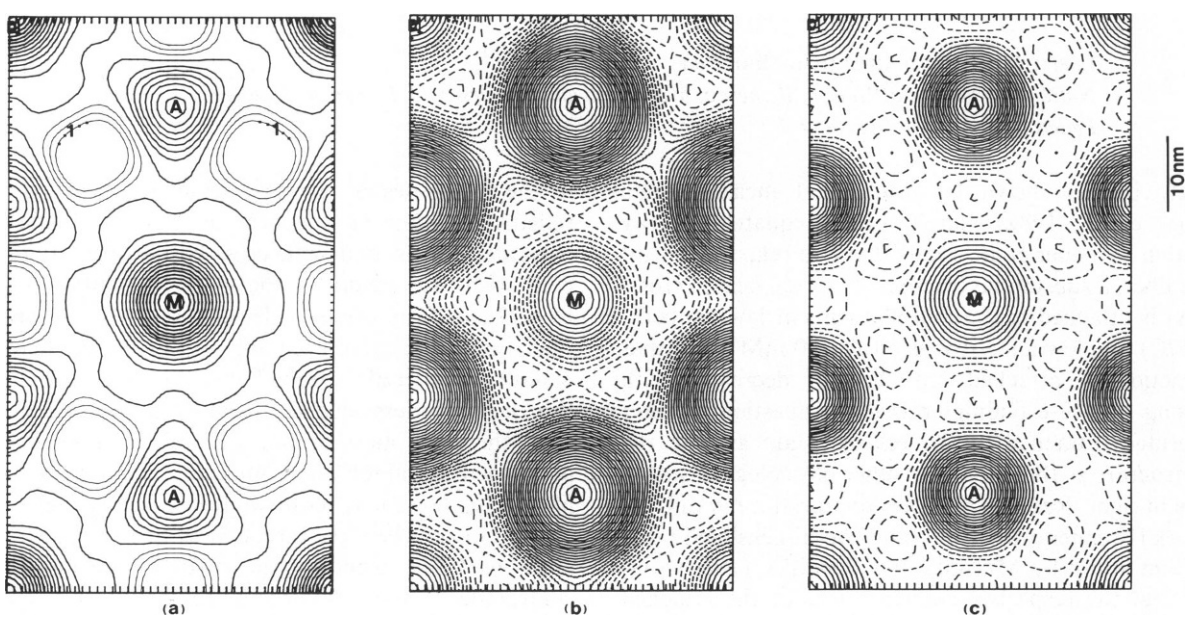

FIGURE 1 (*a*) Two-dimensional electron density map of axially projected myofilament unit cell of single relaxed skinned rabbit psoas fibers at ionic strength of 100 mM and sarcomere length = 2.3 μm. Fourier synthesis was based on experimental amplitudes with appropriate Lorentzian corrections. *M* designates center of the thick filament; *A*, centers of the thin filaments in the unit cell. *1* represents the contour boundary of lowest density levels, which occur in the regions between the thin filaments. (*b*) and (*c*): Difference maps that illustrate the patterns of mass redistribution under different physiological conditions. Redistribution is calculated by subtracting the map of the initial state from the final state. Solid contour lines trace gains of mass; dashed lines trace losses of mass. (*b*) Mass redistribution in transition to rigor state at μ = 100 mM from the relaxed state at μ = 100 mM. (*c*) Transition from the relaxed state at μ = 100 mM to relaxed state at μ = 20 mM.

shows that in transition from the relaxed to the rigor state, the total amount of mass transfer is about twice as that found in Fig. 1 *c* and the loss of mass originates mostly from the region surrounding the backbone of thick filaments. On the other hand, with lowering of μ in the relaxed state (Fig. 1 *c*), loss of mass appears to be evenly distributed in areas surrounding both the thick and thin filaments. Furthermore, in Fig. 1 *b* the attachment of the rigor cross-bridges brings the gain of mass in such a way that the profile of the axially projected thin filament, with cross-bridges attached to it, appears enlarged by 5 nm in its apparent diameter. This behavior is in contrast to the case in Fig. 1 *c* where more relaxed cross-bridges become attached in the final state of lower ionic strength. In this case, there is also a gain of mass at the thin filament, but the apparent diameter is almost unchanged. Preliminary modeling shows that it is the increase in the apparent diameter of the thin filament profile and more severe loss of mass from the area surrounding the backbone that distinguish the rigor-relaxed difference map (Fig. 1 *b*) from the relaxed-relaxed difference map (Fig. 1 *c*). One possible explanation could be that in the relaxed state only a small part of the myosin head is immobilized by attachment and the rest of the head may move around the thin filament freely. This type of attachment could create a rather disordered array of cross-bridges. From an end-on view, at the present spatial resolution, attached cross-bridges would appear hardly different from the detached ones. Therefore, the entire projected profile of the thin filament would appear approximately the same, regardless of the number of attached cross-bridges. On the other hand, in the rigor state Fig. 1 *b*, a larger portion of the myosin head is immobilized. With an orderly array of immobilized myosin heads attached to it, the thin filament should appear enlarged. Such an interpretation is consistent with the earlier suggestion by Matsuda and Podolsky (1984) that the flexibility in binding of attached cross-bridges is different between the relaxed and rigor states.

At the center of the backbone of the thick filament, both difference maps show an increase in density. In Fig. 1 *c* the total mass in the backbone area remains unchanged. This could mean that the thick filament is better ordered as ionic strength is lowered. In Fig. 1 *b*, however, there is a net loss of mass from the backbone. It was suggested that the S2 portion of myosin is probably an integral part of the backbone (Yu et al., 1985). This loss of mass suggests that there is an outward movement of S2 as the muscle goes into rigor.

In summary, we conclude that the differences between redistributions of mass after attachment of the cross-bridges when lowering ionic strength in relaxed fibers and when going into rigor very likely reflect different attachment configurations. Our axially projected two-dimensional difference maps suggest that the part of myosin head immobilized by attachment is smaller in the relaxed state than in rigor state. Furthermore, the attached cross-bridges in relaxed fibers move more freely around the thin filament than those in the rigor fibers.

Received for publication 28 May 1985.

REFERENCES

Brenner, B., M. Schoenberg, J. M. Chalovich, L. E. Greene, and E. Eisenberg. 1982. Evidence for cross-bridge attachment in relaxed muscle at low ionic strength. *Proc. Natl. Acad. Sci. USA.* 79:7288–7291.

Brenner, B., L. Yu, and R. J. Podolsky. 1984. X-ray diffraction evidence for cross-bridge formation in relaxed muscle fibers at various ionic strengths. *Biophys. J.* 46:299–306.

Matsuda, T., and R. J. Podolsky. 1984. X-ray evidence for two structural states of the actomyosin cross-bridge in muscle fibers. *Proc. Natl. Acad. Sci. USA.* 81:2364–2368.

Yu, L. C., A. C. Steven, G. R. S. Naylor, R. C. Gamble, and R. J. Podolsky. 1985. Distribution of mass in relaxed frog skeletal muscle and its redistribution upon activation. *Biophys. J.* 47:311–321.

CROSSBRIDGE AND BACKBONE STRUCTURE OF INVERTEBRATE THICK FILAMENTS

RHEA J. C. LEVINE, ROBERT W. KENSLER, AND PAT LEVITT
Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129

We and others have previously shown that the cross-bridges of the filaments of the chelicerate arthropods (*Limulus*, tarantula, and scorpion) lie at a radius of 4 nm from the filament surface in a highly ordered four-stranded helical array with a repeat every 43.5 nm and an axial rise of 14.5 nm between cross-bridge levels (1–4).

Here we describe the effects of altering environmental conditions on the helical arrangement and radial extension of cross-bridges on *Limulus* thick filaments and present preliminary results of monoclonal antibody localization of paramyosin epitopes in myofibrils, on filaments and filament cores.

MATERIALS AND METHODS

Thick filaments were isolated in relaxing solution from unstimulated *Limulus* telson levator muscles, as previously described (1–3). While still in a thin film of liquid on the grid, the filaments were exposed to the solutions listed on Table I, to determine whether environmental conditions affect the cross-bridge arrangement and if such changes are reversible.

In separate experiments, *Limulus* myofibrils and native and myosin-