THE MYOSIN FILAMENT

IV. Observation of the Internal Structural Arrangement

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ABSTRACT

The subunit organization of the myosin filament of chicken striated muscle has been observed directly in cross-sections in electron microscopy. The organization consists of three centrally located and nine peripherally located subunits in a close-packed arrangement. This arrangement is that predicted by a previously derived model for the detailed molecular organization of the myosin filament (Pepe, 1966 *a*, 1967 *a*, 1971). Each subunit measures approximately 30 A in diameter and the center-to-center distance is approximately 37 A. If these measurements are considered to be on the high side, then they indicate that each subunit represents one myosin molecule. However, it is not possible to determine unequivocally whether one or two myosin molecules per subunit are present on the basis of this work.

INTRODUCTION

The purpose of this work is to observe the internal organization of the myosin filament from cross-sections in electron microscopy. Previous attempts have been made to do this for the myosin filaments from a variety of muscles (Baccetti, 1965, 1966; Gilev, 1966 *a*, 1966 *b*). The structural subunits observed are generally not very well organized which is not surprising considering the treatments involved in fixing and embedding the tissue for electron microscopy. Another approach to elucidating filament organization has been the use of negative staining to observe artificial myosin filaments grown from myosin solutions (Huxley, 1963). These artificial filaments are structurally very similar to the naturally occurring myosin filaments. However, the detailed internal organization of the myosin filament could not be elucidated from these studies. From X-ray diffraction studies on muscle, the helical arrangement of myosin cross-bridges on the surface of the myosin filament was obtained (Huxley and Brown, 1967) but, again, the internal structural organization of the myosin filaments could not be deduced.

Recently, a model for the detailed internal structural organization of the myosin filament was derived (Pepe, 1966 *a*, 1967 *a*, 1971). In this model the myosin molecules are arranged as linear aggregates where successive molecules overlap in a head-to-tail fashion. These linear aggregates, or rows, are arranged in parallel. There are 12 rows of myosin molecules in the model. The 12 parallel rows are arranged so that the nonoverlapping portions are close packed in the core of the filament and the overlapping portions are excluded to the surface of the filament (Fig. 1 a). The close-packed core is such that there are three rows in the center and nine rows peripherally located (Fig. 1 a, 1 b). The overlapping portions from the three central rows are excluded to the surface between two of the peripheral rows. The observations pre-

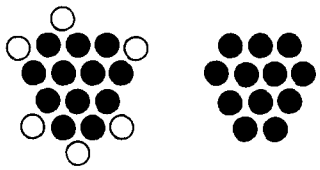

FIGURE 1 Subunit arrangement in cross-sections of the myosin filament predicted by the model (Pepe, 1966 *a*, 1967 *a*, 1971). Fig. 1 a, The solid circles represent cross-sections of the nonoverlapping (LMM) portions of the linear aggregates of myosin molecules arranged in parallel in the myosin filament. The hollow circles represent the position of the overlapping (S_2 part of heavy meromyosin [HMM]) portions of the linear aggregates of myosin molecules. Fig. 1 b, Representation of only the nonoverlapping (LMM) portions of the linear aggregates of myosin molecules in the myosin filament.

sented in this work confirm the predicted relationship of three central rows and nine peripheral rows close packed in the core of the filament in the region of the filament where there is head-to-tail overlap of myosin molecules.

MATERIALS AND METHODS

The pectoralis muscle of white roosters was used in this work. This will be referred to as chicken breast muscle. Both fresh fixed muscle and glycerinated muscle were used. The rooster was anesthetized by intravenous injection of Nembutal and immediately exsanguinated by cutting the jugular veins. The muscle was removed in thin strips along the fiber axis and tied to plastic rods at rest length or slightly more than rest length.

Fixation and Embedding

FRESH FIXED MUSCLE: The freshly excised muscle, on the plastic rod, was placed in a solution containing 5% glutaraldehyde, 8×10^{-2} M KCl, 8×10^{-4} M $MgCl_2$, and 8×10^{-3} M PO_4 buffer at pH 7.0 (buffered 5% glutaraldehyde), at 2–3°C. After approximately 2 hr in buffered 5% glutaraldehyde the muscle was dissected into small fiber segments and placed in 1% OsO_4, prepared in a standard salt solution containing 0.1 M KCl, 1×10^{-3} M $MgCl_2$, and 1×10^{-2} M PO_4 buffer pH 7.0, for 45 min at room temperature. The fiber segments were then washed in the standard salt solution without OsO_4 and dehydrated in steps using 70–100% ethanol and finally embedded in Araldite.

GLYCERINATED MUSCLE: The freshly excised muscle, on the plastic rods, was placed in 50% glycerol solution containing 5×10^{-4} M $MgCl_2$, 5×10^{-2} M KCl, and 5×10^{-3} M phosphate buffer pH 7.0 for 2 days at 2–3°C. The glycerol had previously been passed over Amberlite MB-1 ion exchange resin (Mallinckrodt Chemical Works, St. Louis, Mo.). The glycerol solution was then changed and the muscle strips were stored in glycerol solution at −24°C for at least 3 wk before use. The glycerinated muscle was cut off the plastic rod and transferred to a 25% glycerol solution containing 7.5×10^{-4} M $MgCl_2$, 7.5×10^{-2} M KCl, and 7.5×10^{-3} M phosphate buffer pH 7.0 and allowed to soak for 20 min. It was then shredded by drawing a needle repeatedly through the muscle bundle along the fiber axis. The shredded muscle was transferred to fresh solution and allowed to soak for 20 min. It was then homogenized gently in a Sorvall omnimixer (Ivan Sorvall Inc., Norwalk, Conn.) so that small fiber segments were obtained. These were centrifuged and resuspended in buffered 5% glutaraldehyde for 15–30 min at 2–3°C. They were transferred to 1% OsO_4 prepared in standard salt solution for 15–30 min at room temperature. They were then washed in standard salt without OsO_4 and dehydrated in steps using 70–100% ethanol and finally embedded in Araldite.

Electron Microscopy

Sections were obtained on a Porter-Blum microtome with a diamond knife. They were stained in two steps. The grids containing sections were immersed in a 4% solution of uranyl acetate in a 50:50 mixture of methanol and 70% ethanol for 0.5 hr. They were washed thoroughly in the solvent mixture and dried. They were then stained with Reynolds' lead citrate (Reynolds, 1963) for 0.5 hr followed by vigorous washing in water.

Electron micrographs were obtained with a Siemens Elmiskop I microscope with an accelerating voltage of 80 kv and a 50 μ objective aperature.

Rotations

Plates obtained at a magnification of 40,000 were used. The image of individual filaments was projected onto a small square of photographic paper and the exposure time was determined. For rotating, one third of the exposure time was given at 0°, 120°, and 240° rotation of the paper about the center of the image of the filament. Only plates showing exceptionally good, true cross-sections of the filaments and exceptionally good fixation were used for rotation. Only filaments showing the best structural organization were used. Centering of the rotation at the trigonal point between the three central subunits was critical.

RESULTS

In both fresh-fixed (Fig. 2) and glycerinated muscle (Fig. 3) the cross-sectional profiles of the

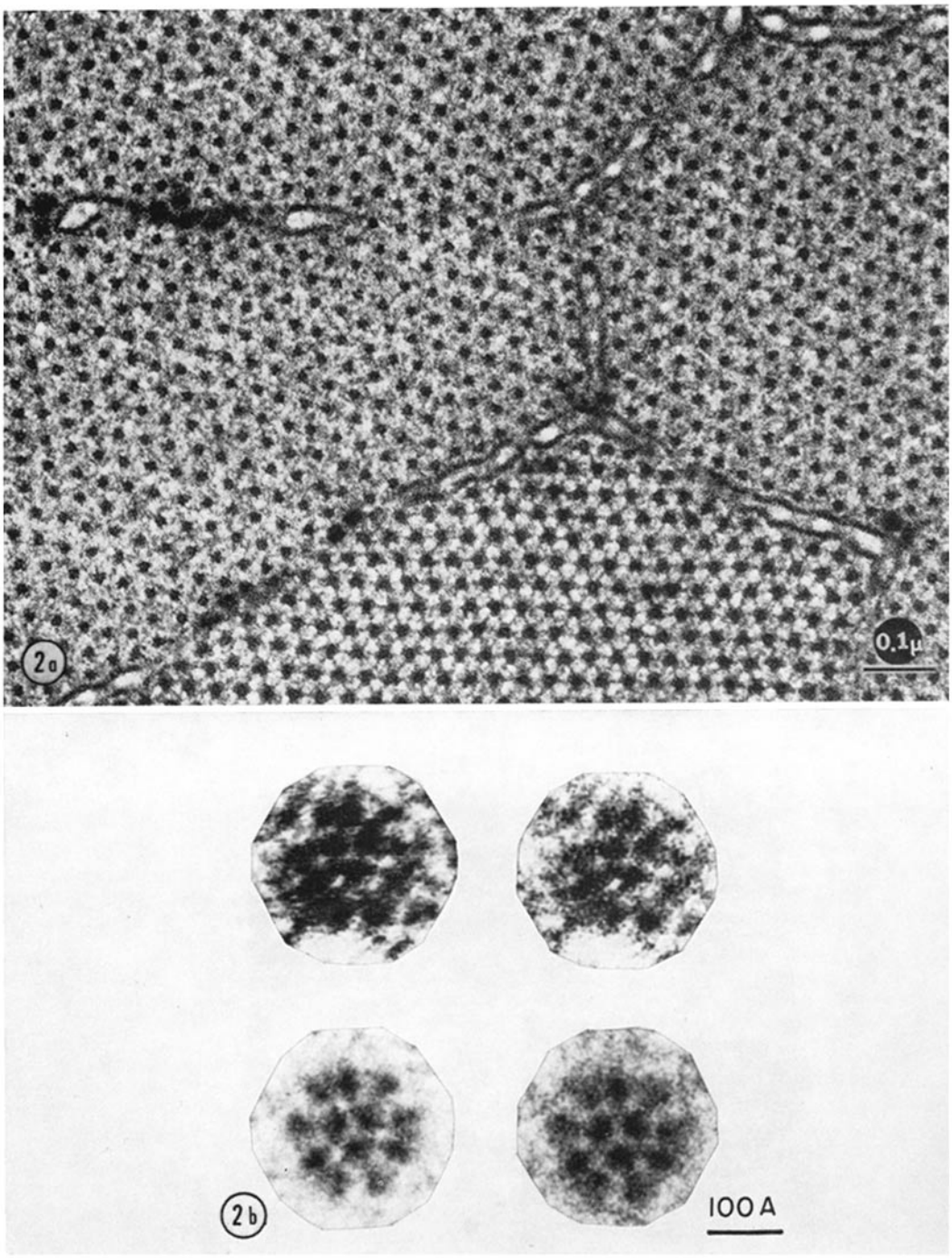

FIGURE 2 Cross-section of the myosin filaments of fresh-fixed muscle taken in the H band (region of the A band where there is no overlap of thin and thick filaments). Fig. 2 a, Area from which the filament shown in Fig. 2 b was taken. Fig. 2 b, The top two images are of the same filament enlarged from the micrograph in Fig. 2 a. The rotated images below were rotated in 120° intervals, recentering the rotation for each of the two images.

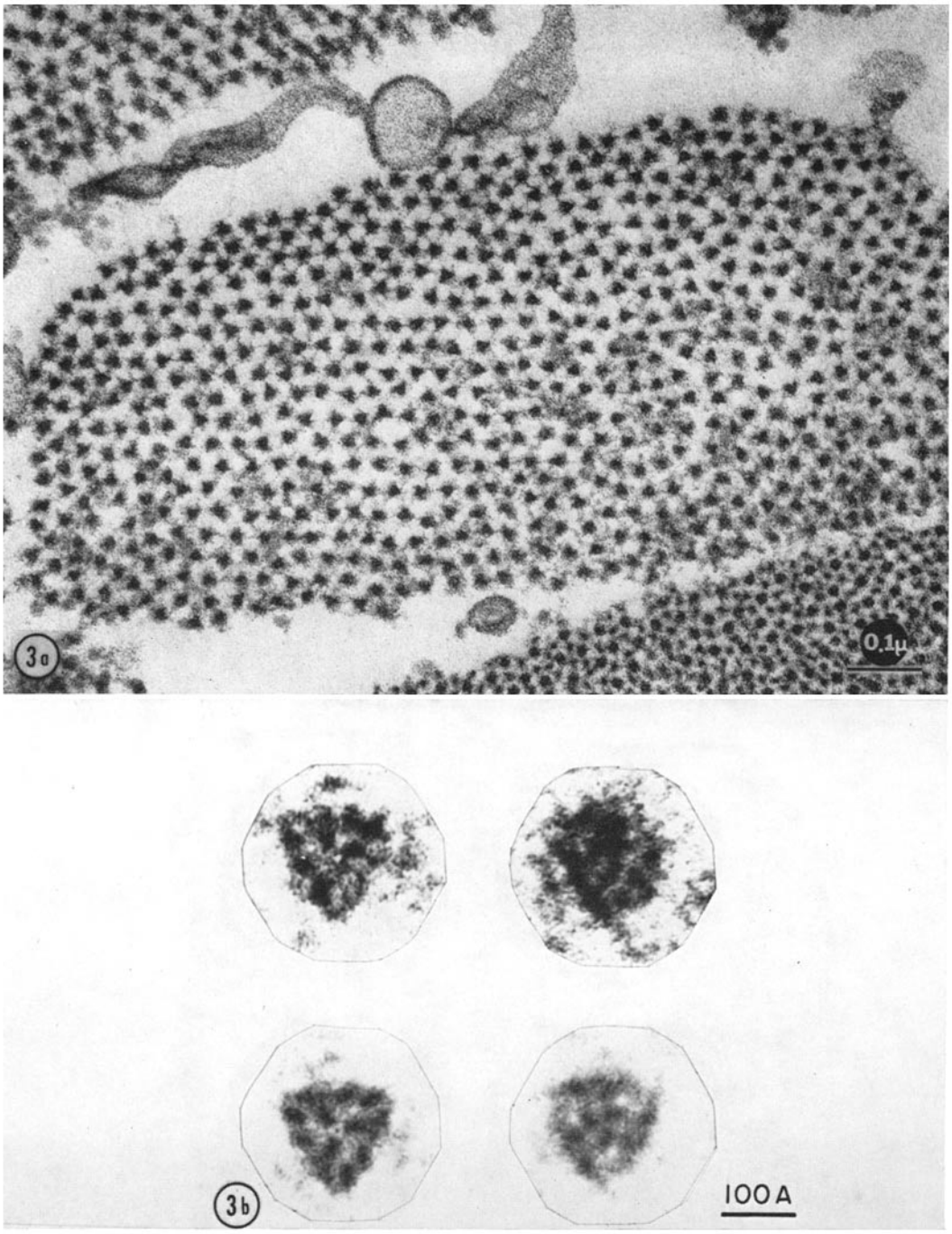

FIGURE 3 Cross-section of the myosin filaments of glycerinated muscle taken in the pseudo–H-zone (region in the middle of the A band where no myosin cross-bridges are present) but not including the M band. Fig. 3 a, area from which the filaments shown in Fig. 3 b were taken. Fig. 3 b, The top two images are of different filaments enlarged from the micrograph in Fig. 3 a. The rotated images below were rotated in 120° intervals.

myosin filaments showed a subunit structure. In general the subunit structure was better perserved in the fresh-fixed muscle. A pattern of close-packed, essentially circular subunits was observed in both cases (top of Figs. 2 b and 3 b.) In general the pattern consisted of three centrally located and nine peripherally located subunits. In most cases one or more of the subunits was either smudged, displaced relative to the others, or missing. In cases where most of the subunits were present and reasonably well organized, rotation printing at 120° intervals intensified the subunit organization relative to the background. The best, close-packed subunit arrangement in the filaments was obtained from cross-sections taken through the H band (region of no overlap of the thick and thin filaments) (see Fig. 2 a), but not including the pseudo–H-zone (region in the middle of the A band where no myosin cross-bridges are present). This is shown in Fig. 2 b where the arrangement can be seen to consist of three centrally located subunits and nine peripherally located subunits, close packed to give the organization previously predicted for the myosin filament (Pepe, 1966 *a*, 1967 *a*, 1971) and shown diagrammatically in Fig. 1. In cross-sections taken in the pseudo–H-zone in the middle of the A band (Fig. 3 a) but not including the M band, the three central subunits were rotated slightly (Fig. 3 b) with respect to the more precise, close-packed arrangement seen in Fig. 2 b.

Measurements of the diameter of the subunits and the center-to-center distance were made on both the rotated and the unrotated images in Figs. 2 b and 3 b. There was essentially no difference in these measurements. The subunits measure approximately 30 A in diameter and the center-to-center distance is approximately 37 A.

DISCUSSION

The observations made in this work (Figs. 2 and 3) confirm the subunit organization of the myosin filament in cross-section as being that predicted by the model (Fig. 1) previously described in detail (Pepe, 1966 *a*, 1967 *a*, 1971). The subunit organization seen in the unrotated images showed the same organization as, though less well defined than, that in the rotated specimens. Recently, the interpretation of Markham rotation images (Markham et al., 1963) was critically evaluated by Friedman (1970), with the conclusion that a rotation image can be relied upon only if it is a more clearly defined image of what can be observed in the less well-defined unrotated image. This eliminates the possibility of artifact introduced by the rotation. The unrotated images in this work had occasional subunits which were missing or smudged. However, the relationships between the existing subunits were not affected by the missing subunits, thus giving the complete subunit arrangement on rotation. In most filaments the subunit arrangement was disturbed or the cross-section was not truly perpendicular to the long axis of the filament, making it impossible to obtain a clear image on rotation. From Fig. 1 a and the observed patterns in Figs. 2 and 3 it can be seen that the observed subunits correspond to the nonoverlapping or light meromyosin (LMM) portions of the myosin molecules. The overlapping parts of the myosin molecules which correspond to the S_2 portion of the myosin molecules are not preserved relative to the LMM portions. This is not surprising since the S_2 and LMM fragments obtained from papain digestion do not interact under conditions where the LMM aggregates (Lowey et al., 1969). Therefore, one might expect that the interactions leading to the relation existing between the LMM and S_2 portions of the myosin molecules seen in Fig. 1 a is a weak one. This is consistent with the results of studies of fluorescent antimyosin staining of the A band (Pepe, 1966 *b*, 1967 *b*, 1968), from which it was concluded that the S_2 portion and, close to the tapered ends of the filament, even a part of the LMM portion of the myosin molecule can bend out of the core of the myosin filament. In order to see the subunits corresponding to the overlap or S_2 portions relative to the core of the filament, conditions which would maximize interaction of the S_2 with the LMM portions would have to be found.

In Fig. 2 b the three central subunits seem to be twisted out of close-packed alignment with respect to the other nine subunits. These cross-sections were taken in the pseudo–H-zone close to the M band. In this region of the myosin filament the myosin molecules are in parallel, but in the immediately adjacent M-band region the myosin molecules are helically wound (Pepe, 1971). The three central molecules come to the surface in the helical region. Therefore, cross-sections of the filament close to the M-band region may include the portion of molecules in transition from the parallel arrangement to the helical arrangement.

The displacement of the centrally located subunits in Fig. 3 may, therefore, represent the beginning of the region where these three central subunits come to the surface in the helical region.

In the detailed model (Pepe, 1966 *a*, 1967 *a*, 1971) for the myosin filament each subunit represents a structural unit which contributes one myosin cross-bridge to the surface of the filament. In the model each structural unit may consist of one myosin molecule or more than one myosin molecule, without altering the model in any way. The question of whether each cross-bridge or structural unit corresponds to one or two myosin molecules in vertebrate muscle has been discussed by Huxley (1960), using values for the amount of myosin in muscle and the number of bridges on the myosin filament. It was not possible to decide unequivocally between one and two myosin molecules per bridge. For insect flight muscle, Chaplain and Tregear (1966) concluded that there are three myosin molecules per cross-bridge. From detailed studies of cross-sections of insect flight muscle in electron microscopy, Reedy (1967, 1968) concluded that each cross-bridge splits into two bridges, thus suggesting that there are two myosin molecules per cross-bridge. In Figs. 2 and 3, each subunit represents a cross-section of a single structural unit. Therefore, it should be possible to decide on the number of myosin molecules making up each structural unit from measurements of the subunit diameter. From electron microscopy of shadowed preparations, the rod portion of individual myosin molecule has generally been reported as having a diameter in the range of 15–20 A (Rice, 1961; Zobel and Carlson, 1963; Huxley, 1963; Carney and Brown, 1966). The diameter of the subunits observed in this work measures approximately 30 A and the center-to-center distance is approximately 37 A. Therefore, once again, it is not possible to unequivocally conclude that either one or two myosin molecules contribute to a single structural unit and, therefore, to a cross-bridge. If we take 15 A as the diameter of the rod portion of a single myosin molecule, then we could have two molecules per subunit; however, a diameter of 20 A for the rod portion of a single myosin molecule would make it difficult to fit two molecules per subunit. Careful inspection of Figs. 2 and 3 shows that each subunit is considerably more dense in the center than at the edges, thus making it difficult to evaluate exactly where the edge of each subunit is in making the measurement of diameter. Also, in general it is difficult to find a filament with precise subunit arrangement. Misalignment would tend to increase the center-to-center distance and would suggest that some swelling or loosening of the organization has occurred during the fixation and embedding. Therefore, if the true center-to-center distance is less than 37 A, a single myosin molecule per subunit is favored. However, it should be emphasized that, from this work, it is not possible to distinguish unequivocally between one or two myosin molecules per subunit, structural unit, or cross-bridge.

This investigation was supported by United States Public Health Service Grant R01 AM04806 and Career Development Award K04 AM07342.

Received for publication 28 May 1971, and in revised form 21 October 1971.

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