A NOTE ON THE STRUCTURE OF CRAYFISH MYOFILAMENTS

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The development of methods for the separation and negative staining of muscle filaments (2, 3) has made it comparatively easy to get detailed information about the structure of these filaments. When these methods were applied to fresh or glycerinated, cross-striated, opener and closer muscles of the crayfish (*Cambarus clarkii*) cheliped, the following results were obtained.[1]

Both thick and thin filaments are present (Fig. 1). The thick filament diameter is about 180 A. A length measurement is difficult to obtain. No clear tapering of the end of the myofilament was apparent and thus breakage could not be exluded. The longest thick filaments measured were 6.1 micra which is consistent with the longest sarcomere lengths of 6.4 micra in sectioned material. A longitudinal period of about 450 to 500 A was sometimes seen. This was enhanced by reacting the fresh filaments on the grid with ATPase substrate containing lead ion, and may represent a cross-bridge period.

At higher magnification a short period was apparent. This is seen on the upper right filament in Fig. 2 and in Figs. 3 and 4. This period consisted of dense transverse lines separated by less dense areas occurring every 40 A. This fine period is taken to be a direct reflection of the molecular architecture of these thick filaments.

On the assumption that the thick filaments are composed of myosin-type, longitudinally oriented molecules, the 40 A spacing would be too short to represent the two ends of the molecule (for example, myosin is about 1600 A long). If the molecules were in lateral register, the period should be equal to the length of the molecule. If the molecules are staggered, one can reason as follows. Since this 40 A period has not been seen in sectioned material, but is seen after negative staining, it may be assumed that it is due to irregularities only on the surface of the myofilament. Therefore, the filament may be treated as a hollow cylinder, and only the outermost layer of molecules need to be considered. When this is done it is clear that, if there is a negative stain deposition at only the ends of the filaments, no matter how the molecules are staggered, one ends up with diagonal lines across the filament between 800 and 1600 A apart, depending on the unit of stagger. Thus, whether in register or staggered, one must postulate multiple loci along the molecules every 40 A, which are capable of being negatively stained, to account for the observed 40 A period.

[1] The appearance of these muscles in fixed and sectioned material is similar to that of the crayfish stretch receptor muscle, RM 1 (5).

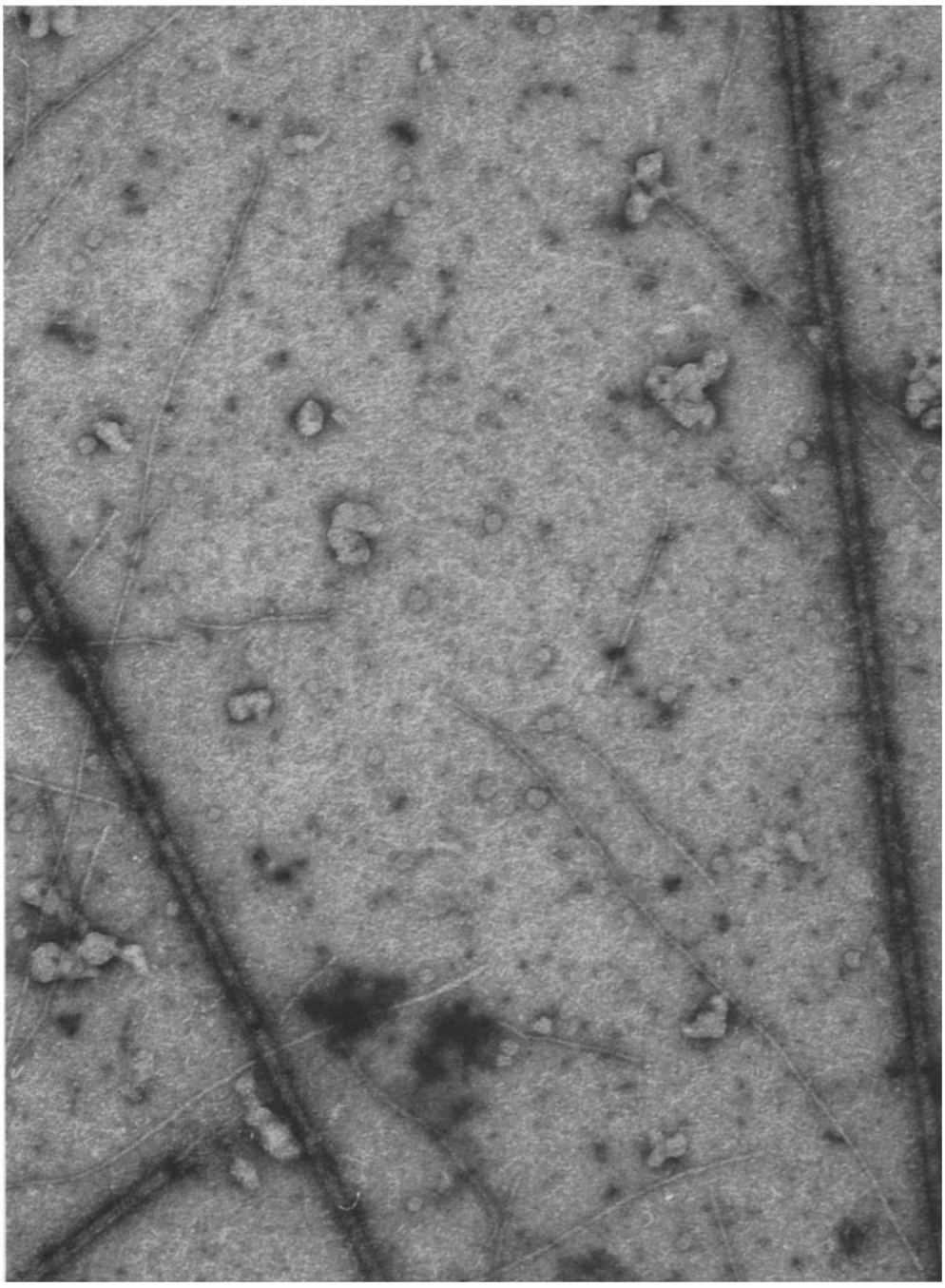


FIGURE 1

Low magnification micrograph of a negatively stained (uranyl acetate, pH 4.2) preparation of myofilaments from the opener muscle of the crayfish cheliped. Parts of three thick filaments and numerous thin filaments may be observed. Globular dark and light masses are probably cell debris. × 90,000.

Additional information comes from the following two observations (Figs. 3 and 4). One is that the lines and the interspaces between them, giving rise to the 40 A period, are of approximately the same width. The second is that consecutive lines are of approximately the same width and the same density. From the first observation it would follow that the negatively stainable loci along the molecule are separated by non-stainable portions of the same dimensions. The regular width and density of the lines would indicate that very probably consecutive loci along the molecule are identical. Thus, along the molecule a unit about 20 A long repeats itself approximately every 20 A.

Confirmation of this analysis, based on the short 40 A period, may be derived from a re-examination of Fig. 2 and Figs. 5 and 6. The other filament in Fig. 2 (the horizontal one) shows longitudinal striations which are also seen in Figs. 5 and 6. These longitudinal striations are due to molecular subunits of the filament which are 20 to 25 A wide and of unknown length. In Fig. 6 these appear to be solid lines. However, in Figs. 2 and 5 it may be seen that there are areas (arrows) where these subunits consist of a row of small nodules. The nodules are about 25 A in diameter with a center to center spacing of approximately 40 A. These are the repeating units mentioned at the close of the preceding paragraph.

From this it is clear that the appearance of longitudinal and transverse striations along the thick filaments arise from the same cause, namely, longitudinal rows of small 25 A nodules. In some areas these nodules are in lateral register and give rise to a 40 A cross-striation. Where this is not apparent it is impossible to tell, from inspection of the micrographs, whether this is due to confusion arising from the image (for example, superimposition of rows of nodules) or whether the nodules are really out of register. The longitudinal subunits of rows of nodules are separated by about the same dimension as the width (25 A) of the subunit. Consideration of this and the circumference (560 A) of the filament would allow an outer shell of about 11 subunits. Since only 3 to 5 longitudinal subunits are seen in the micrographs, it follows that only the upper surface of the filament is being negatively stained. In other words, if the stain had penetrated between the filament and the supporting film, more than 5 longitudinal subunits should be seen due to superimposition. Therefore, since superimposition is not occurring, but there are areas where the 40 A period is not seen, one must conclude that these are areas where the nodules, and therefore the subunits, are not in lateral register. It is very possible that *in vivo* the nodules are in register, but that this order became disrupted in places during preparation.

As stated above, both thick and thin myofilaments are present in these muscles (Figs. 1, 7 to 9). The thin filaments are, again, of variable length, probably because of breakage. However, it was noted that they are considerably longer than the thick ones. The width of the thin filaments varies from a maximum of 95 A to a minimum of 35 A along the same filament. At low magnification they give the appearance of a series of periodic bulges or swellings (Fig. 8). The period of the bulges is about 365 A. Upon closer inspection of these filaments at higher magnification it could be seen that they consist of a series of nodules or dots measuring about 35 A in diameter (Figs. 7 and 9). Where the maximum widths were recorded, at the bulges, there are two rows of nodules which come together to form a single row at the minimum width point. The center to center spacing of the nodules is 65 A. Consideration of the center to center spacing and the bulge period would give a figure of about 6.5 nodules per period per row of granules. Where the nodules are clearly seen, for example Fig. 7, this was confirmed by actual counts. These measurements are summarized in Fig. 10.

With the exception of the size of the nodules, this is consistent with the findings of Hanson and Lowy (1) for actin filaments, and thus permits the same interpretation. This interpretation is that each nodule consists of a molecule of monomeric actin. The actin monomers are polymerized forming a strand. Finally two of these strands are wound together forming a double helix. The thinnest measured width of the filament, 35 A, would represent the cross-over point of the two strands when viewed in two dimensions (Fig. 10). The figure of 6.5 nodules per cross-over period results in 13 monomers per complete turn of one helical strand which is consistent with the findings of Hanson and Lowy (1). The measurement of the nodule diameter of 35 A differs from that of Hanson and Lowy (1) which is about 55 A. Two probable interpretations of this difference are (*a*) that there is a difference in the compactness

FIGURES 2 TO 9

In all of these electron micrographs the contrast has been reversed in printing *via* a lantern slide intermediate. The structures thus appear as if positively stained.

FIGURE 2

Electron micrograph of two thick filaments. These show the two appearances described in the text. The filament at upper right shows predominantly cross-striations at about 40 A intervals. The other, horizontal, filament gives the impression of longitudinal striations. Arrows indicate two places on the latter filament where rows of nodules are seen and are out of register with adjacent subunits, whereas in the upper right filament they are in register. The area indicated at the far left is seen at higher magnification in Fig. 5. × 232,000.

FIGURE 3

This figure shows two thick filaments which were incompletely dissociated during the preparation. Irregular cross-bridges may be noted. In this preparation, despite relatively light negative staining, the cross-striation period of 40 A is seen. × 321,000.

FIGURE 4

At higher magnification, the cross striations (arrows) on a thick filament are apparent. A thin filament crosses obliquely through the figure. This is from a more heavily negatively stained preparation. × 478,000.

FIGURE 5

High magnification micrograph of the area indicated at the far left in Fig. 2. This shows the row of small 20 to 25 A nodules making up the longitudinal subunits of the filament. Where these are in lateral register with adjacent subunits, as in the upper right filaments in Fig. 2, a cross-striation results. × 478,000.

FIGURE 6

Electron micrograph of a thick filament showing the longitudinal striations. Approximately three to five subunits are seen. × 232,000.

FIGURE 7

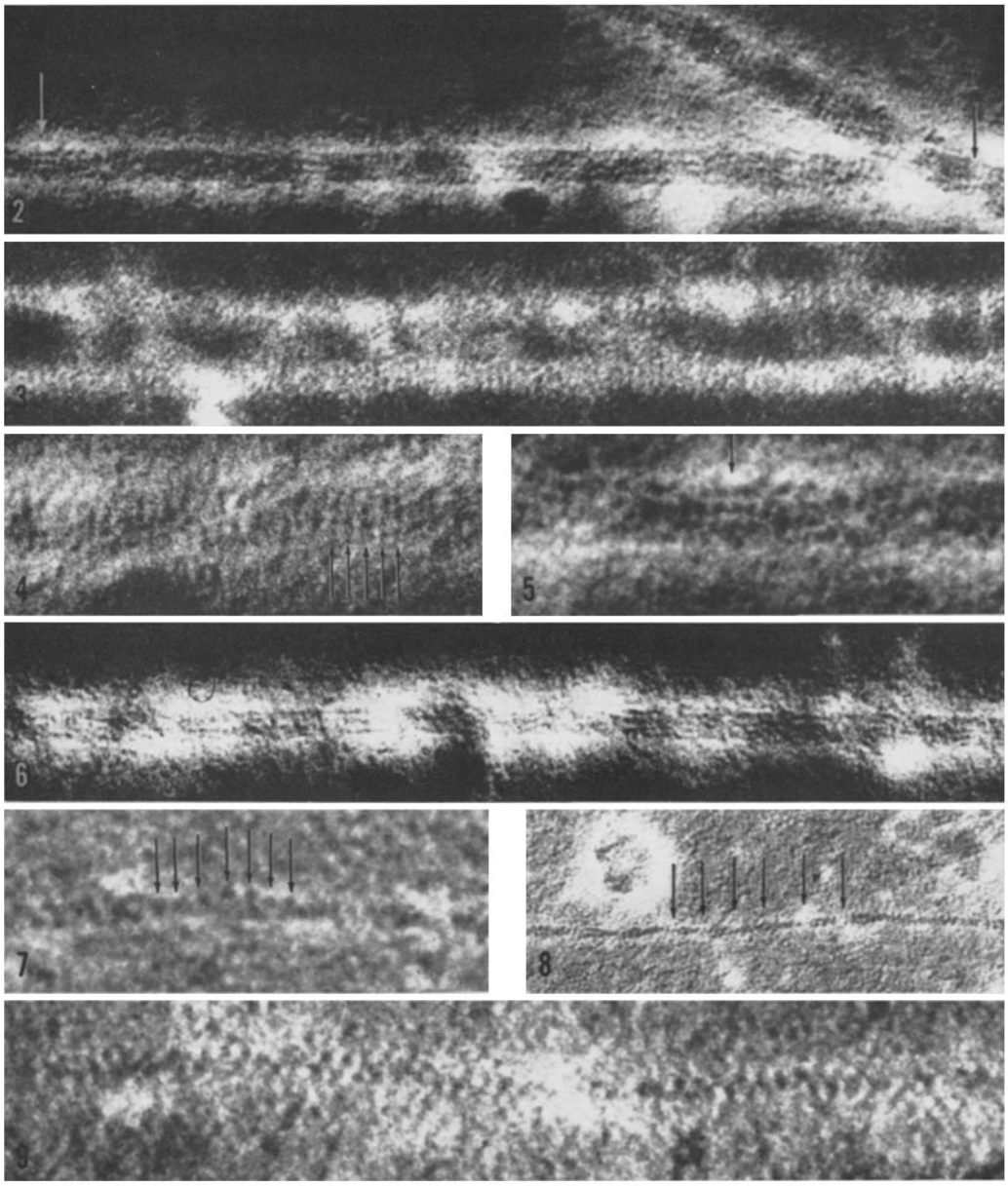
Electron micrograph of a portion of a thin filament. A row of nodules which form one strand is indicated (arrows). The second strand is not clearly seen. This shows how the appearance of bulges is produced. × 478,000.

FIGURE 8

Low power electron micrograph of part of a thin filament. Note the succession of bulges or swellings which are reflections of the cross-over points (arrows) of the double helix as viewed in two dimensions. × 181,000.

FIGURE 9

Electron micrograph of a portion of a thin filament. The appearance suggests that the nodules, and thus the strands, are out of register with each other. × 478,000.



of the molecule, resulting in differences in penetration of the negative stain or (*b*) that this is a different species of actin-type molecule. Either or both are possible and the evidence is insufficient to permit any conclusions. However, it is felt that this is not significant to the basic interpretation.

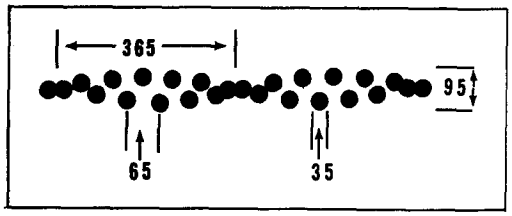


FIGURE 10

Diagram summarizing the description given in the text of the thin filament. All dimensions are in Angstrom units. Whether the two strands are in register or out of register, as indicated, cannot be determined from the micrographs. However, other evidence (4) indicates that they are probably as diagrammed. This may be compared with the electron micrographs, Figs. 7 to 9.

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