UNUSUAL STRIATED THICK FILAMENTS IN *LIMULUS* SKELETAL MUSCLE

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INTRODUCTION

Paramyosin-containing invertebrate muscles display several types of filamentous organization. Very thick (500–700 Å in diameter) filaments in smooth "catch muscles" have a 145 Å periodicity with a 725 Å repeat (Lowy et al., 1964). The thick filaments of obliquely striated muscles containing paramyosin range from 200 Å to 500 Å in diameter but do not exhibit paramyosin periodicity (Hanson and Lowy, 1961), nor do the ordinary thick filaments of cross striated invertebrate muscle from which paramyosin has been extracted, including *Limulus* skeletal muscle (Levine et al., 1972).

However, in the course of our ultrastructural study of both glycerinated and freshly-fixed *Limulus* telson muscles (Dewey, Levine and Colflesh, unpublished observations), we occasionally observed unusually thick, striated filaments or filament aggregates. In this report we describe these structures and discuss the nature of their periodicity.

MATERIALS AND METHODS

Bundles (200–500 μm in diameter) of *Limulus* telson muscle fibers were either fixed *in situ* or tied onto glass rods, removed from the animals, and fixed or glycerinated for up to 6 wk before fixation. Fiber and sarcomere lengths were controlled by maintaining the telson in specific positions during the fixation and/or dissection procedures. Glycerinated bundles were fixed and processed in the same manner as fresh muscle. Fixatives used were any of the following: 5% glutaraldehyde in 0.2 M collidine buffer, pH 7.2 containing 0.5 M sucrose, 5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 containing 0.2 M sucrose, or 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. After rinsing, the tissue was postfixed with 2% osmium tetroxide in the same buffer, stained *en bloc* in 1% aqueous uranyl acetate, dehydrated in graded acetone and propylene oxide, and embedded in Epon-Araldite. Thin sections were cut with glass and diamond knives on LKB Ultrotomes I and III, collected on Formvar-coated grids, stained with lead citrate, and examined and photographed with Hitachi HU-11C and HU-12 electron microscopes at 50 and 75 kV using Kodak EM plates. Magnifications were calibrated using a diffraction grid.

RESULTS

In longitudinal sections, very thick, striated filaments are occasionally found in both freshly-fixed and glycerinated fibers. The smallest very thick filaments are 350 Å in diameter and less than 1 μm long. These often appear in the central A band and have distinct cross striations with a 145 Å periodicity. Larger unusually thick filaments range from 400 Å to > 15,000 Å in diameter and may occupy from 1 μm to 4 μm lengths within the A band (Fig. 1). They taper and fray into smaller diameter branches laterally, although branching can occur anywhere. The thinnest branches resemble ordinary thick filaments (Fig. 2). In the larger filaments the cross striations consist of a series of lighter and darker bands with a 710–760 Å repeat periodicity resembling the DI banding pattern of positively-stained paramyosin paracrystals (Cohen et al., 1971) (Fig. 2).

The number of very thick filaments per sar-

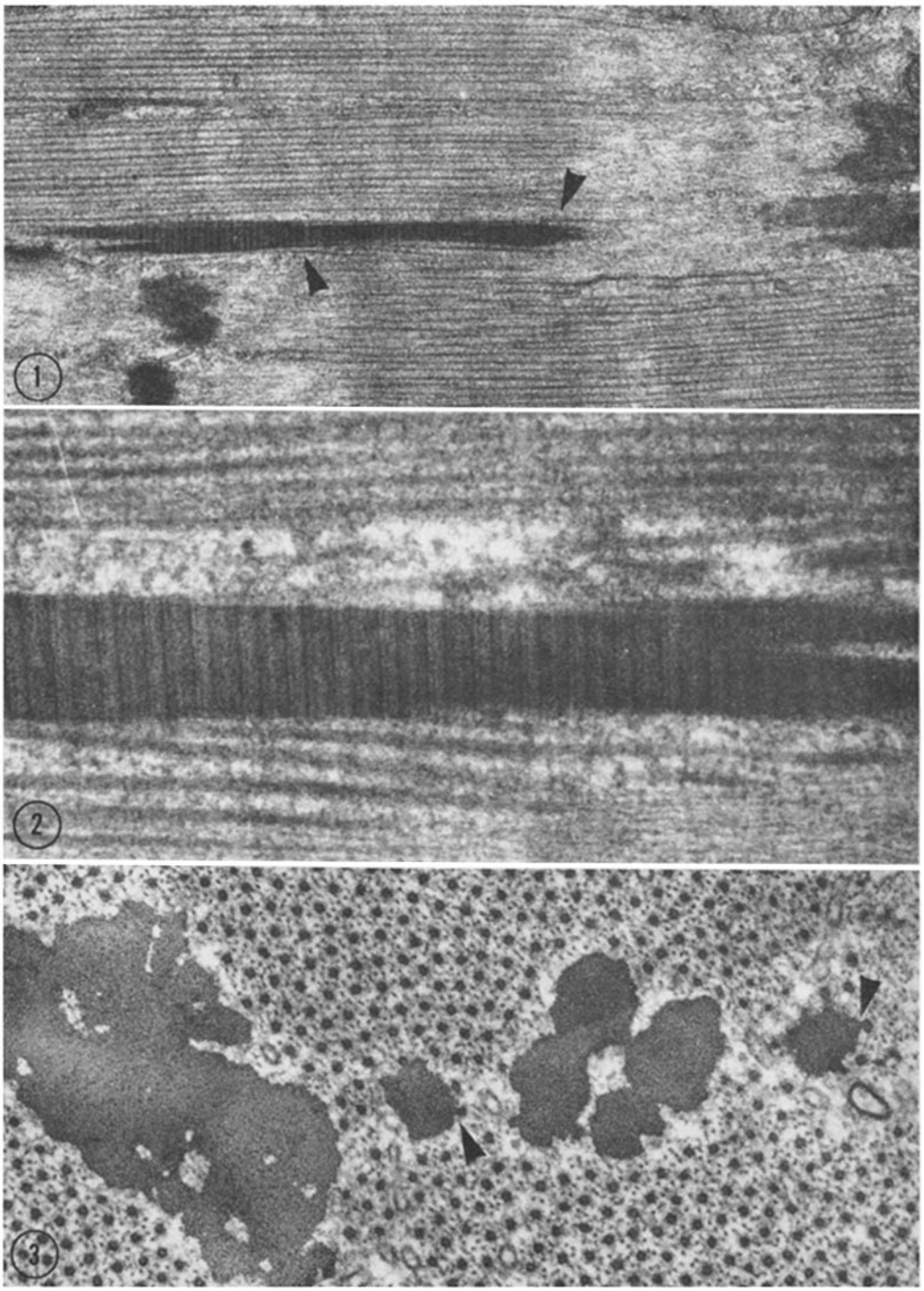

FIGURE 1 Large very thick filament in a longitudinally sectioned sarcomere. Note (arrowheads) branching centrally and laterally into smaller diameter filaments. × 25,000.

FIGURE 2 Area of very thick filament at greater magnification. Note the paracrystalline-like periodicity. Major repeat is every 745 Å. × 90,000.

FIGURE 3 Cross section showing arrangement of very thick filaments in A band. Large very thick filaments are irregular in shape, holey, and (arrowheads) seem to be composed of aggregations of filaments. × 82,500.

comere is seen in cross sections to be greater than the apparent number found in longitudinal sections. These filaments frequently aggregate in groups near the A-I junction. The smaller filaments are at least twice the diameter of the ordinary thick filaments, and have round to ovoid profiles. They remain within the thick filament lattice and are surrounded by and are connected via cross bridges to thin filaments. The larger filaments frequently form irregular masses containing one or more empty regions. The electron opacity of the very thick filaments is similar to that of the ordinary thick filaments (Fig. 3).

DISCUSSION

The occasional presence of unusually thick striated filaments in *Limulus* skeletal muscle is not correlated with the sex of the animals, the season at which they were obtained, the length of time they were stored before use, or any of the fixatives used. They are found in both glycerinated and freshly-fixed fibers. Nevertheless, the possibility remains that these structures may be artifactually produced by solubilization of the paramyosin cores of ordinary thick filaments (Levine et al., 1972) and the subsequent reprecipitation of this protein during our preparative procedures.

Most of the very thick filaments resemble paracrystals with a DI pattern formed by paramyosin precipitated with divalent cations (Cohen et al., 1971). The appearance of true paracrystalline periodicity in myofilaments is most unusual. Selective extraction of myosin from the striated thick filaments of molluscan paramyosin-containing muscles alters their surface features from a pattern having a 145 Å periodicity to a net pattern (Szent-Györgyi et al., 1971). The spontaneously-occurring DI paracrystalline periodicity of *Limulus* very thick filaments, however, resembles that frequently seen in barium paracrystals obtained from purified *Limulus* paramyosin (de Villafranca and Haines, personal communication). According to Cohen et al. (1971), the DI pattern may be generated by the superposition of two oppositely oriented paramyosin molecular arrays, each of which is shifted by approximately 80 Å in relation to each other.

As the branched ends of *Limulus* very thick filaments resemble ordinary thick filaments, it is possible that these striated elements are generated by aggregation of the latter type of filaments which results in the exposure of their paramyosin cores. The observed DI pattern may then reflect the organization of medullary paramyosin molecules in ordinary thick filaments of *Limulus* striated muscle.

We would like to thank Miss Alice A. Sydlowski for her secretarial assistance.

This work was supported in part by grants from The Arcadia Foundation, Norristown, Pennsylvania and the United States Public Health Service (EY 00549).

Received for publication 29 November 1972.

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