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Fraying of thick Filaments from Tarantula Muscle into Subfilaments

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ABSTRACT

Native myosin filaments were isolated in relaxing conditions from tarantula (*Avicularia avicularia*) striated muscle. These filaments were disassembled by suspending them into low ionic strength solutions of imidazole. The way how these filaments disassembled was compared with the reported way in which vertebrate (rat and rabbit) thick filaments frayed. Tarantula thick filaments frayed only near the tips into 4 subfilaments, whereas vertebrate thick filaments frayed into 3 subfilaments completely along the length of the filament, excluding the tip and the bare zone. Tarantula filaments frayed first into two secondary subfilaments; and then each of them frayed into 2 more primary subfilaments. This way of fraying permit to discriminate between the 4 possible models proposed on the basis of ultrathin transverse sections for the backbone of the tarantula thick filament by Guerrero & Padrón: Acta Microsc. 1(2): 63-83, 1992. The selected model, would have a central core (hollow or constituted by a protein different than paramyosin or myosin) surrounded by 4 rounded features (each on constituted of 2 paramyosin molecules) and two concentric rings formed by 8 and 16 rounded features (each one constituted of 2 myosin molecules tails), totalling 48 myosin tails transversally. It is concluded that thick filaments with a central paramyosin core like the tarantula thick filaments are more resistent to dissassembly than filaments (with or without paramyosin content) that do not have such central core.

KEYWORDS:

Muscle, Thick Filaments, Subfilaments, Backbone, Myosin, Electron Microscopy, Paramyosin, Negative Staining, Tarantula.

INTRODUCTION

Muscle sarcomere is mainly constituted by thick filaments (that contain myosin) and thin filaments (that contain actin). To understand how both arrays of filaments interact during muscle contraction it is important to determine their structures, and specifically how the tails of the myosin molecules pack to form the thick filaments. Huxley in 1963 [1] proposed that at physiological conditions the myosin molecules pack in a way that their tails form the backbone of the thick filament and their heads are located regularly on its surface. X-ray diffraction [2, 3] and electron microscopy [4 - 11] have permitted to understand with some detail the helical arrangement of the myosin heads on the surface of the thick filaments of several vertebrate or invertebrate species.

However, it is not clear how the myosin tails assemble to form the backbone of the thick filaments. The presence of long features spaced about 4 nm running parallel to the thick filament axis have been observed on electron micrographs of negatively stained thick filaments from invertebrate muscle [12, 13]. Maw an Rowe [14] have opened a way to disassemble thick filaments by exposing them to very low ionic strength solutions. Using this approach they found that thick filaments of rat psoas muscle frayed out from the central bare zone region into 3 similar subfilaments when exposed distilled water [14]. Similar results were obtained with thick filaments isolated from rat or rabbit psoas muscle but using buffered solutions (pH 7.60 of low ionic strength instead distilled water, to control the pH during the fraying [15]. The subfilaments, aproximately 6 nm in diameter, generally coalesce again at the filament tip, showing an end filament at each filament tip, as have been shown on thick filaments isolated form rabbit [15] or frog [16] muscles. Recently it have been shown that native filaments from scallop striated muscle fray into 5-7 subfilaments of about 10 nm diameter (which probably coil around each other) when exposed to solutions of low ionic strength [17]. The number of subfilaments appear to be close to the sevenfold rotational symmetry fo these filaments [7, 18, 19]. These evidences indicate that myosin

tails do not act individually in the backbone but associating with each others to form subfilaents [16] and provides evidence of the symmetry for the arrangement of myosin heads on the surface of the backbone [4, 7, 8, 19].

We have determined how the myosin heads are arranged in the surface of the backbone of the thick filaments of tarantula muscle by performing a three-dimensional reconstruction [8, 20]. This information together with transverse images of the backbone obtained from ultrathin transversal sections [21] have allowed us to propose 4 models of how the myosin tails pack together to form the backbone of these filaments. To discriminate between thse models it is necessary other independent structural evidences of the backbone substructure. We decided to try the approach of Maw & Rowe [14] with the aim of testing the existence of subfilaments in these invertebrate filaments. The way how thse filament fray, as well as the number of frayed subfilaments and their dimensions could allow to discriminate between the models for the backbone substructure [21]. As tarantula thick filaments appear to fray only partially as different than the vertebrate ones, we have also frayed thick filaments from striated muscle of rat and rabbit, as a control of the fraying procedure. Preliminary reports of this work have appeared elsewhere [22-24].

MATERIAL AND METHODS

Tarantulas (*Avicularia avicularia*, kindly classified by Dr. Sylvia Lucas, Instituto Butatan, Sao Paulo, Brazil) collected at San Jose de Guaribe were kept in a terrarium. Before dissection tarantulas were held at 4°C for 1 hour, then the legs were removed and the muscle dissected as described in [21]. Filament homogenates were prepared from chemically skinned tarantula muscle as described by [7, 8]. Muscles from tarantula (leg) and rat or rabbit (psoas) were chemically skinned by stirring for 3-4 hours at 60 RPM at 5-6 °C in a skinning solution formed by relaxing solution (KCl, 100 mM; $MgCl_2$, 8 mM; EGTA, 5 mM; NaH_2PO_4, 10 mM; NaN_3, 3mM; ATP, 5mM; dithiothreitol, 1mM; pH 7.00) and 0.1% saponin [7, 8,25]. After that the skinned muscles were homogenized in a Sorvall Omnimixer with a modified 5 ml cup (Dr. Roger Craig, personal communication, [7]) during 1 sec in 20 ml of relaxing solution with a "cocktail" of enzyme inhibitors added in order to reduce proteolysis (Dr. John Kendrick-Jones, personal communication, [7]). The "cocktail" of enzymatic inhibitor consisted of 20µl added of phenylmethylsulphonyl fluoride in isopropanol and 200 µl of a freshly thawed solution of 1 mg/ml leupeptin (Sigma, L-2884), 1 mg/ml pepstatin (Sigma, P-4265), 10 mg/ml alpha-N-benzoyl-L-arginine ethyl ester hydrochloride (Sigma, B-4500), 10 mg/ml p-toluenesulphonyl-L-arginine methyl ester hydrochloride (Sigma, T-4626) and 10 mg/ml trypsin inhibitor from chicken egg-white type II-0 (Sigma, T-9253) trypsine inhibitor. The homogenate was centrifugated for 30 sec at 15,000 RPM in a Eppendorf microcentrifuge. Drops from the supernatant (that contain the thick filaments) were put on top of grids coated with Formvar (Ladd # 10835) coated with a very thin layer of carbon, and irradiated with a high intensity uv. light (Mineraligth model R51, to increase the filament adhesion to the grids). Fraying was induced by incubating the thick filaments in a solution of imidazole buffer (pH 7.2 or 7.6) at low ionic strengyth (2 or 4 mM) but extended ranges of pH (4.00 - 9.00) or ionic strength (0-10 mM) were sistematically tested. Thick filaments were then negatively stained with 1% uranyl acetate on the grids. Grids were examined in a Hitachi H-500 electron microscope operated a 80 KV. Photographs were taken using Kodak projector slide plates or film # 4489; and developed with D-19 (diluted 1:2). Measurements of the micrographs were made using a Nikon Profile model 6-C. Electron micrographs were digitized to a final resolution of 1.1 nm by using a Coreco OC-200 digitizing board and a Panasonic video camera model WV-1410 adapted to a Carl Zeiss microscope at 100X magnification. Fourier transform, filtering and image processing were performed as described in [18, 26].

RESULTS

Figure 1 shows negatively stained isolated thick filaments from striated muscles of rat (psoas, fig. 1-a), rabbit (psoas, fig. 1-b) and tarantula (leg, fig. 1-c) in the relaxed state (normal ionic strength, 177 mM). Only for the case of tarantula thick filaments (fig. 1-c) the helical array of myosin heads on the surface of the backbone of the thick filaments is clearly preserved (fig. 1-c, oblique marks), as described in [8, 20]. Thick filaments of rat (fig. 1-a) and rabbit (fig. 1-c) appear to have a more labile array of myosin heads on their surfaces, as they are not preserved (fig. 1-a,b).

The images from tarantula thick filaments present optical diffraction patterns (not shown) with a sharp meridional reflection at 1/14.5 nm^{-1} and strong helical layer line at 1/43.5nm^{-1}.

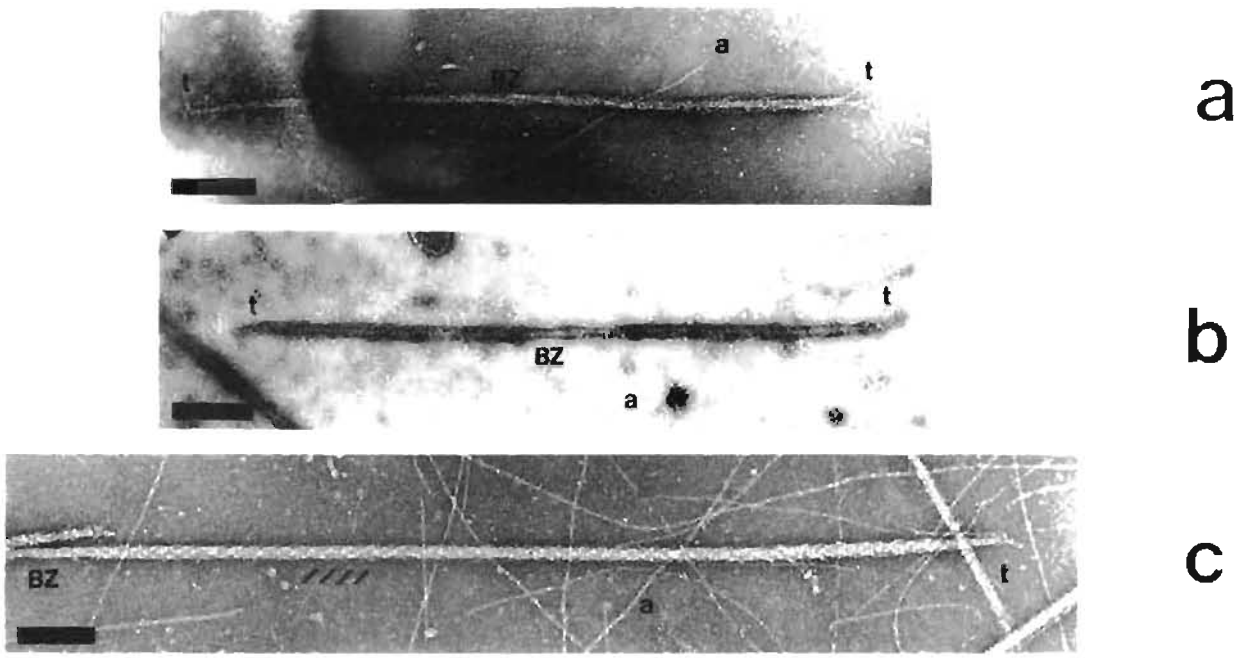

Figure 1. Electron micrographs of native myosin filaments isolated from striated muscles from (a) rat psoas, (b) rabbit psoas and (c) tarantula (*Avicularia avicularia*) leg; maintained in relaxing solution until negatively stained with uranyl acetate. BZ, bare zone; t, tip of the filament; a, actin filaments. In (c) it is shown only one half of the tarantula thick filament as they are very long. As different that with the vertebrate thick filaments (a, b), in the tarantula thick filaments (c) it is possible to see the presence of a helical array (oblique marks) of myosin heads on the surface of the filament (see fig. 2, best seen by viewing at a glacing angle along the direction of the filament axis. Bar 200 nm.

Fourier transforms (fig. 2-b) from selected digitized regions of these thick filaments (fig. 2-a) shows similar features, that includes 5 layer lines (fig. 2-b, marks) with helical layer lines and meridional spots corresponding to an axial spacing between myosin heads of 14.5 nm and a helix repeat of 43.5 nm [8, 20]. These findings clearly indicate that the native structure of *Avicularia avicularia* thick filaments is well preserved in the isolation of these filaments, as demonstrated before for *Eurypelma sp.* [8, 20], but is lost in the case of the more labile vertebrate thick filaments.

Figure 3-a,b shows that vertebrate thick filaments frayed into 3 subfilaments when they were exposed to a low ionic strength solution of imidazole (2 mM) at a pH range 7.0-7.6, confirming previous findings [14, 15, 16]. In the case of rat the subfilaments measured 5.1 ± 0.2 nm (n= 6, mean ± standard deviation of the mean) and sometimes joined at their extremes at the end filaments (fig. 3-a) [15]. Franying of the thick filamets occur along all the filament length with the exception of the bare zone which does not disassembly at all.

As different than the vertebrate thick filaments, in the case of thick filaments from tarantula muscle the fraying occur when the filaments are exposed to a low ionic strength solution of imidazole (range 2-4 mM) at a pH range 7.2-7.6. Fraying of these thick filaments only occur in a segment of 0.13 ± 0.06 μm (n=4) from the tip of the filaments (fig. 3-c). Disassembly occurs first into two secondary subfilaments of 5.7 ± 0.9 nm (n=2) which then frayed into 2 primary subfilaments each one, of 3.7 ± 0.2 nm (n=3).

DISCUSSION

Interaction between myosin tails is mainly electrostatic [27] and any experimental conditions which modify the attractive or repulsive forces (like changes in the ionic strength, pH, dielectric constant) or links (like disulfure bonds) between tails or subfilaments could possibly alter their packing inside the backbone of the thick filament [1]. At low ionic strength the expansion of the electrical double layer around the myosin tails leads to greatly increased repulsive forces which could induce

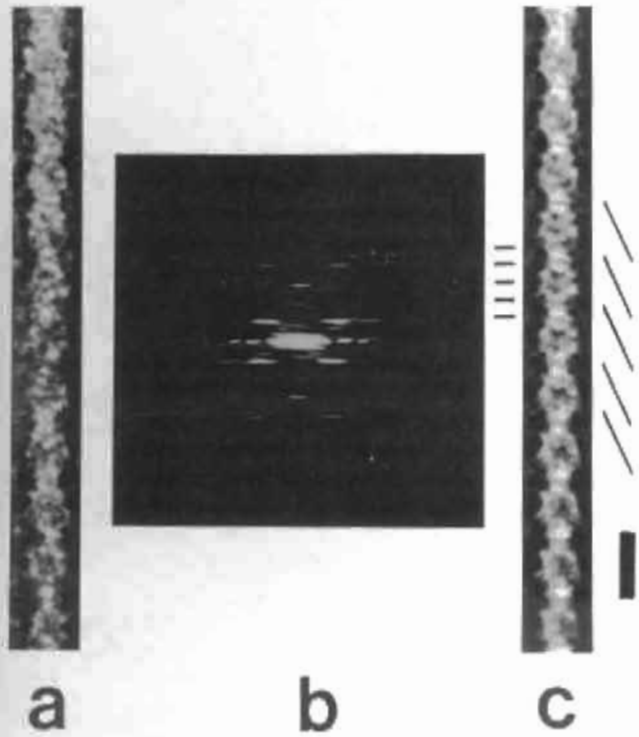

Figure 2. (a) Selected image of negatively stained tarantula (*Avicularia avicularia*) thick filament in relaxing conditions; (b) Fourier transform of the image shown in (a), showing 5 prominent layer lines (marks) corresponding to a 1/43.5 nm^{-1} repeat and 2 strong meridional reflection at 1/14.5 and 1/7.25 nm^{-1}, revealing a helical array of crossbridges; and (c) filtered image of (a) using only these 5 layer lines, the oblique marks indicates the presence of the helical tracks. Bar 43.5 nm.

their disassembly [14] producing intermediate substructures like intermediate filaments [28] or subfilaments [14, 15, 29].

The fraying of vertebrate thick filaments into 3 subfilaments agrees closely with experimental evidences indicating a surface arrangement of myosin heads in a three-stranded helix [4, 11]; and seem to support the subfilaments models of packing proposed by Squire [30, 31]. The way of fraying of tarantula thick filaments into 4 subfilaments agrees with a surface arrangement of myosin heads in a four-stranded helix [8, 13, 20] and is in agreement with one of the 4 models we have proposed recently [21] for the substructure of the backbone of these filaments. These models predicts that the backbone of these filaments have 48 myosin tails and are characterized by a central core (hollow or formed by a protein different than paramyosin or myosin), surrounded by 4 rounded features (formed by two para myosin molecules each one, forming ring I), and two more concentric rings (formed by 8 and 16, 9 and 15, 10 and 14 or 11 and 13 features on ring II or III respectively, with 2 myosin tails per feature) [21]. Figure 4-a shows one of these models, i.e. the one with 8 features (of 3.6 + 0.5 mm diameter) on ring II and 16 features (of 3.8 ± 0.4 nm diameter) on ring III The 24 features shown on figure 4-a are grouped by straight links into 4 subfilaments (each one with 6 features or 12 tails per subfilament) indicated as "1", "2", "3" or "4". In figure 4-c it is shown a schematic diagram drawn at scale to explain how a thick filament with an internal structure like the one proposed in figure 4-a can be frayed into 2 secondary subfilaments "A" and "B" which also fray into two more primary subfilaments "1" and "2" or "3" and "4". It is difficult to compare directly dimensions between the negative stained primary or secondary subfilaments (3.7 and 5.7 nm respectively); with the grouping of 4+2 features per subfilament shown in figure 4-a (3.6 or 3.8 nm for ring II or III respectively), measured on ultrathin cut plastic sections [21]. There is no way to fray the filaments for the other three models ("c", "d" and "e" of reference [21]) in an equal number of features, as their number of features per ring (i.e. 9 and 15, 10 and 14 or 11 and 13) are not a multiple of 4, except by mixing features from rings II and III. As the primary and secondary subfilaments detected by negative staining have similar diameters between them (3.7 or 5.7 nm respectively); we discard, in principle, these 3 models. Only model "b" of figure 7 of Guerrero & Padrón (1992) [21] with 8 features on ring II and 16 features on ring III allows for the results presented on this paper, by considering that a primary subfilament is formed by 6 features (i.e. 8/4 + 16/4 = 2 + 4 as indicated by the straigth links joining groups of 6 features shown in (a). Their models "c" (9 on ring II and 15 on ring III), "d" (10 on ring II and 14 on ring III) and "e" (11 on ring II and 13 on ring III) do not permit fraying them into an equal number of features per subfilament, as is suggested by the similar diameters of the actual subfilaments.

The fact that fraying tarantula thick filaments only occurs near the tips implies that in the rest of the filament structure is someway reinforced against dissasembly. Using poliacrilamide gel electrophoresis it has been quantitatively determined that the molar ratio [Paramyosin] / [Myosin heavy chain] is 0.31 for the tarantula ***Eurypelma sp*** [13] and 0.40 for ***Avicularia avicualria*** (Dr. Nelly Panté, personal communication). Tarantual thick filaments have

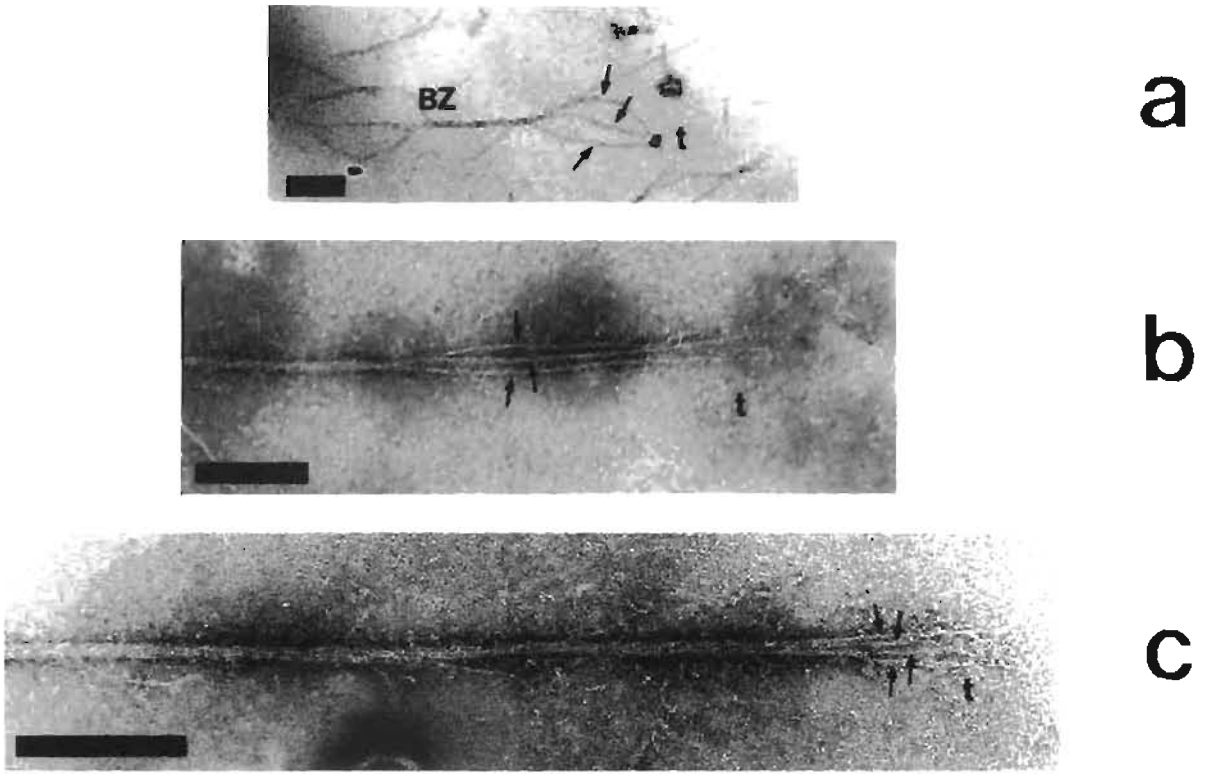

Figure 3. Electron micrographs of myosin filaments isolated from striated muscle of (a) rat psoas, (b) rabbit psoas and (c) tarantula (*Avicularia avicularia*) leg, frayed into subfilaments by rinsing with low ionic strength solution. Rat (a) an rabbit (b) thick filaments were frayed with a low ionic strength solution of imidazole (2 mM0 at pH 7.6 and then negatively stained. They frayed into 3 subfilaments (arrows) of similar diameter along all their length, with the exception of the central bare zone (BZ) which remain intact. In the case of rat, the presence of the end filaments [15] was sometimes seen at the tip (t) of the frayed filaments, which remain also intact. The tarantula thick filaments (c) were frayed with a low ionic strength solution of imidazole (mM) at pH 7.2 and then negatively stained. As different than the vertebrate ones, they frayed only near the tips, into 4 subfilaments of similar diameter (arrows, see text). t: position of the tip of the filaments. Bar 200 nm.

this paramyosin inside [13]. From ultrathin sections it have been proposed that paramyosin run along almost all the filament length forming a central core as the filaments gave solid dark features in transverse sections except near the tip which is hollow because centers near the thin filaments (I band) are clear [21]. There is a difference between the way disassembly occurs as observed between vertebrate (rat, fig. 3-a, rabbit fig. 3-b) and invertebrate (tarantula, fig. 3-c). This difference can be attributed to a different assembly of the myosin tails between vertebrate and invertebrate muscle, as well as to the presence or not of a central core of paramyosin molecules. There is not paramyosin content in the vertebrate thick filaments, whereas in the tarantula thick filaments there is a central core of paramyosin [13] which is relatively stable [32-33] under the fraying conditions studied here. Apart of exploring fraying with solutions of ionic strengths outside the range 2-4 mM and with a pH outside the range 7.2 - 7.6 (see material and methods), we have also try without success to dissasemble these filaments by modifying the dielectric constant of the solvent in the range 2.3 - 80; by using agents like urea at 0.5 - 8 M; and by breaking disulfure bonds using β -mercaptoethanol. Probably myosin interacts strongly with paramyosin on the rest of the filaments (about 99% of its total length), avoiding total fraying, and only in the 0.13 μm segment from the tips dissasembly is possible. It have been reported that scallop thick filaments fray progressively into more subfilaments and with a longer fraying length if the pH is increased over 7.5 at low ionic strength (18 mM). The reported fraying for the scallop thick filaments start at the end and proceeds towards the central bare zone [17]. In our hands, the fraying of thick filaments from tarantula muscle occurs only in the range of pH 7.2 - 7.6 at a ionic strength range 2-4 mM, as different that for scallop thick filaments [17]. One possibility is that there are strong myosin paramyosin interactions all along the tarantula thick filament [33] that avoids filaments disassembly, and that only near the tip (0.13 μm,

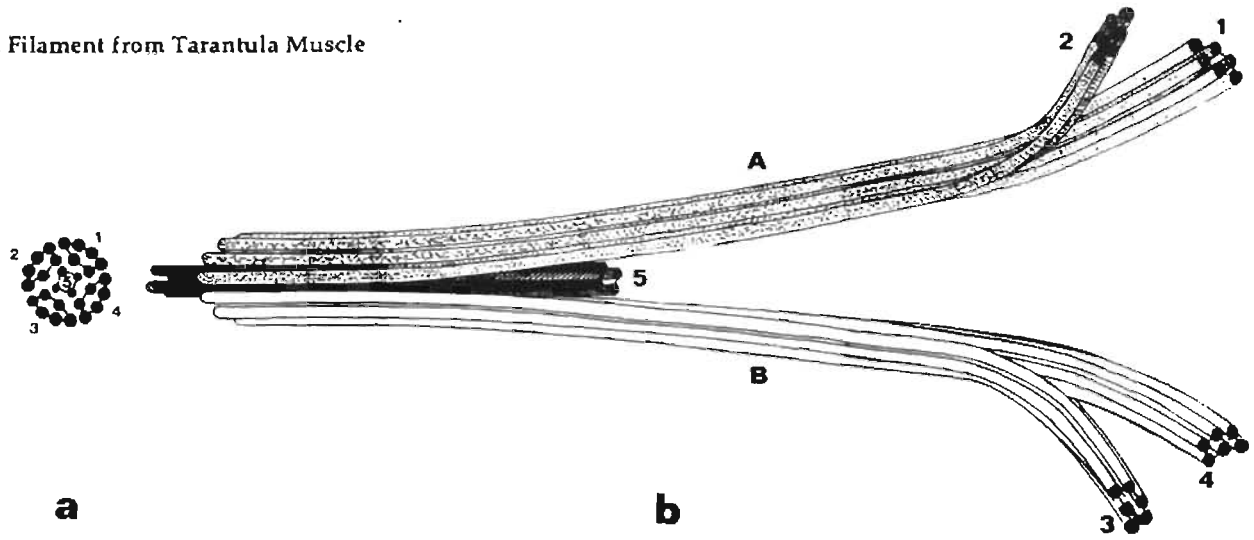

Figure 4. (a) Transverse section (drawn at scale) of one of the 4 most feasible models suggested by [21] (model "b", figure 7, Table VI; [21]) for the backbone of the thick filaments of tarantula (*Avicularia avicularia*) striated muscle. This model presents a core (hollow or constituted by a protein different than paramyosin or myosin) surrounded by 4 rounded features (constituted of paramyosin, indicated as "5", the *central core of paramyosin*), and have 48 myosin tails transversally (16 tails on the 8 rounded features of ring II and 32 tails on the 16 rounded features of ring III), forming an internal substructure of the thick filament that can be related with the surface array of myosin heads described from the three-dimensional reconstruction of tarantula muscle thick filaments [8, 20]. (b) Schematic diagram drawn at scale of a thick filament of tarantula striated muscle frayed into two secondary subfilaments "A" and "B", which also frayed each one into two more primary subfilaments "1" & "2" and "3" & "4". The corresponding features in model shown in (a) are joined by straigth links, and indicated also as subfilaments "1", "2", "3", "4" and paramyosin central core "5".

as observed) fraying can occur (Fig. 3-c). Filaments from rat and rabbit without paramyosin content fray easily from the tip to the bare zone. For filaments from scallop striated muscle, on the other hand, with a smaller amount of paramyosin (6-12% that of myosin in a molar basis), the fraying begins at the filament tips and proceeds toward the bare zone [17]. It have been proposed [34] that for these filaments paramyosin appears to be associated with each of the reported 5-7 subfilaments [17], and may not be confined to a central core domain. This could explain the difference in fraying with tarantula and the similitarity with vertebrate thick filaments. Crab [35] muscles have thick filaments which are solid in the bare zone and hollow on the rest of their lengths as is apparent from its negatively stained images which show a central stained core (Rodríguez & Padrón, unpublished results). We have evidences (Rodríguez and Padrón, unpublished results) that thick filaments from crab muscle fray into a variable number of subfilaments from the tip to the bare zone. We concluded that thick filaments that have a central paramyosin core, like the thick filaments of tarantula striated muscle, are more resistent to dissassembly than filaments that do not have such core, like the thick filaments from vertebrate striated muscle (rat and rabbit) or invertebrate striated muscles (scallop and crab) in spite of their paramyosin content.

In conclusion, our results support a model for the substructure of the backbone of tarantula thick filaments [21] which have a central core (hollow or formed by a protein different than paramyosin or myosin), surrounded by 4 rounded features that constitutes the central core (formed each one by two paramyosin molecules), and two concentric rings of 8 and 16 features (each one formed by 2 myosin tails) totalling 48 myosin tails in transverse section. Thick filaments that have a paramyosin central core like this one are more resistent to dissasembly than filaments (with or without paramyosin content) which do not have it.

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RESUMEN

Se aislaron filamentos de miosina nativos en condiciones de relajación a partir de músculo estriado de tarántula (*Avicularia avicularia*). Estos filamentos gruesos fueron deshilachados suspendiéndolos en soluciones de imidazol de baja fuerza iónica. La manera como se deshilachan estos filamentos fueron comparados con la reportada para los filamentos de vertebrados (rata y conejo). Los filamentos gruesos de tarántula se deshilachan en 4 subfilamentos sólo cerca de sus puntas; mientras que los filamentos gruesos de los vertebrados de deshilachan en 3 subfilamentos a lo largo de toda la longitud del filamento, excluyendo la zona desnuda central. Los filamentos de tarántula se deshilachan primero en 2 subfilamentos secundarios; y luego cada uno de estos se deshilachan en 2 subfilamentos primarios más. Este modo de deshilachamiento permite discriminar entre los cuatro modelos posibles propuestos para la estructura interna del esqueleto de los filamentos gruesos de la tarántula, en base a selecciones transversales ultrafinas, por Guerrero & Padrón: Acta Microsc. 1(2): 63-83, 1992. El modelo seleccionado tendría un núcleo central (hueco o constituido por una proteína diferente a la paramiosina o la miosina) rodeado de 4 estructuras circulares (cada una constituida por 2 moléculas de paramiosina) y dos anillos concéntricos formados por 8 y 16 estructuras circulares (cada una constituida por 2 colas de moléculas de miosina), totalizando 48 colas de miosina transversalmente. Concluimos que los filamentos gruesos con un núcleo central de paramiosina, como ocurre con los filamentos gruesos de tarántula, son mas resistentes al deshilachamiento que filamentos (posean contenido de paramiosina o no) que no tienen tal núcleo central.

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