New Myofibrillar Proteins

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During the past five years, there have been a large number of reports on new proteins which have been found in muscle myofibrils. Many of these proteins are minor in quantity and the majority appear to play primarily a structural role. Also, in most cases, only one or two papers have been published on each protein so the characterization work is far from complete. Thus, the information in this paper should be accepted with a degree of skepticism since some of it will probably be shown to be incorrect in the future.

The new protein which has received the most attention is connectin. This protein, which was originally discovered by Maruyama and coworkers (1976), constitutes the major part of the residue after all the rest of the myofibrillar proteins are extracted. A typical purification flow chart is shown in Figure 1. Part of this protein can be solubilized in sodium dodecyl sulfate. SDS electrophoresis on 10% polyacrylamide gels gives a major band that barely enters the top of the gel and minor bands with apparent subunit weights of 45,000 and 90,000 daltons (Maruyama et al., 1977c; Maruyama et al., 1980). Electrophoresis on lower concentration acrylamide gels allows the high molecular weight protein to enter and gives a double band with apparent molecular weight in the 700,000-1,000,000 dalton range. Proteins of this size range have been observed in SDS extracts of whole muscle and purified myofibrils (Wang et al., 1979; King and Kurth, 1980; Maruyama et al., 1981). Wang and coworkers (1979) purified this high molecular weight protein by chromatography of SDS extracts on a gel filtration column and named it "titan." It has subsequently been shown that the high molecular weight connectin fraction and titan are identical in regards to electrophoretic mobility, amino acid composition, and immunofluorescence localization (Maruyama et al., 1981).

Connectin and titan antibody studies suggest that the protein(s) is located throughout the sarcomere with the exception of the Z lines (Wang et al., 1979; Maruyama et al., 1981). There appears to be a higher concentration in the region of the A-1 junction. Thin filaments (about 2 nm in diameter) are also seen in myofibrils from which the myosin and actin have been extracted (dos Remedios and Gilmour, 1978; Takahashi and Saito, 1979) and in muscles stretched beyond the thick filament-thin filament overlap (Locker and Leet, 1975, 1976a, b). These latter filaments have been termed "gap" filaments and presumably contain connectin.

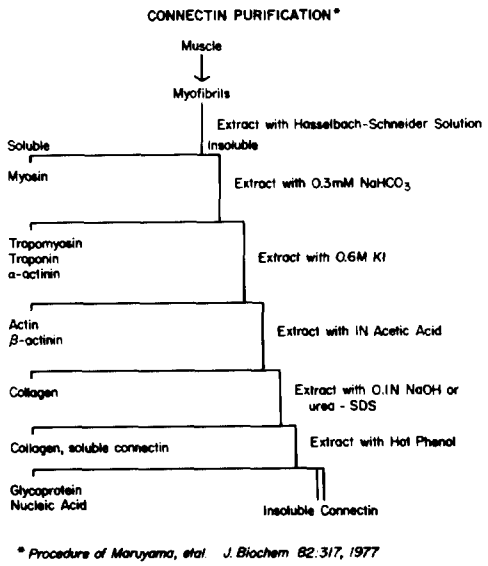

Figure 1: Purification procedure for connectin.

Insoluble connectin (Maruyama et al., 1976) as well as the pellets formed upon addition of ammonium sulfate to SDS soluble connectin are rubbery and elastic in nature (Figure 2). Electron microscopy of the ammonium sulfate precipitates reveals a complex, anastomosing network of filamentous aggregates (Figure 3). There appear to be subfilaments with diameters of approximately 2 nm which align side to side. Micrographs of insoluble connectin have a similar appearance.

Another high molecular weight protein has been identified during the course of studies on titan and which has been called the N_2-line protein (Wang and Williamson, 1980). It has an SDS subunit weight of approximately 600,000. Antibodies against this protein stain the N_2-lines (structures found parallel to the Z lines in the I bands). The N_2-line protein may bind to the titan network since the N_2-line does not remain a constant distance from the Z line with varying sarcomere lengths. The function of this protein and the N_2-line are not known.

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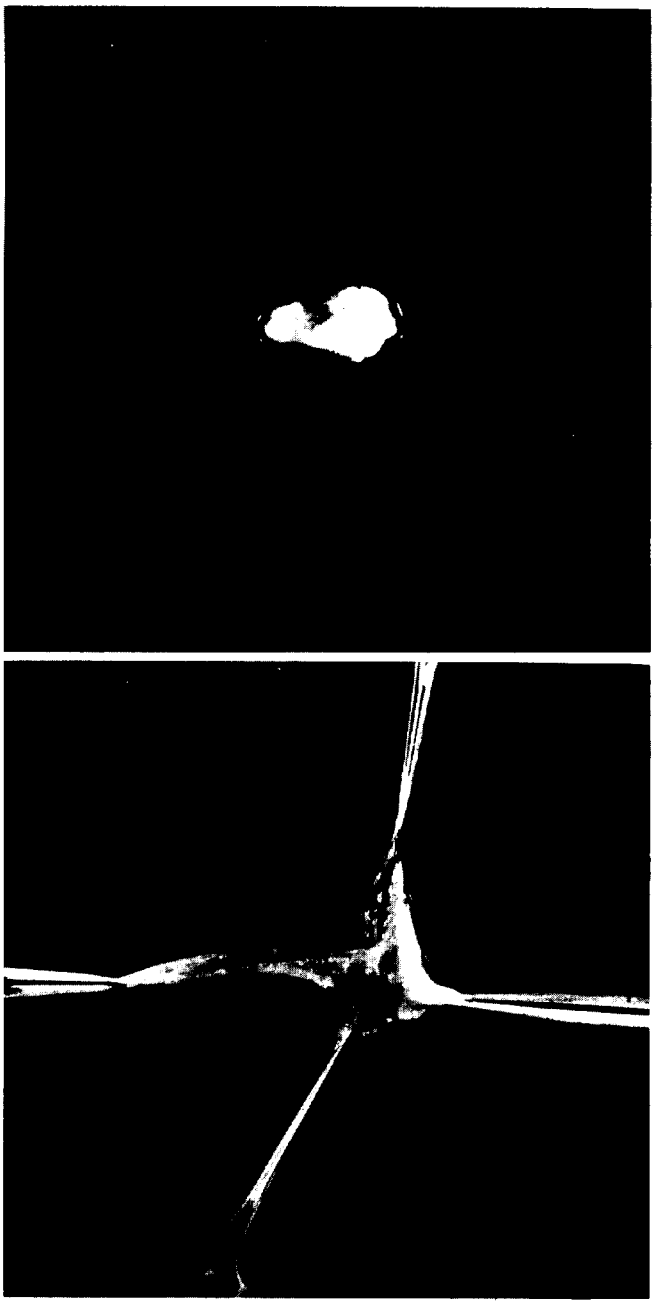

Figure 2. Chicken skeletal muscle connectin. Muscle was extracted with Hasselbach-Schneider solution and KI to solubilize most of the protein. The residues were further extracted with 8 M urea—3% SDS, and insoluble material was removed by centrifugation. Ammonium sulfate addition to 30% saturation resulted in the formation of a large elastic aggregate. A. Unstretched. 1.2X. B. Stretched. 1.2X.

Figure 3. Electron micrograph of bovine cardiac connectin. The SDS soluble connectin was mixed with ammonium sulfate to make a 30% saturated solution. The insoluble pellet formed was fixed in glutaraldehyde and osmium tetroxide, stained *en bloc* with uranyl acetate, embedded in Epon, and sectioned. Sections were also stained with uranyl acetate and lead citrate. The protein appears as large, interconnecting networks made up of different sizes of strands. The strands are composed of filamentous material with filament diameters of about 2 nm. 24,000X.

The M line has been identified as the location of creatine kinase and myomesin. Antibodies against creatine kinase (an enzyme composed of two 42,000 dalton subunits and involved in the transfer of high energy phosphate groups from creatine phosphate to ADP or from ATP to creatine) bind specifically to the M line (Wallimann et al., 1978). Furthermore, monovalent Fab' fragments of these antibodies solubilize most of the creatine kinase activity bound to myofibrils and at the same time remove most of the electron dense material from the M line. Myomesin, also referred to as M-protein, has a subunit weight of 165,000 daltons (Masaki and Takaiti, 1974; Trinick and Lowey, 1977; Eppenberger et al., 1981). This protein binds to myosin and creatine kinase *in vitro* (Mani and Kay, 1978; Mani et al., 1980). Myomesin apparently does not contribute significant electron density to the M line since it is present in heart myofibrils which lack a visible M line (Sommer and Johnson, 1969; Strehler et al., 1979). The proteins phosphorylase and glycogen debranching enzyme are extracted from myofibrils concomitantly with M line removal but antibodies against these proteins do not bind specifically to the M line (Heizmann and Eppenberger, 1978, 1979; Trinick and Lowey, 1977).

F-protein was first noticed as a band on SDS gels and has an apparent subunit weight of 110,000 daltons (Starr and Offer, 1971). It is extracted with myosin (Offer, Moos, and Starr, 1973) and can be purified by ammonium sulfate precipitation and DEAE-Sephadex chromatography. F-protein binds to myosin, but this binding is inhibited by C-protein (Miyahara et al., 1980). F-protein is presumably located in the thick filaments, but its role in structure and function remains to be determined.

I-protein has been purified from KI extracts of rabbit and chicken myofibrils (Ohashi et al., 1977a). The protein has a subunit weight of 50,000 daltons and has a high content of aspartic and glutamic acids. Antibody localization studies have shown that the protein is found throughout the A band with the exception of the center (Ohashi et al., 1977b). I-protein inhibits the Mg activated ATPase activity of actomyosin in the absence of Ca^{++} but not in its presence (Maruyama et al., 1977b). It also will bind to myosin *in vitro*.

Some confusion exists regarding the nature of β-actinin, a protein originally purified by Maruyama (1965). Two different proteins have been given this name, one a monomeric protein with a subunit weight of 60,000 and the other a dimer of 37,000 and 34,000 dalton subunits. The 60,000 dalton β-actinin (Maruyama, 1971; Heizmann and Haupte, 1977) has recently been shown to be identical to serum albumin (Heizmann et al., 1981). The dimeric β-actinin binds to F-actin and inhibits the recombination of fragmented F-actin filaments (Maruyama et al., 1977a). Antibody localization studies suggest it binds to the free ends of the thin filaments (Maruyama et al., 1977a). It therefore may be involved in regulating thin filament length.

γ-actinin has been purified from native thin filaments of rabbit skeletal muscle (Kuroda and Maruyama, 1976a). It has a subunit size of 35,000 daltons and is rich in non-polar and neutral amino acids. γ-actinin binds to F-actin *in vitro* and inhibits G-actin polymerization (Kuroda and Maruyama, 1976b).

A very recently discovered protein in the "actinin" series is Eu-actinin (Kuroda and Masaki, 1980; Kuroda et al., 1981). It was observed that the properties of the 42,000 proteins extracted from I-Z-I brushes were different depending on whether the low ionic strength extraction was conducted at pH 6.5 or pH 8.0 (Kuroda and Masaki, 1980). The pH 6.5 extract contained a 42,000 dalton protein which was more acidic than actin as determined by isoelectric focusing (Kuroda et al., 1981). Antibodies against Eu-actinin did not cross-react with actin or α-actinin. Immunofluorescence microscopy indicated that Eu-actinin was located in the Z line (Kuroda et al., 1981).

The protein filamin was originally purified from smooth muscle where it is found in relatively high concentrations (Wang et al., 1975; Shizuta et al., 1976). It is a dimer of 240,000 dalton subunits. Small quantities of this protein have recently been found in skeletal muscle and it appears to be localized at the Z line (Bechtel, 1979). It has also been shown to bind to F-actin.

Another Z line protein has been purified from KI extracts of chicken myofibrils by Ohashi and Maruyama (1979). It has a subunit weight of 55,000 (identical to desmin), but its solubility, amino acid composition, and immunochemical properties were different from desmin. The protein will form lattices *in vitro* which were similar in appearance to cross sections of the Z line.

Two other Z line proteins in skeletal muscle myofibrils have been detected using antibody methods. Antibody against vimentin, a 58,000 dalton protein which is the primary constituent of intermediate filaments in fibroblasts, stains the periphery of the Z line in myosin and actin extracted Z disc scaffolds (Granger and Lazarides, 1979). These results suggest that at least a small amount of this protein is found in skeletal muscle and that it, like desmin, may function to hold the Z lines from adjacent myofibrils in register. Synemin, a 230 dalton protein which co-purifies with desmin and vimentin through cycles of depolymerization and polymerization, also produces antibodies which stain skeletal muscle Z lines (Granger and Lazarides, 1980).

A diagram of a myofibril sarcomere is shown in Figure 4. The current ideas regarding the location of the different proteins is indicated. It should be realized that in spite of the large number of different proteins that have been identified, many of them contribute only a small amount of the myofibril's mass (Table 1).

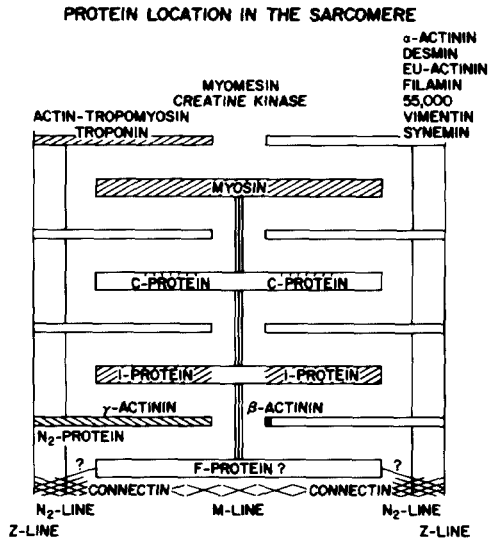

Figure 4. Protein location in the sarcomere.

Table 1. Protein content of the myofibril - 1981

Component	*%*
Myosin	45
Actin	20
Tropomyosin	5
Troponin	5
Connectin (titan)	6
N_2-line protein	3
C-protein	2
Myomesin	2
α-actinin	2
β-actinin	<1
γ-actinin	<1
Eu-actinin	<1
Creatine kinase	<1
55,000 dalton protein	<1
F-protein	<1
I-protein	<1
Filamin	<1
Desmin	<1
Vimentin	<1
Synemin	<1

Discussion

Max Judge, Purdue, University: Is there some evidence that connectin also might attach to the inside of the sarcolema?

Greaser: Yes, there is. There is some thinking that it may provide some continuity between the end of the myofibrils and the inside of the sarcolema. It's found in the inner surface of a number of other cells as well, just inside the cell membrane.

Tom Bidner, Louisiana State University: What's the purpose of this N_2 line? I'm not familiar with this.

Greaser: Well, I'm not sure what the purpose of it is. One can see it in certain kinds of muscle preparations. It may be involved in sort of 'regulating' the spacing of the thin filaments apart; it may be something the connectin can, in fact, attach to. In terms of the whole structure, it's just not clear what the function really is.

R. G. Kauffman, University of Wisconsin: Has this changed our view of the physiological functioning of muscle or the sliding filament theory? Is there anything that we do know now that we didn't know before about this?

Greaser: Actually, of all the proteins that I've talked about today, about the only one that might possibly modulate the function of the muscle is the I protein, which tends to supress the actomyosin ATPase in the absence of calcium. Most of the other proteins I've talked about are structural—at least at this point—or may be involved in the assembly process, regulating the assembly of thick and thin filaments. But, in terms of most of the other ones, the essential story about how muscle contraction works and the sliding filament theory, I think, is pretty much intact.

R. M. Robson, Iowa State University: Does it bother you, Marion, that creatine kinase seems not to bind very well with the light meromyosin? It seems to bind better, I think, to SI.

Greaser: Yes, that bothers me. But, it just may mean that there's something—that the binding to myosin, per se, may not be the direct one. But, in fact, they can demonstrate binding to myosin and, I don't really know if there is another intermediate protein that may be involved or not. It seems that there's been work done where people have, in fact, taken myofibrils in which the M-line has been removed, added creatine kinase, and gotten a structure reconstructed on it. That's still a little shakey, because we don't know what was still left there.

Elgasum Ali Elgasim, Oregon State University: In terms of rigidity and flexibility, how do you characterize these proteins? Do they add to the flexibility of the muscle, or to the rigidity of the muscle?

Greaser: That's a good question. The connectin may, in fact, provide some elastic continuity to the whole sarcomere, and may be involved in preventing things from getting pulled out too far, or whatever. I don't think it probably contributes any to the force of shortening, but it may be involved, say in the force of restoring muscle back to its original length after contraction. Again, this is speculation. We don't really know and understand these things too well yet. But, most of the other proteins that I've talked about are either minor in amount or play only a minor role in terms of the contractile process.

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