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BIOCHEMISTRY AND STRUCTURE OF DESMIN AND THE RECENTLY DISCOVERED MUSCLE CELL CYTOSKELETON

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INTRODUCTION

IT HAS been established (for review see Goll et al., 1977a) that striated muscle cells are literally packed with myofibrils, the long protein threads that are composed of about ten proteins; namely, myosin, actin, tropomyosin, troponin, α-actinin, M-proteins (possibly two), C-protein and probably about two to four very recently discovered, as yet poorly characterized, proteins. These proteins are assembled into a highly ordered, structural complex composed of a double array of interdigitating thick and thin filaments (Huxley, 1957, 1963, 1969). The thin filaments are anchored at one end to the transverse Z-line and extend between the thick myosin filaments at the other end. Earlier studies in several laboratories, including our own (reviewed in Goll et al., 1972, 1974, 1977b; Parrish, 1978), have shown that, aside from the variable degree of shortening and overlap of actin and myosin filaments (depends in turn upon factors such as degree of muscle restraint, temperature, species, fiber type, and antemortem handling), the major structural alteration that occurs in muscle postmortem is centered around the Z-line structure. The Z-line gradually disintegrates with postmortem storage. Loss of Z-line integrity postmortem may, in turn, be due to the action of proteases, such as the Ca^{2+}-activated, endogenous muscle protease (termed CAF) that we have recently characterized (Dayton et al., 1976a, 1976b), or perhaps to a combination of muscle proteases, including CAF, working in concert (Bird et al., 1980; Okitani et al., 1980). Because of the importance of the Z-line region in maintaining overall integrity of the myofibril and muscle cell, we have carefully examined the structure and biochemistry of Z-line and Z-line-like structures (Robson et al., 1970; Robson and Zeece, 1973; Dayton et al., 1976a, 1976b; Schollmeyer et al., 1976; Stromer et al., 1976; Suzuki et al., 1976; Singh et al., 1977; Davies et al., 1978; Yamaguchi et al., 1978; Zeece et al., 1979). During some of these experiments, which involved preparations of fractions enriched in Z-line material from myofibrils of skeletal and smooth muscle, we observed an increase in the proportion of a new protein having a subunit molecular weight of 55,000. As described later, we think that this new protein is an important part of the muscle cell cytoskeleton; namely, 10-nm diameter filaments.

Within the past two to three years, it has become evident that essentially all vertebrate cells contain, in addition to microtubules and microfilaments (actin filaments), a third class of cytoplasmic filaments (often referred to as "intermediate" filaments) with diameters of approximately 10 nm (Ishikawa et al., 1968; Uehara et al., 1971; Dahl and Bignami, 1975; Steinert et al., 1976; Hynes and Destree, 1978; Starger et al., 1978; Franke et al., 1978; Goldman et al., 1979). Although these 10-nm filaments from a wide variety of cell types share some common structural and chemical properties, analysis by electrophoresis and immunofluorescence microscopy suggests there are at least as many as five major classes of 10-nm filaments composed of distinct subunit proteins (see reviews by Goldman et al., 1979 and Lazarides, 1980); namely, differentiated muscle cell intermediate filaments, intermediate filaments in mesenchymally derived cells (e.g., fibroblasts), tonofilaments and epidermal keratin filaments of epithelia-derived cells, glial cell filaments, and neurofilaments. Each class is, in general, characteristic of a particular cell type, although some cells may have two (Franke et al., 1979; Gard et al., 1979) or even three (Wang et al., 1980) co-existing classes.

Relatively little is yet known about the properties and function of the 10-nm filaments present in muscle cells. Studies in our laboratory (Schollmeyer et al., 1976; Huiatt et al., 1978, 1980) and in others (Cooke, 1976; Lazarides and Hubbard, 1976; Small and Sobieszek, 1977; Bennett et al., 1978; Fellini et al., 1978) have suggested that a protein having a subunit molecular weight of approximately 55,000 (termed desmin by Lazarides and Hubbard, 1976, and skeletin by Small and Sobieszek, 1977; desmin will be used herein) is a major component of the 10-nm filaments present in vertebrate smooth muscle cells. By immunofluorescence (using antibodies produced against the smooth muscle protein) (Lazarides and Hubbard, 1976; Bennett et al., 1978, 1979; Campbell et al., 1979; Richardson et al., 1980[1]) and by two-dimensional gel electrophoretic analysis (Izant and Lazarides, 1977), desmin also has been identified in differentiated skeletal muscle cells. The immunofluorescent studies indicate that desmin is located primarily at or near the Z-line structure, and it is likely that desmin plays important roles in muscle Z-lines, in alignment of myofibrils with respect to each other and to the rest of the muscle cell, and in maintaining overall integrity of the muscle cell (i.e., desmin is an important functional part of the muscle cell cytoskeleton).

The major problem that has hindered work on desmin has been the lack of a satisfactory method for preparing the protein in a reasonably pure form. We have recently succeeded in preparing highly homogeneous preparations of desmin from both avian smooth muscle (Huiatt et al., 1978, 1980) and from adult mammalian (porcine or bovine) skeletal muscle (O'Shea et al., 1978, 1979, 1980) and have been able to partially characterize some of the properties of desmin.

The major purposes of this paper are to 1) review our recent biochemical and structural studies on muscle desmin, and 2) briefly discuss the potential importance of desmin to the muscle cell *in vivo* and implications for meat quality of postmortem changes that occur in desmin.

MATERIALS AND METHODS

DETAILED procedures for preparation of crude desmin extracts and purification of desmins from avian smooth muscle and from porcine skeletal (semitendinosus and biceps femoris) muscle are described in Huiatt et al. (1980) and O'Shea et al. (1980), respectively. Purification of the two desmins included successive chromatography on hydroxylapatite and DEAE-Sepharose CL-6B in the presence of urea. The Ca^{2+}-activated muscle protease was prepared according to the procedure of Dayton et al. (1976a). Descriptions of the isolation of crude desmin and subsequent purification will be given in the Results and Discussion section.

[1] F. Richardson, M. Stromer, T. Huiatt and R. Robson, manuscript in preparation.

Electrophoresis in the presence of sodium dodecyl sulfate (SDS) was done either on cylindrical polyacrylamide gels essentially according to the methods of Weber and Osborn (1969) or on polyacrylamide slab gels using a discontinuous Tris-glycine buffer system (Laemmli, 1970). Preparation of all insoluble samples (myofibrils, native intermediate filaments, etc.) for electrophoresis was done as described in Huiatt et al. (1980) and O'Shea et al. (1979, 1980). For two-dimensional electrophoresis, the isoelectric focusing was done in the first dimension according to the method of O'Farrell (1975) in 4% acrylamide gels, and the SDS- polyacrylamide gel electrophoresis in the second dimension was done on 10% polyacrylamide slab gels according to the method of Allen et al. (1978) using the Laemmli (1970) buffer system. Porcine skeletal (α-variant) and turkey gizzard (primarily the γ-variant) actins used for standards in two dimensional gels were extracted and purified from acetone powders by the method of Spudich and Watt (1971). Peptide mapping comparisons of purified desmin from porcine skeletal, porcine smooth, and avian smooth muscle were done as described in O'Shea et al. (1979).

Negatively stained preparations of native and synthetic 10-nm filaments were prepared according to the procedures described in Huiatt et al. (1980) and O'Shea et al. (1980) and examined in an RCA EMU-4 electron microscope operated at 100 KV. Protein concentrations, quantitation of percent desmin in fractions at selected steps during isolation and purification, sedimentation velocity experiments, CD spectra, and amino acid analyses were done as described in O'Shea et al. (1979, 1980) and Huiatt et al. (1980).

RESULTS AND DISCUSSION

BECAUSE desmin (subunit molecular weight of 55,000) makes up approximately 5.0% of the total protein in homogenized gizzard muscle but only about 0.18% of the total protein in homogenized skeletal muscle, we first developed procedures for isolation and purification of desmin from smooth muscle. This information was then used to help us in the difficult task of developing procedures for isolating the small amount of desmin from mature mammalian skeletal muscle. Because previous studies (Cooke and Chase, 1971; Cooke, 1976; Schollmeyer et al., 1976) showed that intermediate filaments in smooth muscle remained insoluble after extraction of actomyosin at high ionic strength from myofibrils, a similar procedure was used in our experiments on avian smooth and porcine skeletal muscle desmin to first obtain a fraction enriched in desmin before solubilization and further purification by chromatography in urea. For either the skeletal or smooth preparation, well-washed myofibrils (including washes with solutions containing Triton X-100 to remove membranous material) were first prepared. The myofibrils were then exhaustively extracted with actomyosin solvents. Examination of the actomyosin-extracted residues from smooth muscle with the electron microscope demonstrated that they contained large numbers of native intermediate filaments, together with dense bodies (Z-line analogs), insoluble collagen fibrils and some residual membranes (Huiatt et al., 1980). Studies now are under way to try to unambiguously identify 10-nm filaments in similar extracts prepared from skeletal muscle.

We have found that smooth muscle desmin can be quantitatively solubilized by overnight extraction of these crude intermediate filament preparations with 6 M urea-containing solutions (Huiatt et al., 1980). For preparation of crude desmin from skeletal muscle, the actomyosin-extracted residues were also extracted with KI (primarily to remove more of the actin contamination) and were subjected to one cycle of desmin-solubilization in acetic acid followed by precipitation of desmin by pH neutralization (adapted from Small and Sobieszek, 1977) in order to further enrich the fractions in desmin prior to solubilization in urea (O'Shea et al., 1980). Desmin comprises approximately 25% and 20% of the total protein in the urea-solubilized, crude desmin fractions of the smooth and skeletal muscle preparations, respectively. Major contaminants present in the urea solubilized, crude desmin from smooth muscle have molecular weights on SDS-gels of 42,000 (actin) and 200,000 (myosin). Minor contaminants correspond in molecular weight to 130,000 [putative vinculin/focsin (Geiger et al., 1979; Burridge and Feramisco, 1980)] and 100,000 (α-actinin). The urea-solubilized, crude desmin extracts from porcine skeletal muscle contain a large amount of poorly resolved, high-molecular-weight components, plus significant quantities of actin and α-actinin, as well as several other minor contaminants (O'Shea et al., 1980).

We tried a variety of chromatographic methods for purification of desmin from the urea-solubilized crude desmin extracts. The best success has been achieved with successive chromatography of the crude desmin on hydroxylapatite (phosphate salt gradient) and DEAE-Sepharose CL-6B (NaCl-gradient) in the presence of urea. The hydroxylapatite column, in particular, was effective in removing actin (does not stick to column and elutes before initiation of the phosphate salt gradient) from desmin. Typical preparations of the DEAE-Sepharose-purified desmins contained no actin contamination, as judged by electrophoresis done by the Laemmli (1970) or Weber and Osborn (1969) methods. A small amount (less than 2% of protein) of a 48,000-dalton component, which is a putative proteolytic breakdown product of desmin (Huiatt et al., 1980), was generally present in the purified desmin. Typical yields of purified desmin averaged 150-200 mg from preparations started with 100 g minced gizzard (Huiatt et al., 1980) and 6 mg from 100 g minced porcine skeletal muscle (O'Shea et al., 1980). These comparative figures reflect the much higher concentration of desmin in smooth muscle (desmin makes up 8% of avian smooth muscle myofibrils, but only 0.35% of skeletal muscle myofibrils).

Examination of the purified desmins by two-dimensional gel electrophoresis demonstrated that the avian smooth muscle desmin is composed of two isoelectric variants, α- (more acidic) and β-desmins. β-desmin is the more prominent of the two, constituting approximately 70 to 80% of the protein. Purified porcine skeletal muscle desmin consists almost entirely of one major desmin variant. The two desmins (avian and mammalian) are clearly resolved from each other on 2-D gels. Analysis of our purified smooth and skeletal desmins by one- and two-dimensional gels also has demonstrated clearly that we have no fibroblastic intermediate filament protein (termed vimentin by Franke et al., 1978) in our preparations.

Purified desmin remains soluble after removal of urea by extensive dialysis against 10 mM Tris-acetate, pH 8.5, and clarification at 183,000 x g for 1 hr. Analytical ultracentrifugation showed that purified avian smooth desmin sedimented as a single peak (S^{o}_{20} = 5.2) when examined in 10 mM Tris-acetate, pH 8.5. The ultraviolet absorption spectrum of desmin, measured in 10 mM Tris-acetate, pH 8.5, showed a maximum at 278 nm and a measured $A^{1\%}_{278}$ of 5.57 (protein conc. measured by biuret) and a ratio of absorbance at 278 nm to that at 260 nm of 1.64. The ultraviolet circular dichroism spectra of soluble desmin (in 5 mM sodium phosphate, pH 8.5) is typical of an α-helical protein, with two negative extrema at 208 and 222 nm. The protein contains approximately 45% α-helix, suggesting that the purified desmin adopts a native structure after removal of urea (Huiatt et al., 1980).

Amino acid analyses of avian smooth and porcine skeletal desmins indicate a similar amino acid composition (O'Shea et al, 1979). Comparative peptide maps of the two desmins after selective cleavage by CAF or trypsin (O'Shea et al., 1979) suggest some homol-

ogy between the avian and mammalian desmins, but also some distinct differences, a result consistent with our two-dimensional gel electrophoresis experiments. A notable property of desmin is its marked sensitivity and susceptibility to partial proteolytic breakdown.

To determine whether our purified desmin would form "synthetic" filaments in vitro, solutions of either soluble porcine skeletal or avian smooth desmin were dialyzed against 100 mM NaCl, 1 mM $MgCl_2$, 10 mM imidazole-HCl, pH 7.0, (O'Shea et al., 1980) or 150 mM NaCl, 10 mM imidazole-HCl, pH 7.0, (Huiatt et al., 1980). The resulting, transparent, viscous suspension was negatively stained and examined in the electron microscope. Both desmins had assembled into filaments that appeared as long, flexible strands with a diameter very close to 10 nm. The morphology of these synthetic filaments is very similar to that of native 10-nm filaments present in crude intermediate filament fractions before urea extraction (Huiatt et al., 1980).

To ascertain the location of desmin in muscle cells, we have prepared antibodies to DEAE-Sepharose-purified desmin that has been further purified by preparative electrophoresis. Indirect immunofluorescence localization studies[1] demonstrated localization of desmin near the Z-lines of striated muscle cells. A connecting link between Z-lines of adjacent myofibrils often can be seen. In general, the immunofluorescent studies indicated that desmin was located around the periphery of the Z-line, as opposed to being in the Z-line proper as is α-actinin. Immunoelectron microscope experiments using the horseradish peroxidase technique demonstrated electron-dense end product, primarily between adjacent myofibrils at the level of the Z-line[1], in harmony with the immunofluorescent localization of desmin. The localization studies strongly suggest a cytoskeletal role for desmin in muscle cells.

SUMMARY

THE PRESENCE of significant amounts of actin and other contaminants in desmin preparations (Fellini et al., 1978; Hubbard and Lazarides, 1979) has severly hampered interpretations of previous studies on desmin. In contrast, our studies have demonstrated that highly purified desmin can be routinely prepared that is free of electrophoretically detectable quantities of actin. Even the small amount of desmin known to be present in skeletal muscle cells (Izant and Lazarides, 1977; Fellini et al., 1978) can be successfully prepared and studied. The results described herein provide a purified system that permits a detailed examination for potential interactions of desmin and desmin filaments with other cytoskeletal and myofibrillar components such as actin and α-actinin, and a simple, well-defined in vitro system for study of the self-assembly process and structure of synthetic desmin filaments.

The only reliable, currently known functional test for desmin is that it forms 10-nm filaments. We have demonstrated formation of 10-nm filaments from highly purified smooth or skeletal muscle desmin at near physiological conditions of pH and ionic strength. The synthetic filaments are similar to native 10-nm filaments from smooth muscle. Although antibodies to desmin bind to (or at the periphery of) skeletal muscle Z-lines, 10-nm filaments have never been unambiguously identified in adult skeletal muscle cells (see discussion in Bennett et al., 1979). They have been observed in cells of the working myocardium (Ferrans and Roberts, 1973; Behrendt, 1977). Lack of identification of 10-nm filaments in skeletal muscle cells may reflect the small number of such filaments present. We cannot, however, rule out the possibility that the skeletal muscle desmin is not present in the assembled form of 10-nm filaments. That the highly purified skeletal muscle protein will self-assemble into 10-nm filaments argues, albeit indirectly, for their presence in skeletal muscle cells.

Although studies on the muscle cell cytoskeleton are still in their infancy, it now seems clear that desmin and 10-nm filaments are going to be an important part of this system. Our studies indicate that desmin is the major subunit of mature muscle 10-nm filaments and ties adjacent myofibrils together at their Z-line levels and also to surrounding parts of the cell such as the cell membrane and nucleus (i.e., it ties myofibrils into the cell cytoskeleton). It is likely that desmin plays an important role in ordering of the muscle subcellular components, in maintaining myofibrillar integrity, and perhaps even in maintaining overall muscle cell size and shape.

In preliminary studies (O'Shea, Robson and Stromer, unpublished) on postmortem changes in bovine skeletal muscle desmin, we have found that desmin is degraded at about the same rate as troponin-T. We suggest that specific postmortem changes in elements of the cytoskeleton, which are responsible for both the lateral association between myofibrils and for integrating the myofibril into the three-dimensional meshwork of the entire muscle cell, may have profound effects on certain quality attributes of meat.

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