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PURIFIED DESMIN FROM ADULT MAMMALIAN SKELETAL MUSCLE: A PEPTIDE MAPPING COMPARISON WITH DESMINS FROM ADULT MAMMALIAN AND AVIAN SMOOTH MUSCLE*

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SUMMARY

Comparative one-dimensional peptide maps were prepared by the electrophoresis of digests derived from treatment of desmins with Ca^{2+}-activated muscle protease, trypsin, Staphylococcus aureus V8 protease, and cyanogen bromide. Desmins from adult mammalian skeletal and smooth muscles were very similar. Avian smooth muscle desmin, although homologous with respect to many peptides, was different from the mammalian smooth and skeletal desmins. The amino acid compositions of the three desmins were quite similar.

INTRODUCTION

Many, if not all, animal cells contain a cytoplasmic filament class with diameters of about 10 nm (1-6). The degree of relatedness, however, of 10-nm filament classes from different cell types and of their constituents is not yet clear (4, 7-10). Ten-nm filament proteins, with a subunit molecular weight of approximately 55,000, have been isolated from mammalian and avian smooth muscle (11) . Although Lazarides and Hubbard (12) have used immunofluorescence to localize 10-nm filament protein, which they have termed desmin, at or near the Z-line of skeletal muscle myofibrils, identification of 10-nm filaments has not been achieved at the electron microscope level.

As part of our continuing studies on the structural composition of the Z-line and Z-line-like structures (13-18), we have examined the properties of a 55,000-dalton protein that we have isolated from mature mammalian skeletal muscle. The results in this paper indicate that the 55,000 molecular weight protein from skeletal muscle is desmin, that it

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is very homologous to mammalian smooth desmin, and that it shares considerable homology with avian smooth desmin.

MATERIALS AND METHODS

Source of Muscles and Protein Preparations: Fresh porcine semitendinosus and biceps femoris muscles were used as the source of adult mammalian skeletal muscle. Smooth muscle of the stomach from the same animals was used as the source of mammalian smooth muscle tissue. Turkey gizzards were used for avian smooth muscle. Full details of the protein extraction and preparation of the desmins will be presented elsewhere. Steps used for solubilization of the two mammalian samples are similar to those described by Small and Sobieszek (11). Purification of the three desmins included successive chromatography on hydroxylapatite and DEAE-Sepharose CL-6B. The Ca^{2+}-activated muscle protease was prepared according to the procedure of Dayton et al. (19).

Preparation of Peptide Digests: Specific conditions used for the enzyme digests are given in the figure legends. Optimum conditions were selected following time-course and concentration-dependence experiments. Commercial proteases used were trypsin (Type XI, Sigma Chemical Company, St. Louis, MO) and Staphylococcus aureus V8 protease (Miles Laboratories Inc., Elkhart, IN). Cyanogen bromide digests were done by dissolving 1 mg of freeze-dried desmin in 2 ml of 70% formic acid containing 1% phenol (20). After adding 15 mg of cyanogen bromide, samples were incubated under nitrogen for 18 h in the dark at room temperature. Distilled water was added to bring the total volume to 25 ml and the mixture was lyophilized. All peptide digests were repeated a minimum of three times on each of three different protein preparations. Protein concentrations were determined by the biuret method (21) as modified by Robson et al. (22) or by the Folin-Lowry procedure (23).

One-Dimensional Electrophoretic Analysis: Electrophoresis of peptides was performed on SDS-polyacrylamide slab-gels (17 X 16 X 0.15 cm) using a discontinuous Tris/glycine buffer system (24). The concentrations of acrylamide (ratio of bisacrylamide/acrylamide was 1/30) in both separating and stacking gels were varied to achieve maximum resolution and are given in the figure legends. Gels were stained overnight in 50% methanol, 7% acetic acid, and 0.1% Coomassie Brilliant Blue R250 (C.I. No. 42660). The amount of protein loaded onto each well was adjusted so that minor bands could be seen and is given in the figure legends. Molecular weights were estimated by extrapolation using proteins with known subunit molecular weights (α-actinin, actin, tropomyosin, carbonic anhydrase, chymotrypsinogen, lysozyme).

Amino Acid Analysis: Analyses were carried out on a Durrum D-400 Amino Acid Analyzer (Durrum Instruments, Sunnyvale, CA). Proteins were hydrolyzed in vacuo for 24 h in 6N hydrochloric acid at 110° C. Cystine and cysteine were measured as cysteic acid (25). Five μl of 10% phenol solution was added to protect tyrosine.

RESULTS

To facilitate nomenclature of proteins in this section, the 55,000-dalton protein isolated from mature skeletal muscle will be referred to as desmin. The control (0 time) and 2, 10, and 30 min digests with Ca^{2+}-activated muscle protease of desmins from porcine skeletal (tracks a-d), porcine stomach (tracks e-h), and avian smooth (tracks i-l) muscles

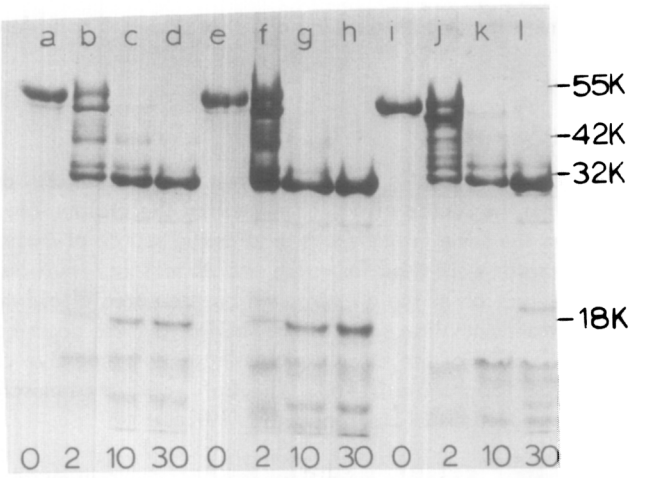

Fig. 1. Digestion of desmins with Ca^{2+}-activated muscle protease. Porcine skeletal, a-d; porcine smooth, e-h; avian smooth, i-l. The enzyme to substrate ratio was 1/50 (w/w). Desmins were first extensively dialyzed against 20 mM Tris·acetate, pH 7.5, 10 mM 2-mercaptoethanol, 0.1 mM EDTA. Digestion was done at 25° C and was initiated by addition of $CaCl_2$ to a final concentration of 5 mM. Aliquots were removed at 0, 2, 10, and 30 min and immediately boiled in the presence of 1% SDS, 1% 2-mercaptoethanol for 5 min. For the controls (a, e, i), 5 μg of protein was loaded, and for the digests, 25 μg. Electrophoresis was done with an 8% acrylamide stacking gel and a 12½% acrylamide separating gel.

are shown in Fig. 1. The subunit of the avian smooth muscle desmin migrated with a molecular weight of approximately 53,000, about 2,000 less than the two mammalian samples (compare track i with a and e), a result similar to that noted previously (10). Patterns generated from digestion of desmins from porcine skeletal and smooth muscles were very similar. These two desmins also were essentially the same in terms of the rate of digestion with regard to breakdown and appearance of peptides. The pattern of peptides from the avian smooth muscle sample suggested some homology with the mammalian proteins. In particular, the digestion of all three desmins led to accumulation of a major peptide product having a molecular weight of approximately 32,000. Differences between digests from the avian desmin and those from the mammalian proteins were evident in the 2-min digests as additional intermediate degradation steps between 32,000 daltons and

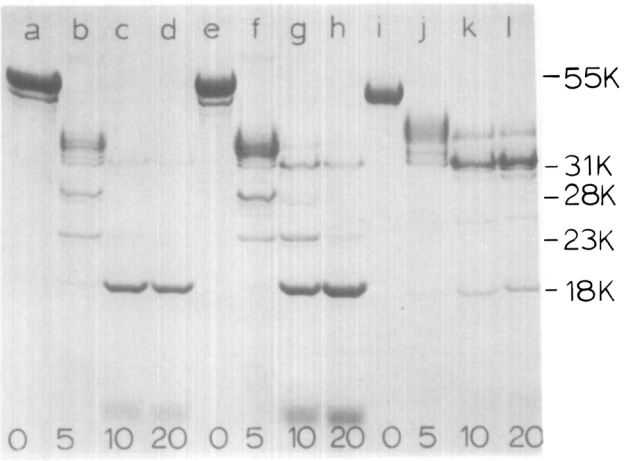

Fig. 2. Digestion of desmins with trypsin. Porcine skeletal, a-d; porcine smooth, e-h; avian smooth, i-l. The enzyme to substrate ratio was 1/250 (w/w). All proteins were first extensively dialyzed against 10 mM Tris·acetate, pH 8.5, 5 mM EDTA. Digestion was done at 25° C for 2, 10, and 20 min and terminated by boiling of removed aliquots in 5% SDS, 1% 2-mercaptoethanol for 5 min. For the controls (a, e, i), 10 μg of protein was loaded, and for the digests, 25 μg. Electrophoresis was done with an 8% acrylamide stacking gel and a 15% acrylamide separating gel.

the intact subunit. With further digestion, an 18,000-dalton peptide accumulated in the mammalian samples, but was absent or nearly so from the avian sample.

Peptide maps of the three desmins produced by digestion with trypsin are shown in Fig. 2. Again, the pattern of fragments produced are quite similar for the two mammalian desmins, but differences between the mammalian and avian proteins were evident in peptides in the molecular weight range of 31,000 to 39,000. Peptides of 28,000 and 23,000 daltons were major intermediates in the mammalian samples, but were not in the avian samples. With continued digestion, a fragment having a molecular weight of about 18,000 became a major breakdown product in the mammalian system, but to a lesser degree in the avian desmin. Also, several small peptides were generated from the mammalian desmins, which were absent or nearly so in the avian digests.

The results of digesting the three desmins with Staphylococcus aureus V8 protease are shown in Fig. 3. The fragment pattern from mammalian desmins in the 30,000- to 55,000-

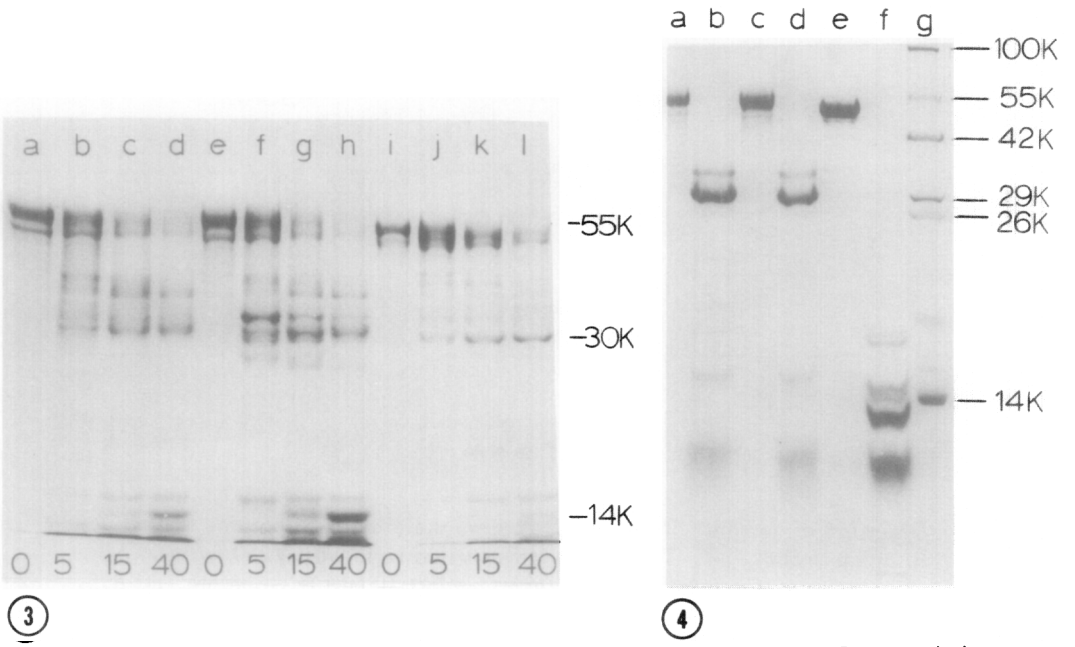

Fig. 3. Digestion of desmins with Staphylococcus aureus V8 protease. Porcine skeletal, a-d; porcine smooth, e-h; avian smooth, i-l. The enzyme to substrate ratio was 1/250 (w/w). All proteins were first extensively dialyzed against 20 mM ammonium bicarbonate, pH 8.0, 5 mM EDTA. Digestion was done at 35° C for 5, 15, and 40 min and terminated by boiling removed aliquots in 5% SDS, 1% 2-mercaptoethanol for 5 min. For the controls (a, e, i), 7 μg of protein was loaded, and for the digests, 25 μg. Electrophoresis was done with an 8% acrylamide stacking gel and a 12½% acrylamide separating gel.

Fig. 4. Digestion of desmins with cyanogen bromide. Porcine skeletal, a-b; porcine smooth, c-d; avian smooth, e-f. For the control (a, c, e), 7 μg of protein was loaded, and for the digests, 20 μg. Electrophoresis was done with an 8% acrylamide stacking gel and a 12½% acrylamide separating gel. The labeled standards in track g were α-actinin, skeletal desmin, actin, carbonic anhydrase, chymotrypsinogen, and lysozyme.

dalton range was quite similar, but the rate of digestion was slightly different in that the mammalian smooth muscle desmin more quickly generated a larger amount of peptides in the 26,000 and 34,000 molecular weight regions. Although there were some differences in the patterns from the mammalian and avian desmins, they were not striking except for a peptide of about 14,000 daltons that accumulated at about the same rate in the two mammalian samples, but to a lesser extent in the avian desmin. Again, a 30,000-dalton peptide was a major product of each of the three samples.

Table 1. Amino acid analysis of desmin

Amino acid	Porcine skeletal	Porcine stomach	Chicken gizzard
Asp	9.7±0.2	9.7±0.1	8.6±0.2
Thr	5.3±0.1	5.1±0.1	5.6±0.1
Ser	7.1±0.3	7.0±0.0	6.0±0.1
Glu	17.6±0.3	18.4±0.1	19.9±0.4
Pro	3.6±0.2	2.5±0.1	1.8±0.1
Gly	6.4±0.5	5.5±0.2	4.0±0.1
Ala	9.2±0.1	9.6±0.0	9.3±0.2
Cys	0.3±0.0	0.3±0.0	0.3±0.0
Val	6.0±0.1	6.0±0.1	5.2±0.3
Met	1.8±0.1	1.8±0.1	2.6±0.1
Ile	4.1±0.1	4.1±0.0	4.4±0.1
Leu	9.4±0.2	10.1±0.1	10.8±0.4
Tyr	2.8±0.1	2.8±0.1	2.9±0.2
Phe	3.0±0.1	2.9±0.1	2.8±0.1
Lys	5.1±0.2	4.5±0.1	4.9±0.2
His	1.4±0.0	1.5±0.1	1.6±0.1
Arg	7.7±0.5	8.7±0.2	9.3±0.2

*Results are expressed in mole percent as means plus or minus standard error of analyses on three different preparations run in quadruplicate.

Treatment of the desmins with cyanogen bromide and subsequent electrophoresis resulted in the peptide map shown in Fig. 4. The patterns for the two mammalian samples are very similar, but markedly different from that of the avian desmin. In the mammalian samples, there was a major peptide product at about 30,000 daltons that was nearly absent in the avian desmin. Amino acid analysis of the digested material revealed the equivalent of 2 residues of residual, undigested methionine in the mammalian samples compared with only half a residue in the avian desmin. In the absence of any measured modification of methionine by amino acid analyses, it is suggested that the inability of the cyanogen bromide to cleave at all methionyl residues may be due to the presence in the mammalian desmins of specific sequences that are known to be more difficult to cleave by cyanogen bromide (26-30).

The amino acid analyses of the three desmin samples are shown in Table 1. The results indicate that all three desmins have very similar amino acid compositions. These analyses agree closely with those published by other workers for 10-nm filament proteins

of chicken gizzard (11), hog stomach (11), human astrocyte (2), bovine epidermis (5), and baby hamster kidney cells (6),suggesting that all these proteins share some properties in common.

DISCUSSION

The peptide maps and amino acid analyses show that the 55,000 dalton protein from mature mammalian skeletal muscle is very similar to the analogous control protein prepared in this study from mammalian smooth muscle. Our mammalian smooth protein is, in turn, essentially identical to that described (and called skeletin) from the same source by Small and Sobieszek (11). Considering 1) that skeletal muscles used contained a relatively small amount of vascular smooth muscle tissue, 2) that any small amount of contaminating smooth muscle myofibrillar proteins (which would contain desmin) present in this original, homogenized skeletal muscle sample would have been preferentially discarded during very low-speed centrifugation steps used in washing the myofibrils before extraction of skeletal muscle desmin, and 3) that Lazarides and associates (12, 31) have shown by immunofluorescence that vertebrate skeletal muscle cells do contain desmin, it is likely that the desmin isolated in this study from skeletal muscle samples originated from the skeletal muscle cells and not from contaminating vascular smooth muscle cells.

The results of the peptide digests suggest that the desmins contain a core of material having a subunit molecular weight of about 30,000 to 34,000 that is fairly resistant to proteolysis. Among other proteins that contain regions of structure resistant to proteolysis are myosin (32), tropomyosin (33, 34), paramyosin (35), and bovine epidermal keratin (36). The resistant regions in these proteins are in the form of coiled-coil, α-helical structure. It recently has been shown by X-ray diffraction that 10-nm filaments of baby hamster kidney (BHK-21) cells and epidermal keratin filaments have similar structures (9). The desmins, when digested with trypsin, generated essentially the same subunit molecular weight fragments at the same rate as reported for trypsin digestion of bovine epidermal

keratin filaments (36). Thus, it is likely that the muscle desmins share at least some structural properties with the proteins making up 10-nm filaments in BHK-21 and epidermal cells. Although there may be five or more overlapping groups of 10-nm filaments (4, 7-10), which represent different gene products, all may share common properties such as having similar regions of proteolytically resistant α-helical cores. Although desmins do contain cores or regions that are fairly resistant to proteolysis, the intact molecules were very susceptible to initial cleavage steps. The susceptibility to partial proteolytic breakdown was a feature of all three desmins and has been noticed previously (11). It has been suggested that one of the proteases, the Ca^{2+}-activated muscle protease, used in this study may play a role in turnover of myofibrillar proteins and structure in vivo (19). The muscle desmins and 10-nm filaments likewise are certainly susceptible to this endogenous muscle protease.

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