COPURIFICATION OF ACTIN AND DESMIN FROM CHICKEN SMOOTH MUSCLE AND THEIR COPOLYMERIZATION IN VITRO TO INTERMEDIATE FILAMENTS

BRUCE D. HUBBARD and ELIAS LAZARIDES

From the Division of Biology, California Institute of Technology, Pasadena, California 91125

ABSTRACT

Desmin is a 50,000-mol wt protein that is enriched along with 100-Å filaments in chicken gizzard that has been extracted with 1 M KI. Although 1 M KI removes most of the actin from gizzard, a small fraction of this protein remains persistently insoluble, along with desmin. The solubility properties of this actin are the same as for desmin: they are both insoluble in high salt concentrations, but are solubilized at low pH or by agents that dissociate hydrophobic bonds. Desmin may be purified by repeated cycles of solubilization by 1 M acetic acid and subsequent precipitation by neutralization to pH 4. During this process, a constant nonstoichiometric ratio of actin to desmin is attained.

Gel filtration on Ultrogel AcA34 in the presence of 0.5% Sarkosyl NL-97 reveals nonmonomeric fractions of actin and desmin that comigrate through the column. Gel filtration on Bio-Gel P300 in the presence of 1 M acetic acid reveals that the majority of desmin is monomeric under these conditions. A small fraction of desmin and all of the actin elute with the excluded volume. When the acetic acid is removed from actin-desmin solutions by dialysis, a gel forms that is composed of filaments with diameters of 120–140 Å. These filaments react uniformly with both anti-actin and anti-desmin antiserum. These results suggest that desmin is the major subunit of the muscle 100-Å filaments and that it may form nonstoichiometric complexes with actin.

KEY WORDS 100-Å filaments · immunofluorescence · α-actinin · Z disk

A group of filaments with diameters of ~100 Å has been described in several widely differing cell types. They are known as "neurofilaments" in neurons (31, 43), "glial filaments" in glial cells and astrocytes (43), "10-nm filaments" in cultured nonmuscle cells (3, 11, 13), and "intermediate filaments" in muscle (1, 5, 6, 39).

The putative subunits of 100-Å filaments have been isolated from nerve cells (9, 30), glial cells (2, 7, 28), cultured cells (13, 34), and smooth muscle cells (5, 22, 33). Immunological and biochemical comparisons have indicated that the subunits of neurofilaments, glial filaments, and intermediate filaments are chemically distinguishable (2, 7, 8, 21), and that they are apparently unrelated to the keratin which makes up epidermal tonofilaments (4, 35). 100-Å filaments thus form a heterogeneous group, although the extent of this heterogeneity has not yet been determined.

Biochemical characterization of the subunit of intermediate filaments has recently become possible with the demonstration that the extraction of smooth-muscle actomyosin at high ionic strength leaves an insoluble residue which is enriched in 100-Å filaments (6). There are two predominant

J. CELL BIOLOGY © The Rockefeller University Press · 0021-9525/79/01/0166-17$1.00
Volume 80 January 1979 166-182

proteins in this residue: actin and a 50–55,000-mol wt protein (22). This latter protein has been characterized as the major subunit of muscle intermediate filaments by several researchers (5, 22, 33). We have isolated it from chicken gizzard as a 50,000-mol wt protein which we call desmin (22).

In smooth muscle cells, one of the most characteristic morphological features of the intermediate-sized filaments is their insertion into cytoplasmic and membrane-bound dense bodies and their intimate associations with actin filaments at these sites (1, 5, 39). In skeletal muscle, immunofluorescence reveals that desmin is localized at the Z lines and where the Z lines come into apposition with the plasma membrane. Desmin is also found at the Z lines and intercalated disks of cardiac muscle (22). These are all sites where actin structures are linked either together or to membranes. From these distributions, we concluded that desmin forms a network in muscle cells which interlinks individual myofibrils, at their Z disks, into a single integrated mechanical unit and also functions in the linkage of this unit to the plasma membrane.

In this paper, we present evidence that actin and desmin copurify from extracts of chicken gizzard and copolymerize into 100-Å-like filaments. These results suggest that desmin and actin may form a stable complex and provide an insight to the molecular basis of how desmin may function to link actin filaments in muscle cells.

MATERIALS AND METHODS

Materials

From Eastman Organics (Rochester, N.Y.): acrylamide (No. 5221) for slab gels, acrylamide (No. X5521) for isoelectric focusing gels, and N,N'-methylene bisacrylamide (No. 8383) were used as supplied without further purification. Sarkosyl NL-97 (Sarkosyl) is a trademark of CIBA-GEIGY (Greensboro, N.C.); Nonidet NP-40 is a trademark of Shell Chemical Co. (New York). Sodium dodecyl sulfate (SDS) was obtained from Serva (Heidelberg, West Germany); Ultrogel and Ampholines were products of LKB Produkter (Bromma, Sweden); urea was the ultrapure grade from Schwarz/Mann (Orangeburg, N.Y.); Bio-Gel was a product of Bio-Rad (Richmond, Calif.). Fluoresceine-labeled IgG fraction of goat anti-rabbit IgG was obtained from Miles Laboratories (G5-173-1; Miles Labs Inc., Elkhart, Ind.). Chicken gizzard was obtained from Pel-Freeze (Rogers, Ark.). Plus-X film (Kodak) was developed in Diafine (Accufine Corp., Chicago, Ill.). Elvanol is a water-soluble polyvinyl alcohol (Du Pont 51-05, E. I. Du Pont de Nemours, Wilmington, Del.). All other chemicals were of reagent quality. Dialysis tubing was number 8 from Union Carbide (Chicago, Ill.). Ethylene glycol-bis-(β-aminoethyl ether)*N,N'*-tetra acetic acid is EGTA.

Analytical Methods

Protein concentration was determined relative to a bovine serum albumin (BSA) standard by an elevated temperature modification of the Lowry method (29). All buffer pH values were determined at 20°C. Electrophoretic recipes are all wt/vol.

Dialysis

Dialysis tubing was prepared by simmering it in 0.1 M NaOH and 10 mM EDTA for 8 h. The tubing was then neutralized with Tris-HCl, pH 6.8, extensively rinsed with water, and stored in water at 5°C.

One-Dimensional SDS Slab Gel Electrophoresis (SDS-PAGE)

One-dimensional electrophoretic analysis of proteins was performed on high-resolution SDS-polyacrylamide slab gels (SDS-PAGE) by a modification of the discontinuous Tris-glycine buffer system (18). The stacking gel contained: 5% acrylamide, 0.13% N,N'-methylene bisacrylamide, 0.125 M Tris-HCl, pH 6.8, and 0.1% SDS. The quantities of acrylamide and of bisacrylamide in the analytical (lower) gel were provided by a hyperbolic relationship: % acrylamide × % bisacrylamide = 1.3. Gels containing 12.5% acrylamide were used most often because of their high resolution in the molecular-weight range of actin and desmin. A 12.5% analytical gel contained: 12.5% acrylamide, 0.107% bisacrylamide, 0.386 M Tris-HCl, pH 8.7, and 0.1% SDS. Polymerization was catalyzed by the addition of 100 μl of 10% ammonium persulfate and 10 μl of N,N,N',N'-tetramethylethylenediamine/30 ml of gel solution. The same running buffer was used in both the upper and lower reservoirs: 0.025 M Tris base, 0.112 M glycine, and 0.1% SDS, final pH 8.5. Sample buffer (2 ×) contained 0.1 M dithiothreitol, 0.08 M Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, and bromophenol blue. After electrophoresis, gels were stained overnight in 50% ethanol, 10% acetic acid, and 0.05% Coomassie Brilliant Blue R-250. Gels were destained by three changes of 10% ethanol, 5% acetic acid, and photographed over a light box with Polaroid PN-55 film (Polaroid Corp., Cambridge, Mass.), using an orange-colored filter to enhance contrast. In the figures, most of the well-stained, compact bands represent 2–4 μg of protein, while faint bands may represent <0.1 μg. In our experience, SDS-electrophoresis systems other than discontinuous Tris buffered slab PAGE do not adequately resolve or visualize significant minor components in desmin preparations.

The effluents of gel-filtration columns were analyzed

by SDS-PAGE. For fractions containing Sarkosyl NL-97, aliquots were mixed directly with 0.2 vol of 5 × SDS sample buffer and heated briefly before loading on gels. For fractions containing acetic acid, aliquots were dialyzed first against distilled water, lyophilized if necessary, and then mixed with SDS sample buffer.

Two-Dimensional Isoelectric-Focusing SDS-Gel-Electrophoresis (IEF/SDS-PAGE)

Two-dimensional electrophoresis was carried out according to the system of O'Farrell (24). The first dimension (isoelectric focusing) was prepared and pre-run as described (15), but with the following modifications: the gels (2.5 × 120 mm) contained 0.2% Ampholines, pH range 3.5–10; 0.8% Ampholines, pH range 4–5; and 2% Ampholines, pH range 5–7 (each supplied as 40% solution). Ampholines were not added to overlay solutions nor to lysis buffers. Samples (see below) were loaded, overlayed directly with 0.02 M NaOH, and run at 450 V for 16 h and then at 800 V for 1 h. Samples of native proteins were dissolved in 8 M urea at room temperature for 1 h. They were then made 1% in Nonidet NP-40 and 0.5% in 2-mercaptoethanol. The samples were subsequently isoelectric focused as described above, but without being heated.

Microscopy

Unless otherwise specified in the text, all desmin gels for microscopy were induced from cycle-2 acetic acid extracts (see below). Samples for electron microscopy were placed on carbon-coated 400-mesh copper grids. Because many of the samples were bulky, insoluble gels, it was found helpful to place a drop of water on the grid and then wipe the gel across it. These preparations were stained for 2 min with a 2% aqueous solution of uranyl acetate, excess stain was drawn off with a filter paper, and the grids were then air dried. The specimens were observed in a Philips EM 201 electron microscope operated at 80 kV, and photographed with a 35-mm camera. Final magnifications were determined from the calculated values for the 35-mm camera.

Samples of desmin gels for fluorescence microscopy were placed on glass coverslips and spread by gentle flattening against a slide. This causes some of the gel to adhere to the coverslip in a thin layer. The coverslips were then dehydrated for 10 min in 95% ethanol, rehydrated in calcium- and magnesium-free phosphate-buffered saline at pH 7.4 (PBS), and stained for indirect immunofluorescence as described (19). Antibodies against actin (19) and desmin (22) were the same as those described. They were prepared against proteins purified to apparent homogeneity from smooth muscle (chicken gizzard). The coverslips were mounted on slides with a drop of Elvanol as mounting medium. Photomicroscopy was performed on a Leitz microscope equipped with a fluorescence epi-illumination system and Leitz FITC filter module H. Samples were photographed through a × 100 oil immersion phase objective. Plus-X film was exposed at Din 28 and developed in Diafine. Magnification was determined by photographing the lines ruled on a hemacytometer.

Enrichment of Gizzard Preparations for Desmin (see Scheme I)

Desmin was extracted from chicken smooth muscle by modifications of previous methods (6, 22, 33). All procedures were performed at 5°C. The details are presented in Scheme I. During the extractions, gelatinous masses formed and were discarded. They were not analyzed. The final KI-insoluble residue (KI-residue) was washed with water to reduce the KI concentration to below 1 mM and stored as a thick slurry in the presence of 10 mM NaN_3. KI-residue stored as an actual pellet tended to solidify with time. Freezing (at −20°C) hastened this process.

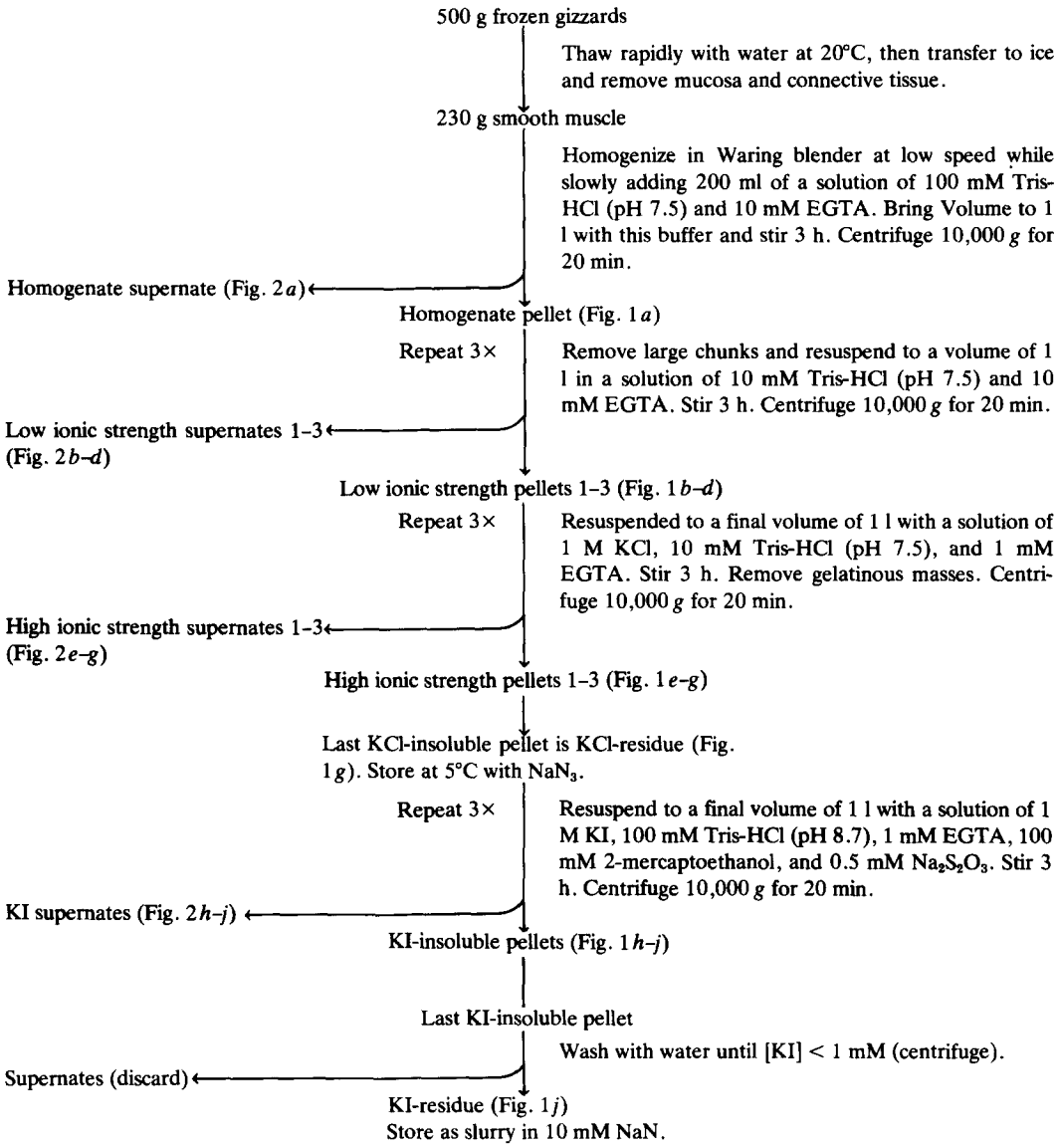
Water-washed KCl- or KI-residue pellets were made into acetone powders (KCl-AP, KI-AP) by suspending them in an equal volume of water to prevent clumping. This suspension was then mixed with 3–5 vol of cold acetone, stirred for 1 h, and spun out. This was repeated with more acetone until the final suspension contained <5% of the original water. The final acetone-insoluble residue was air dried overnight and stored at −20°C.

Purification of Desmin from KCl- and KI-residues (see Scheme II)

Desmin was extracted from KCl- or KI-residues with acetic acid at low temperatures, because this resulted in the least number of artifactual charge modifications observable by IEF/SDS-PAGE (see Results). The details of this procedure and the notations used for the various desmin extracts are given in Scheme II. Cycle 2 desmin was used in most experiments.

Desmin was extracted from acetone powders as described in Scheme II but with the following modification: the acetone powder was first extracted at room temperature with water and then washed on a Büchner funnel to remove soluble actin and tropomyosin. The washed material was then extracted with acetic acid as described above.

Purified desmin was occasionally stored by dissolving it in 1 mM HCl and keeping this solution frozen at −80°C. Desmin was stable for up to 2 wk under these conditions. If desmin was stored as a precipitate at pH 4 in acetic acid/acetate or at pH 7.5 in Tris-HCl, it tended to become acid insoluble with time. Freezing either KI-residues or desmin precipitates for several weeks also caused desmin to become acid insoluble. In the cyclic purification procedure of Scheme II, desmin was found to be soluble in acid only at low ionic strength. Desmin was not solubilized away from contaminating proteins if



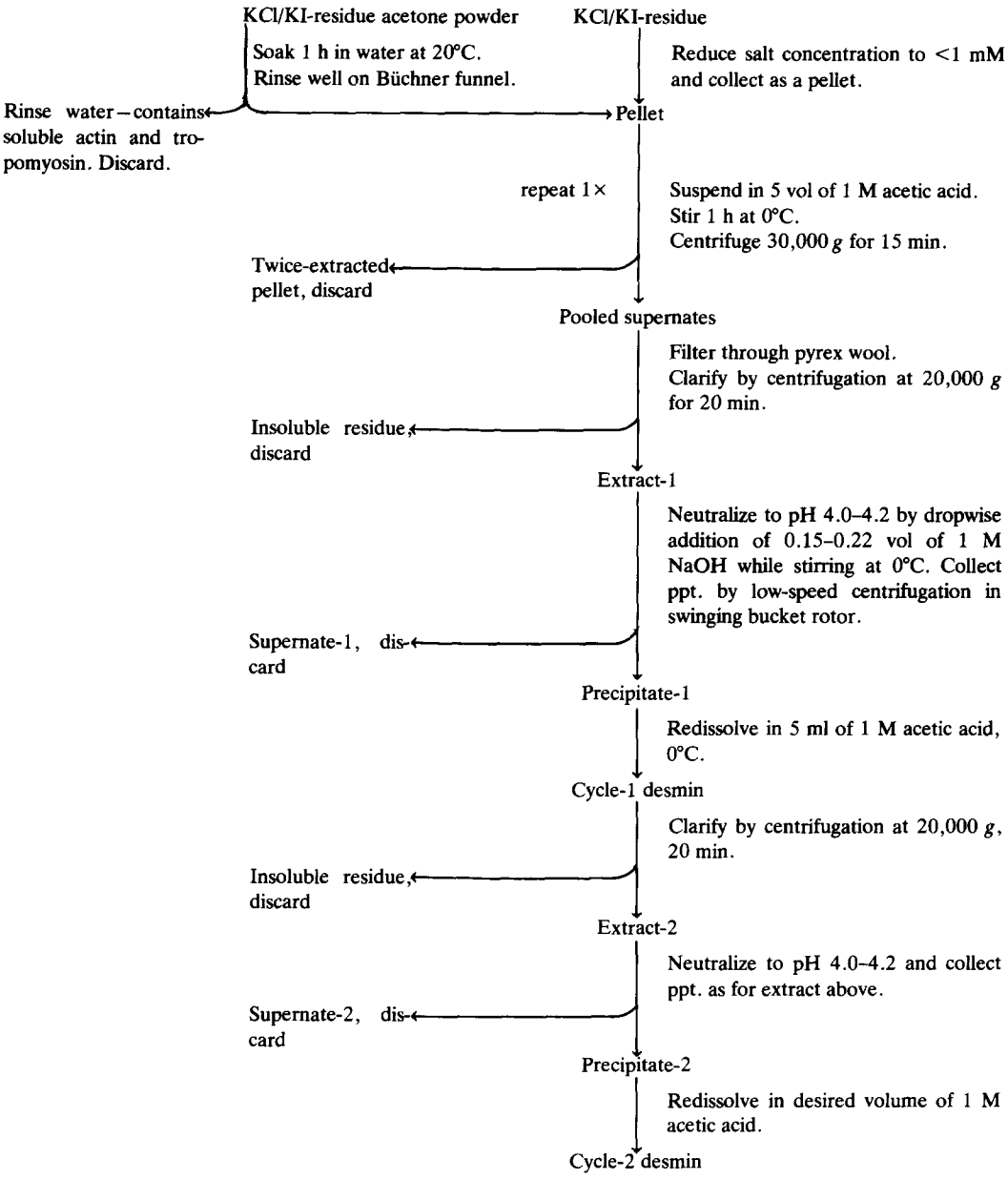
SCHEME I
Preparation of KCl- and KI-Insoluble Residues of Gizzard

too much salt remained in the precipitate. Desmin precipitates are very sticky, especially towards glass, and the manipulation of them was minimized to reduce losses.

RESULTS

Copurification of Actin and Desmin

PREPARATION OF EXTRACTED CHICKEN-GIZZARD RESIDUES THAT ARE ENRICHED IN DESMIN (SCHEME I): Figs. 1 and 2 show the proteins that are extracted (Fig. 2) and that remain insoluble (Fig. 1) at each step of the extraction procedure described in Scheme I. The presence of 100 mM Tris-HCl in the homogenization buffer releases some actomyosin (Fig. 2*a*). During the next three low ionic strength (LI) extractions (20 mM Tris-HCl), actin and tropomyosin, but not myosin, are released (Fig. 2*b–d*). As the LI extractions proceed, α-actinin, desmin,

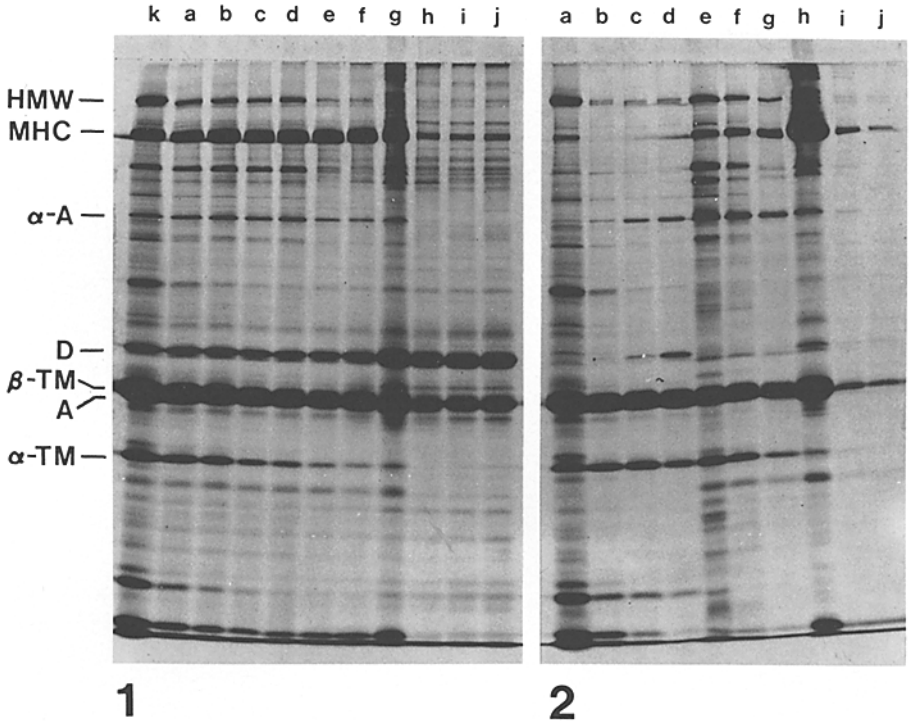


This cyclic procedure may be continued for an arbitrary number of steps.

SCHEME II

Cyclic Purification of Desmin Extracted with Acetic Acid

and a high molecular weight protein (HMW) begin to appear in the supernate, especially in LI supernate 3 (Fig. 2*d*). Two-dimensional gel electrophoresis of LI supernate 3 (Fig. 3) reveals the presence of the α and β components of desmin (15), the presence of β- and γ-actin (10, 15, 27, 36, 42), a pair of spots designated * (shown most clearly in Fig. 6), and three isoelectric variants of α-actinin. The HMW does not appear on our two-dimensional gels. We have adopted the conversion of labeling the actin isoelectric variants of smooth muscle as β and γ, with γ denoting the most basic



FIGURES 1 AND 2 The extraction of chicken gizzard proteins at low and high ionic strength, showing, respectively, the insoluble (Fig. 1) and soluble (Fig. 2) proteins at each step of the extraction. See Materials and Methods for details, and Results for discussion. The protein loads in the various wells have been roughly equalized to aid viewing and do not represent the concentrations actually present at that step. (1*k*) shows the initial homogenate in 100 mM Tris-HCl, pH 7.5. (1*a*) Pellet and (2*a*) supernate from the initial homogenate. Three successive extractions with 10 mM Tris-HCl, pH 7.5, produced insoluble pellets (1*b–d*) and soluble supernates (2*b–d*). Three subsequent successive extractions with 1 M KCl produced insoluble pellets (1*e–g*) and soluble supernates (2*e–g*). Finally, three subsequent successive extractions with 1 M KI produced insoluble pellets (1*h–j*) and soluble supernates (2*h–j*). The final KI-insoluble residue that is enriched in 100-Å filaments is (1*j*). Actin and β-tropomyosin form a doublet on less heavily loaded gels. Actin (*A*), α-actinin (*α-A*), desmin (*D*), high molecular weight protein (*HMW*), myosin heavy chain (*MHC*), tropomyosin (*TM*).

variant (10, 15).

High ionic strength (HI) extraction of the LI-insoluble residue with 1 M KCl (Fig. 2*e–g*) and then with 1 M KI (Fig. 2*h–j*), releases most of the actomyosin, α-actinin, HMW, and tropomyosin. The remaining KI-insoluble residue (Fig. 1*j*) contains mostly actin and desmin. We have not quantitated yields or fold purification during this purification because of the lack of a suitable quantitative assay for desmin. Comparison of Fig. 1*a* and *k* and examination of the solubilized proteins (Fig. 2), however, indicates that the purification is substantial. The total amount of recoverable desmin in gizzard is quite small, however, and 200 g of gizzard muscle will typically yield 100–200 mg of moderately pure desmin (such as the cycle-2 desmin shown in Figs. 5 and 6).

The actin and desmin that remain in the KI-insoluble residue are still associated with a considerable bulk of SDS-insoluble matrix, the composition of which is unidentified. Two-dimensional electrophoresis of the KI-residue reveals the presence of α- and β-desmin, of $*_1$ and $*_2$ (brackets), and of γ-actin (Fig. 4). A band is seen next to desmin on one-dimensional gels of some KI-residue preparations. It has never been unambiguously observed on the corresponding two-dimensional gels, however, and is most likely either the α- or β-component of desmin. We have not observed any marked tendency for desmin to be proteolyzed during our extraction procedures.

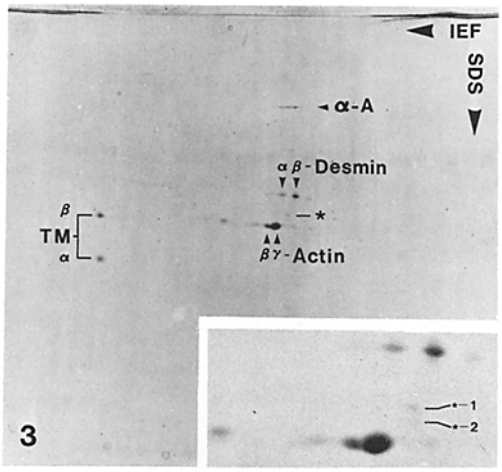


FIGURE 3 Two-dimensional gel of the low ionic strength supernate-3 (Fig. 2*d*). Indicated are α-actinin, α- and β-desmin, proteins $*_1$ and $*_2$, β- and γ-actin, and gizzard tropomyosin (α, β *TM*). α-Actinin focuses into three isoelectric variants. The acid side is on the left. The samples were loaded at the upper right (base side) for the first (isoelectric, *IEF*) dimension. The second (*SDS*-PAGE) dimension is top to bottom. The very high molecular weight proteins generally do not resolve on these gels.

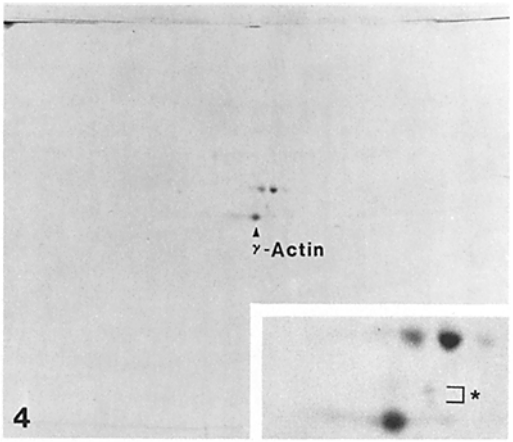


FIGURE 4 Two-dimensional gel of the KI-insoluble residue (Fig. 1*j*). α,β-desmin, proteins $*_1$ and $*_2$, and γ-actin are present.

The desmin from KI-extracted muscle has the same molecular weight and isoelectric variant composition as desmin from fresh muscle (15). Desmin, however, is slowly degraded at low pH.

SOLUBILIZATION OF DESMIN: Crude desmin may be solubilized from KCl- or KI-extracted gizzard residues by 1 M acetic acid (reference 33 and this paper), concentrated ethylene diamine, 0.5% Sarkosyl NL-97, 3 M sodium trichloroacetate, or 3 M urea. Of these, 1 M acetic acid at 0°C was chosen for routine extraction of desmin because it is easy to work with and to remove, is reasonably selective, and does not appear to denature or rapidly damage desmin. Although acetic acid at elevated temperatures is reported to give better yields of desmin (33), its use was avoided because it produced extensive charge heterogeneity in both actin and desmin, and also solubilized considerable quantities of collagen and myosin. The major difference between the KI-residue and the KI-residue acetone powder is that the latter yields much purer desmin.

CYCLIC PURIFICATION OF DESMIN SOLUBILIZED BY ACETIC ACID (SCHEME II): When the first acetic acid extract (0°C) is neutralized, a fine precipitate containing desmin forms at about pH 4.0. As the pH is increased to 4.2, the precipitate coalesces into small flocculent masses. These are extremely sticky and easily trap air bubbles. A desmin-containing precipitate also forms if a 1-M acetic acid extract is brought to 0.3–0.4 M in NaCl.

The first acid extract of a KI-residue (Fig. 5*a*) was purified by four cycles of precipitation and solubilization as described in Materials and Methods (see Scheme II). The supernate from each precipitate was dialyzed against water and lyophilized. Precipitates 1–4 are shown by one-dimensional SDS-PAGE in Fig. 5*b–e*, and concentrated supernates 1–4 in Fig. 5*f–i*. During this process of cyclic precipitation, a constant protein composition is attained, with >90% of the actin and desmin precipitating in each cycle. The small amount of actin and desmin which remains in the final supernate-4 are in the same relative proportion as the actin and desmin in precipitate-4. Desmin (mol wt 50,000) isolated in this manner is associated with four other proteins: myosin (mol wt 210,000), two intermediate-sized proteins (mol wt $*_1$: 45–47,000; mol wt $*_2$: 43–45,000), and actin (mol wt 42,000) (Fig. 6). Other proteins sometimes appear in the region between actin and desmin, but $*_1$ and $*_2$ predominate.

Precipitate-4 (Fig. 5*e*) was analyzed by two-dimensional electrophoresis. It contained both α- and β-desmin, $*_1$ and $*_2$, and actin. The actin has an isoelectric-focusing mobility corresponding to the γ-variant of this molecule (15). It can be

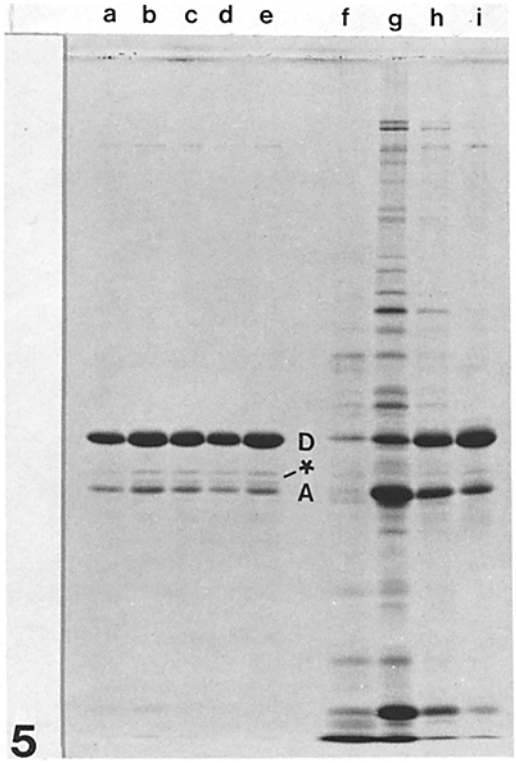


FIGURE 5 Proteins extracted from a KI-residue by 1 M acetic acid. The initial extract (extract-1) made with 1 M acetic acid at 0°C for 1 h. Extract-1 was cyclically precipitated with NaOH (at pH 4) and redissolved in acetic acid (see Scheme II) to yield precipitate-1 (5*b*), supernate-1 (5*f*), precipitate-2 (5*c*), supernate-2 (5*g*), precipitate-3 (5*d*), supernate-3 (5*h*), precipitate-4 (5*e*), and supernate-4 (5*i*). By cycle 4, a constant ratio of actin to desmin is attained both in the precipitate (5*e*) and in the supernate (5*i*).

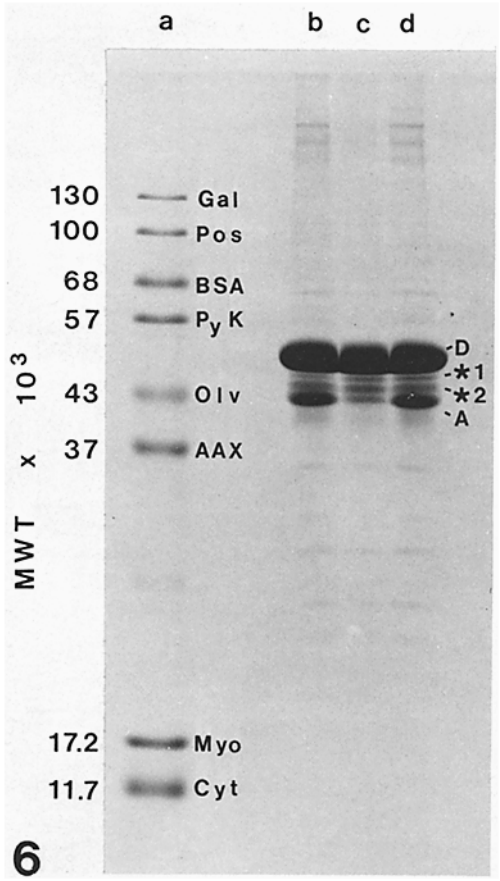


FIGURE 6 Cycle-2 desmin extracted by acetic acid from a KCl-residue acetone powder (KCl-AP; 6*b*), and from a KI-residue acetone power (KI-AP; 6*c*). Molecular weight (*MWT*) standards (6*a*) are β-galactosidase (*Gal*; 130,000), phosphorylase *a* (*Pos*; 100,000), bovine serum albumin (*BSA*; 68,000), pyruvate kinase (*PyK*; 57,000), ovalbumin (*Olv*; 43,000), D-amino acid oxidase (*AAX*; 37,000), myoglobin (*Myo;* 17,200), and cytochrome *c* (*Cyt*; 11,700). The SDS-PAGE is a 20% acrylamide gel. Calculated mol wt are: 50,000 for desmin; 47,000 for $*_1$; and 45,000 for $*_2$. Actin is given to have mol wt 42,000. Note that more actin is extractable from a KCl-acetone powder. When a KI-AP is washed with 1 M KCl before the preparation of a cycle-2 extract, the high-salt treatment reverses the effect of acetone powdering (6*d*).

further shown to be actin by reaction with anti-actin antibody (see below). The amount of myosin that is observed with desmin is variable and is virtually absent from desmin extracted from KI-residue acetone powders. Every nondenaturing purification scheme that we have investigated so far has failed to selectively solubilize desmin away from either the * proteins or from actin. It is of interest that both desmin and the proteins that copurify with it have P_I's of ~5.71 in 9 M urea/1% Nonidet NP-40.

Although there is a certain amount of variability in the relative proportions of α- and β-desmin in purified desmin, we have never observed the purification of one desmin variant without the other. At least some of this variability appears to be artifactual. α-Desmin is particularly susceptible to conversion to a more acidic variant. Urea, heating, and the use of pH 4–6 Ampholines will all promote this conversion. In the presence of the pH 4–6 Ampholines, the α-desmin spot splits into

two, with one remaining in the old position and one running ~0.05 P_I units more acidic (data not shown).

GEL CHROMATOGRAPHY OF ACTIN AND DESMIN: The consistent copurification of actin and desmin suggested that they might be associated together as a complex. Because purified desmin is insoluble under conditions of low or high ionic strength, we studied desmin that had been solubilized with the medium-strength anionic detergent Sarkosyl NL-97. This detergent does not appear to denature desmin, because desmin preparations that have been solubilized with Sarkosyl will form 100-Å filaments when the Sarkosyl is dialyzed away (see below). When Sarkosyl-solubilized desmin is chromatographed by gel filtration on a column of Ultrogel AcA 34 (range 20,000–340,000 mol wt), it is fractionated into two populations: one containing actin, desmin, and HMW that is excluded from the column; and one containing actin, desmin, $*_1$, and $*_2$ that is barely included in the column (Fig. 7). Two-dimensional electrophoresis (not shown) revealed the presence of α- and β-desmin and of γ-actin in both protein populations (fractions 17 and 37).

When desmin is chromatographed on Bio-Gel P300 in the presence of 1 M acetic acid and 0.05 M NaCl (Fig. 8), the vast majority of desmin elutes in what is probably a monomeric position and is not associated with actin under these conditions. A small fraction of the desmin elutes with the excluded volume. The actin present does not appear to be monomeric, and most of it also elutes with the excluded volume.

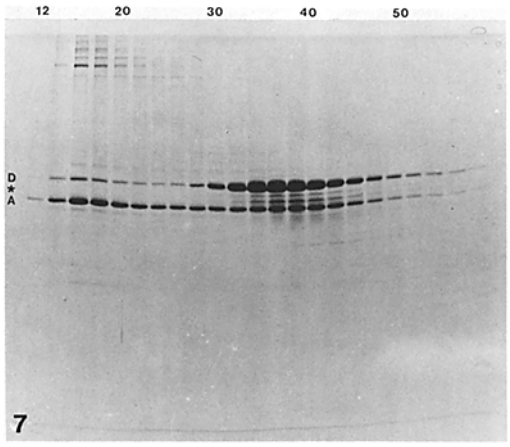
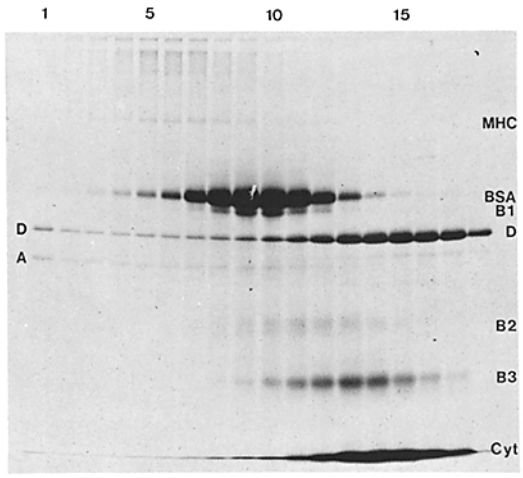


FIGURE 7 SDS-PAGE analysis of the fractions produced by the gel filtration of desmin and associated proteins on Ultrogel AcA 34 in the presence of 0.5% Sarkosyl NL-97. A KI-AP was washed with 1 M KCl and then with water. This resolubilizes some of the high molecular weight proteins. The washed pellet was extracted with 1 M acetic acid and this was cycled as described in Materials and Methods to produce a precipitate-2 (cycle-2 desmin). This was rinsed with 0.1 M Tris-HCl, pH 7.5, and then solubilized with 0.5% Sarkosyl NL-97, 100 mM NaCl, 10 mM Tris-HCl, pH 7.5, 10 mM 2-mercaptoethanol, and 10 mM NaN_3. Ultrogel AcA 34 (range 20,000–350,000 mol wt) was equilibrated in this buffer and poured as a column of 2 × 40 cm. Blue dextran-2,000 eluted with a peak at fraction 17, and an included dye marker eluted with a peak at ~fraction 120. In the actual run, all of the protein eluted between fractions 12 and 55. Fractions were heated with 0.2 vol of 5 × SDS-sample buffer before analysis on SDS-PAGE. We have not determined whether the micell structure of Sarkosyl NL-97 had any effect on the elution profile.

Copolymerization of Actin and Desmin

FORMATION OF DESMIN GELS: When purified desmin is recovered from 1 M acetic acid (pH 2.4) by neutralization to pH 4.1, it generally precipitates as cohesive, cottonlike flakes. However, if the acetic acid is instead removed by dialysis against several changes of distilled water, three different and alternate phenomena are observed: (*a*) the spontaneous formation of a clear gel; (*b*) a clear solution; or (*c*) a cohesive precipitate. All three represent different states of desmin and of the proteins that copurify with it, as no differential participation of any of them is observed. A spontaneous gel is the most frequently observed state. The gels are extremely sticky, especially to themselves and to glass, but they will also coat the insides of plastic pipette tips. Desmin gels are strong enough to hold their shape when extruded from the dialysis bag. This includes any notches and wrinkles that resulted from their association with the nonuniform contours of the dialysis membrane. If they are left undisturbed, the clear gels contract slowly and become translucent over a period of several days. This spontaneous contraction can be speeded up, so that it is complete within 1 h, if the ionic strength or divalent cation concentration of the dialysis medium is raised. Fig. 9*a* shows an uncontracted gel in the dialysis bag in distilled water. Fig. 9*b* shows the same gel 1 h after the addition of 1 mM $MgCl_2$ to the dialysis medium.

The dialysis of cycle-purified desmin from acetic acid into water occasionally results in a metastable, nongelled solution (state 2). If left undis-



8

FIGURE 8 SDS-PAGE analysis of the fractions produced by gel filtration of desmin and its associated proteins on Bio-Gel P300 in the presence of 1 M acetic acid and 0.05 M NaCl. Blue dextran-2,000 eluted in fractions 1 and 2. BSA and Cyt were mixed with the desmin before it was loaded on the column. The majority of desmin elutes as a monomer while a small fraction of desmin elutes with the excluded volume. All of the actin chromatographs in a manner similar to the partially excluded desmin. The elution profile for desmin is retarded relative to that expected for a 50,000 mol wt protein; one possible reason is that desmin may interact with Bio-Gel P300 under the above conditions. If this is so, caution should be exercised in assigning a monomeric molecular weight to this desmin fraction. The proteins *B1*, *B2*, and *B3* are most likely BSA degradation products and they are not present when desmin alone is run on the column. Other abbreviations are as in Figs. 1, 2, and 6.

turbed in the dialysis membrane, these solutions will remain in this state for days at 5°C. However, if even a small amount of an ionic substance (Table I) is dialyzed into the metastable solution, gelation is initiated and is complete within 1 h (state 1 above). Glass will also trigger gelation (Fig. 10). Syneresis of the gel occurs subsequently. While we have not measured the relative effectiveness of various ions in inducing gelation, we have not noticed any obvious requirements for any particular ions. All of the above gelation and syneresis phenomena are apparently passive in the sense that an external source of energy is not required.

Finally, the gelation phenomenon is not reversed by the removal of any of the substances listed in Table I (by extensive dialysis against water). The gels may be resolubilized in 1 M acetic acid, however, and the gelation procedure repeated.

Sarkosyl NL-97 solutions of desmin will also form spontaneous gels when the Sarkosyl is removed by extensive dialysis against water. These gels appear to be similar to the acetic acid gels, but were not investigated extensively. They are composed of fibrils with ~100-Å diameters (data not shown).

LIGHT MICROSCOPY OF DESMIN GELS: Phase microscopy of semicontracted desmin gels reveals a tangled network of branching fibers that are often embedded in an amorphous matrix (Fig. 11). These fibers are <1 μm wide and appear to be bundles of many smaller fibrils. No differences in morphology are seen when spontaneous gels or ion-initiated gels (10 mM KCl or 10 mM $MgCl_2$) are compared. The matrix and fibril bundles are intimately associated with each other (Fig. 11). We were unable to selectively solubilize either matrix or fibers at any of several concentrations of urea between 0.1 and 1.0 M. Desmin precipitated from acetic acid by rapid neutralization to pH 4.1 is usually amorphous, but occasionally exhibits fibril bundles similar to those observed from desmin gels produced by dialysis (data not shown).

In indirect immunofluorescence, both the fibrous and matrix components of the desmin gels are uniformly reactive with anti-desmin (Fig. 12) and anti-actin (Fig. 13) at the level of resolution of the light microscope. No periodicities or differential reactivity has been observed. Control preimmune antisera were unreactive.

ELECTRON MICROSCOPY OF DESMIN GELS: The insoluble gizzard residue which remains after extraction with 1.0 M KCl was investigated by negative staining to determine what fiber morphologies it contained before the extraction of desmin with acetic acid. High magnification pictures show tangled groups of well-preserved 100-Å filaments (Fig. 14*a–c*). The 100-Å-fiber configurations of Fig. 14*a–c* are similar to those seen in association with dense bodies (1, 5, 39). The measured diameters of the fibers in Fig. 14 range from 120 to 140 Å.

The most characteristic microscopic feature of desmin gels is that the long, tangled fibers seen at low magnification (Fig. 15*a*) actually represent a network of extensively intertwined fibrils that become visible at high magnifications (Fig. 15*b*, *c*). Neither microtubules nor F-actin, both of

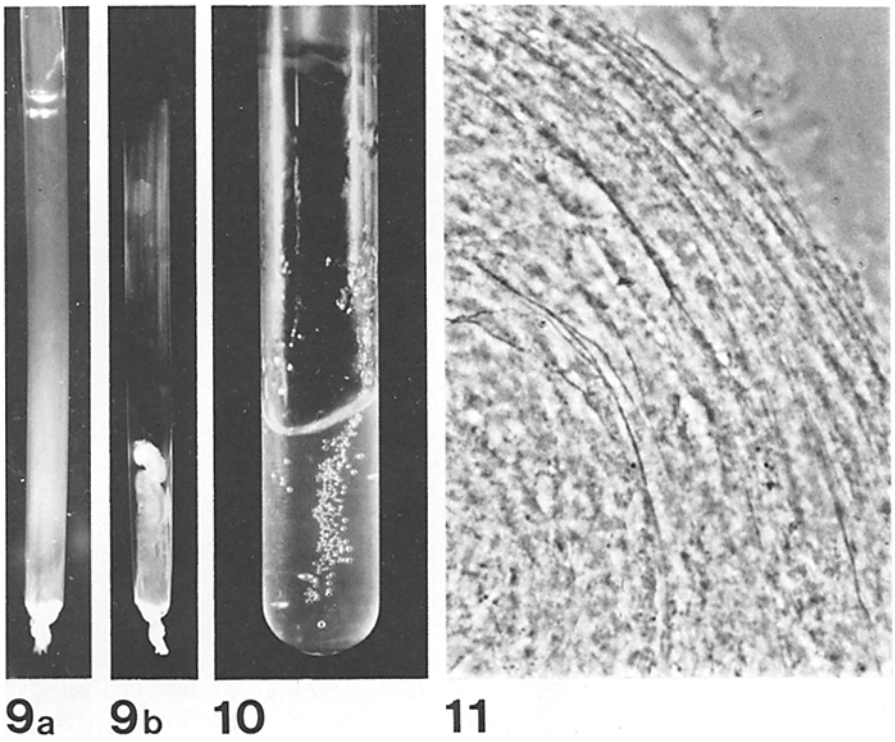


FIGURE 9 The formation of a spontaneous desmin gel. Acetic acid-solubilized cycle-2 desmin was dialyzed against several changes of water for 2 days at 4°C. The gel that resulted was photographed in the dialysis membrane (Fig. 9*a*). Dialysis was continued and 1 mM $MgCl_2$ was added to the dialysis medium to initiate contraction of the gel. The gel was rephotographed 1 h after the addition of $MgCl_2$ (Fig. 9*b*).

FIGURE 10 Gelation of metastable aqueous desmin upon contact with glass. A 1-M acetic acid extract of cycle-2 desmin was dialyzed against water to yield a metastable solution. The solution was removed from the dialysis membrane by inverting the open end of the membrane directly into a glass test tube. Gelation occurred within seconds, trapping air bubbles which appear suspended in the gel. The gel was photographed horizontally.

FIGURE 11 Light micrograph (phase optics) of a desmin gel similar to that depicted in Fig. 9*b*. Phase contrast dense fibers are seen embedded in an amorphous matrix. The fibers were probably oriented while the preparation was being flattened. Final magnification is × 1,000; 10 μm/cm.

which can form gel-like solutions, show this mode of twisting self-interaction. The fibril morphology of these gels is not uniform and a single gel may contain fibrils which exhibit regular profiles, twisted ribbon-like profiles, and intertwined profiles (Fig. 15*c*). The most consistent interpretation of these profiles is that they are actually flat ribbons of 120–140 Å width which have an inherent tendency to twist. The thin regions (60–80 Å; Fig. 15*c*, arrow) would correspond to nodes where the ribbon is parallel to the electron beam. We have not observed any profiles which can be interpreted as being strictly cylindrical along their entire lengths.

DISCUSSION

Solubility Properties of Desmin

Desmin is present in smooth muscle in two forms: a "soluble" one that is released during extraction at LI with EGTA, and an "insoluble" one that remains after extraction of the muscle cells at HI. In both cases, desmin is associated with actin.

The HI-insoluble desmin is solubilized either at low or high pH, or by agents that dissociate hydrophobic bonds. Two solubilizing agents were investigated in detail: acetic acid (1 M) and Sarkosyl NL-97 (0.5%). Acetic acid solubilizes des-

TABLE I

Conditions Causing the Gelation and Subsequent Contraction of Metastable Desmin Solutions

Salt	Concentrations tested
	mM
$MgCl_2$	0.1–100
ATP	0.1–10
Mg^{2+} + ATP	0.1, each
Mg^{2+} + K^+ + ATP	0.01, each
$CaCl_2$	0.1–50
EDTA pH 7.5	0.02–0.1
PIPES pH 6.9	100
Tris-HCl pH 6.9	10
KCl	10
NaCl	10
Glass	–

min along with a variety of other proteins from KCl- or KI-insoluble residues. Of these, only desmin, actin, and two proteins designated * precipitate quantitatively at pH 4.0 (Fig. 5). The pH-dependent precipitation of desmin is probably not a simple isoelectric phenomenon, however. First, desmin does not resolubilize above pH 4 until a very high pH is reached (e.g., with ethylene diamine). Second, the dialysis of desmin from acetic acid into water sometimes results in a metastable soluble state. Exposure of these solutions to an ionic environment causes the immediate coprecipitation of both actin and desmin. These properties are unlike any previously described for actin (26).

Desmin from avian muscle is resolved into two major isoelectric variants by two-dimensional electrophoresis (15, 21). Both variants are always present in every preparation and appear to behave identically in each of the purification schemes that we have employed (i.e., salt extractions, acetic acid cycling, and gel filtration in the presence of Sarkosyl). There is usually an excess of β-desmin over α-desmin in gizzard, however. One reason for this is the tendency of α-desmin to become modified and focus as two or more species. It is presently unknown whether α- and β-desmin are distinct gene products or if one arises by modification of the other.

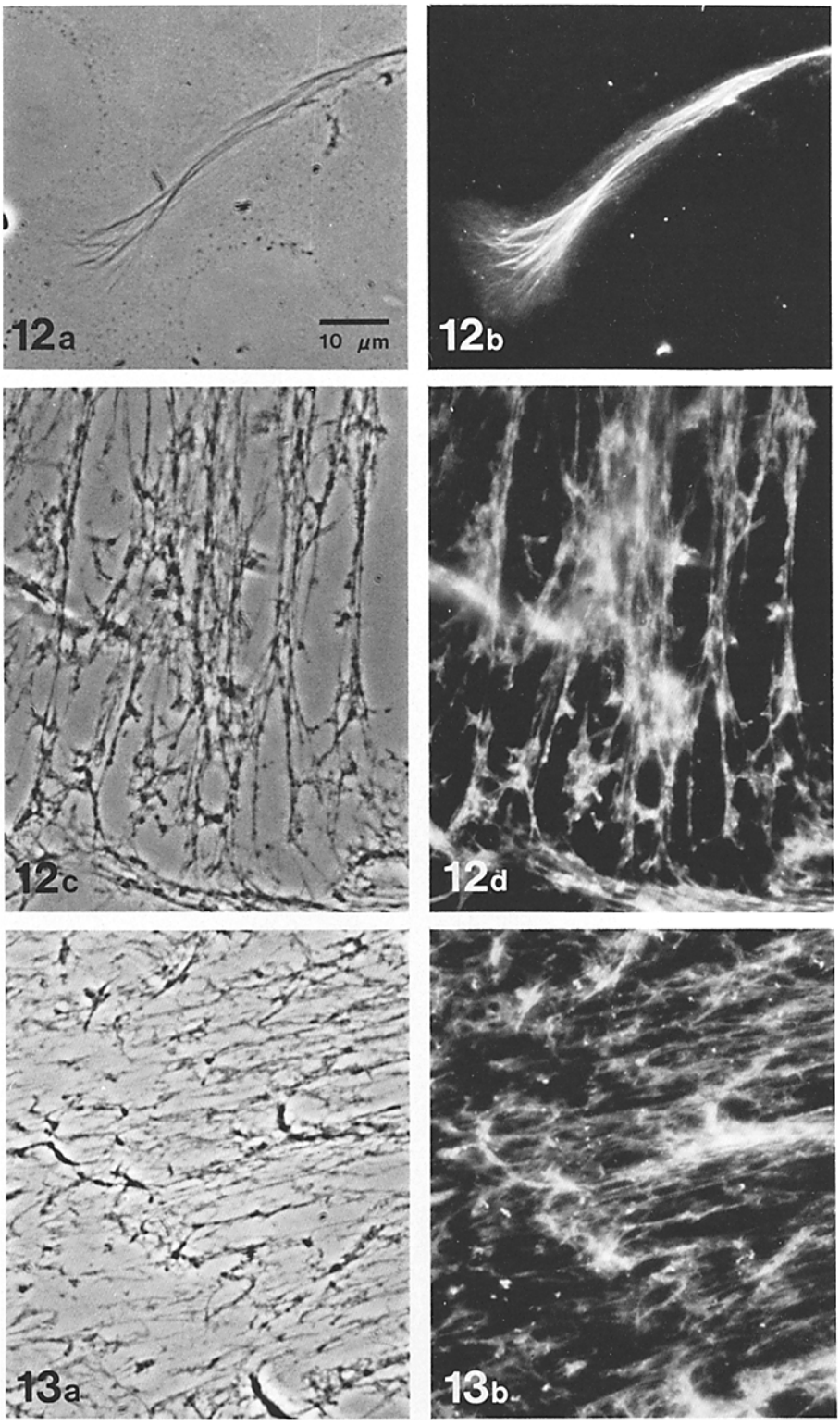
Copurification of Actin and Desmin

The most significant finding of this research is the suggestion that desmin and actin form nonstoichiometric complexes. The evidence for this is as follows: (*a*) A small fraction of gizzard actin has solubility properties that are different from the bulk of the actin but which are the same as for desmin. (*b*) Both actin and desmin copurify during repeated cycles of acetic acid solubilization and pH 4 precipitation. A constant ratio of actin to desmin is attained and this ratio is found in both the pH 4 precipitate and the supernate (Fig. 5*e* and *i*). (*c*) Gel filtration in the presence of 0.5% Sarkosyl NL-97 reveals an included fraction of actin and desmin that comigrate through the column. (*d*) Both actin and desmin appear to copolymerize from a metastable soluble state to form a single species of 100-Å-like filaments in which they are homogeneously distributed (see discussion below). The simplest interpretation of this is that actin and desmin are able to form stable, nonstoichiometric complexes with each other. We have hypothesized that desmin functions in muscle to bind separate actin-containing structures together into mechanically integrated units (22). The formation of an actin, desmin-containing polymer may provide a molecular basis for this hypothesis. It is important to note, however, that we have not excluded the possibilities of nonspecific interaction of actin and desmin or of separate populations of actin and desmin that simply have similar solubility properties.

Copurification of other Proteins with Desmin

At least two other proteins, termed $*_1$ and $*_2$, appear to be associated with desmin in a manner that is similar to that discussed for actin above. We wish to avoid giving these proteins names until we can determine whether they are specifically associated with desmin and whether or not they are cleavage fragments of desmin. This latter is a strong possibility. $*_2$ Comigrates with a known proteolytic fragment of desmin (in preparation), and both $*_1$ and $*_2$ are seen occasionally as isoelectric doublets on two-dimensional gels (data not shown). This further suggests that they may be derived from the cleavage of α- and β-desmin. It is intriguing to note that some preparations contain similar amounts of actin, $*_1$, and $*_2$ (Fig. 6*c*).

The remaining proteins that associate with desmin are HMW, myosin, α-actinin, and tropomyosin. These are all proteins that are known to bind to actin in the absence of desmin and are probably bound to the actin that copurifies with desmin. The HMW protein may be filamin, an actin-binding protein from smooth muscle (40, 41). How the cell chemically specifies the interactions of



actin with these proteins remains a matter for speculation.

Copolymerization of Actin and Desmin to 100-Å-like Filaments

Desmin appears to exist as an insoluble hydrophobic polymer under physiological conditions. These polymers may be solubilized by conditions of low or high pH (at low ionic strength) or by agents which dissociate hydrophobic bonds (5, 22, 33). Acetic acid-solubilized desmin forms either spontaneous gels or metastable solutions when the acetic acid is replaced with water by dialysis. The metastable solutions rapidly convert to gels if the

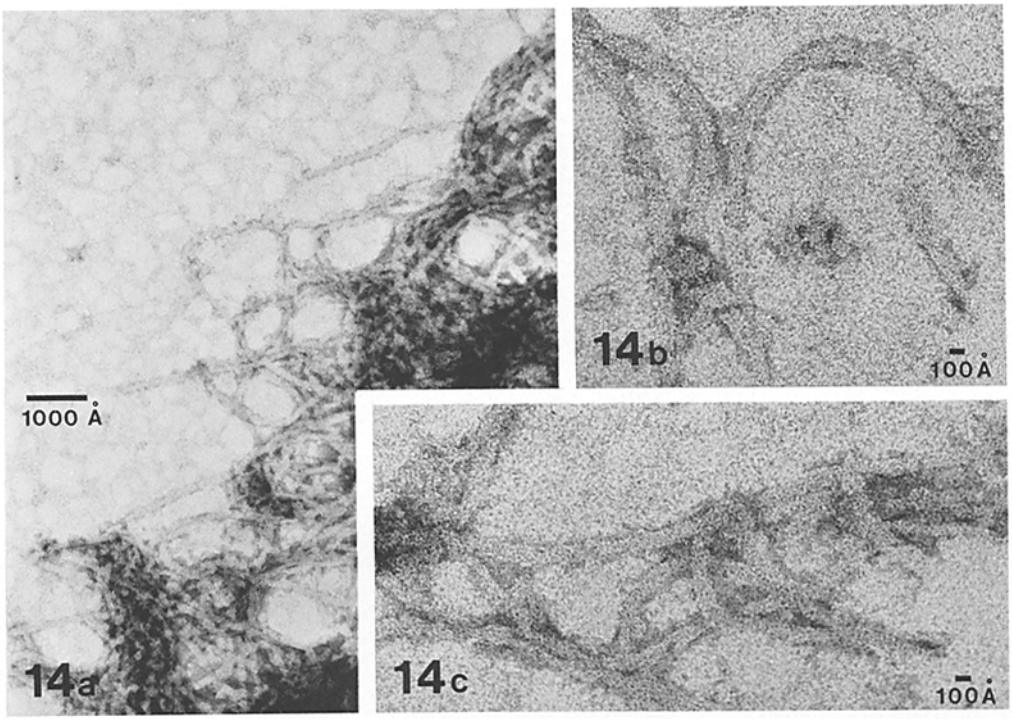


FIGURE 14 100-Å-diameter fibers in a KCl-residue. Fig. 14*a–c* shows tangled groups of 100-Å fibers with a well-preserved substructure and diameters of 120–140 Å.

FIGURE 12 Indirect immunofluorescence of desmin gels using desmin specific antibodies. Desmin fibers (described in Fig. 13) were reacted with anti-desmin and observed with phase contrast (Fig. 12*a* and *c*) and epifluorescence (Fig. 12*b* and *d*) optics. Fig. 12*b* shows a fine fluorescent network, between the fluorescent-filament bundles, that is nearly invisible in 12*a*. The larger fibers thus appear to be aggregates of thinner fibers. The fluorescence is uniformly distributed throughout the whole length of the fibers. Preimmune antisera (not shown) were completely negative. × 100 oil-immersion objective, NA 1.32; final magnification is × 1,000; 10 μm/cm.

FIGURE 13 Indirect immunofluorescence of desmin gels using actin specific antibodies. Desmin fibers from a gel were reacted with anti-smooth-muscle-actin and viewed with phase contrast (Fig. 13*a*) and epifluorescence (Fig. 13*b*) optics. As in the case with anti-desmin (Fig. 12), the fluorescence is uniformly distributed throughout the whole length of the fibers. No periodicities or differential staining of matrix or fibers is seen with either anti-actin or with anti-desmin. Preimmune antisera (not shown) were uniformly negative. The gels used for Figs. 12 and 13 were cycle-1 acetic acid extracts of KI-residue. The extracts were dialyzed against water and the resulting metastable solutions were induced to gel with 10 mM $MgCl_2$. These extracts are relatively rich in desmin-associated actin. × 100 oil-immersion objective, NA 1.32; final magnification × 1,000; 10 μm/cm.

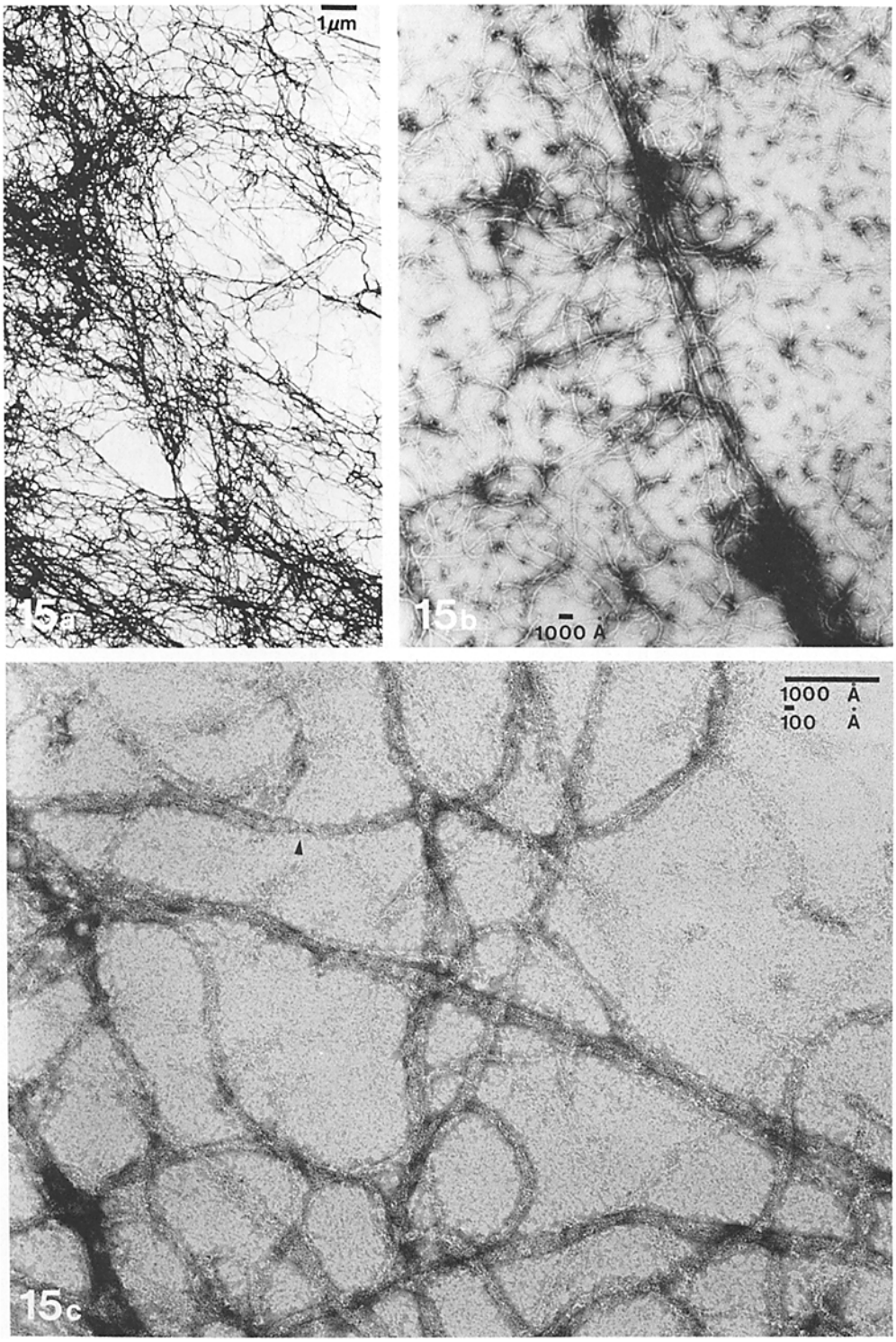


FIGURE 15 Typical gels produced by the dialysis of cycle-2 acetic acid extracts of KI-residue acetone powders against water. Many long, straight fibers can be seen in Fig. 15*a* and *b*. Most of these are composed of twisted and intertwining fibrils of 130 Å diameter (15*b*). Ribbonlike characteristics are evident in Fig. 15*c*. Occasional 11–15-Å profiles, which may be protofilaments, are visible in Fig. 15*c*. The substructure in Fig. 15*c* resembles that of the 100 Å filaments shown in Fig. 14*b* and *c*. The arrow indicates a region of apparent twisting. Final magnifications: (15*a*) × 5,200, 2 μm/cm; (15*b*) × 21,000, 4,750 Å/cm; (15*c*) × 140,000, 700 Å/cm.

concentration of ions rises above roughly micromolar values or if they are exposed to ionic surfaces (e.g., glass). These gels are characteristically composed of a network of highly intertwined fibrils which measure 120–140 Å in diameter. Most of the negatively stained fibril images are consistent with an interpretation of the fibrils as flat ribbons. These ribbons appear to intertwine to build up the macroscopic fibers that are visible in the light microscope, although it is possible that the fibers are an artifact of the negative staining procedure.

Immunofluorescence indicates that desmin and the actin that copurifies with it are uniformly distributed in desmin fibers at a resolution limit of 2,500 Å (Raleigh criteria for self-luminous points at λ of 530 nm and NA of 1.32; 32). Similarly, there is no overt evidence at the electron microscope level of separation into distinct filament morphologies. The gels also contain the same ratios of actin and desmin that the ungelled solution did. In addition, gel filtration in acetic acid indicates that most of the actin and desmin are unassociated under these conditions (Fig. 8). If the high molecular weight actin and desmin are not already associated in acetic acid, then they must become associated once it is removed. Thus, actin and desmin appear to copolymerize from solution.

Comparison of the Gelation of Desmin and Actin

A variety of cytoplasmic extracts have been discovered to undergo gelation and subsequent syneresis in vitro, and it is of interest to compare these with the gelation and syneresis of desmin. Typically, an extract that is capable of undergoing gelation is produced by homogenizing cells at 0°C in a buffered solution containing ATP, EGTA, and sucrose or glycerol. Upon being warmed, these extracts gel and then undergo syneresis if the gelled state is maintained. The gel-forming components of *Acanthamoeba* (23, 25), *Dictyostelium* (38), pulmonary macrophages (12, 37), and sea urchin eggs (16, 17) have been fractionated. While these are not identical systems, gel formation generally appears to depend upon the polymerization of G-actin to F-actin and on the subsequent cross-linking of this F-actin by one or more accessory proteins. In most cases, syneresis is magnesium-ATP dependent and is based upon the interaction of the cross-linked F-actin with myosinlike proteins. Exceptions to this include the ATP-independent syneresis of sea urchin egg gels (16, 17) and F-actin-filamin gels (41).

The formation of desmin gels does not depend upon any conditions which are known to stabilize F-actin: gel formation can be triggered by subphysiological concentrations of many different ions; neither ATP nor calcium is required; gel formation is not reversible except by resolubilization in acetic acid or Sarkosyl NL-97; and syneresis occurs in the absence of myosin or ATP. The adhesiveness of desmin for itself suggests that the syneresis of desmin gels may result from an autoaggregation process. It thus appears that the gelation of extracts based predominantly on actin is significantly different from the gelation of extracts that are predominantly desmin. The physiological significance of gelation and syneresis remains to be determined.

Are Desmin Filaments Related to 100-Å Filaments?

Two lines of evidence indicate that desmin is a major subunit of the 100-Å filaments of muscle. The first is based on the solubility properties of these filaments. Smooth muscle that has been extracted at high ionic strength is enriched in 100-Å filaments and contains actin and desmin as its major protein constituents (5, 6, 22, 33. See also above). Urea solubilizes these two proteins and also removes the 100-Å filaments from extraction enriched muscle (5). Second, the subsequent removal of solubilizing agents from desmin by dialysis has resulted in the production of ~100-Å-sized filaments from urea (5), acetic acid (reference 33 and this paper), and Sarkosyl NL-97 (data not shown). We have shown that most of the actin can be removed from desmin and that it will still polymerize to intermediate-sized filaments. These filaments are very similar to in vivo 100-Å filaments.

There are apparently two major differences between in vitro desmin fibrils and in vivo muscle 100-Å filaments. First, if desmin fibrils are in fact ribbonlike, then they differ from in vivo 100-Å filaments, which are shown to have cylindrical cross sections in the vast majority of preparations (1, 6, 39). It is possible, however, that the negative staining and drying procedures induced artifactual flattening and twisting in otherwise cylindrical desmin fibrils. Second, the adhesiveness of in vitro desmin fibrils and precipitates is unex-

pected because the 100-Å filaments of smooth muscle are not aggregated under normal circumstances. This phenomenon may result from either our in vitro assembly conditions or from an interaction of desmin with one of the proteins that copurifies with it. However, the formation of 100-Å-filament aggregates in colcemid-treated cultured cells has been reported for a variety of cell types, including striated muscle (14), cardiac muscle (20), and smooth muscle (unpublished observations). Although the mechanism of this aggregation remains unknown, it may reflect the unmasking of adhesive properties in vivo which are similar to those shown by the desmin fibrils in vitro.

We would like to thank the two reviewers for their time and effort and their many helpful comments. This work was supported by grant PHS-GM 06965-18 from the National Institutes of Health, and also by grants from the Muscular Dystrophy Association of America and the American Cancer Society.

Received for publication 16 March 1978, and in revised form 11 July 1978.

REFERENCES

1. Ashton, F. T., A. V. Somlyo, and A. P. Somlyo. 1975. The contractile apparatus of vascular smooth muscle: Intermediate high voltage stereo electron microscopy. *J. Mol. Biol.* **98**:17–29.
2. Benitz, W. E., D. Dahl, K. W. Williams, and A. Bignami. 1976. The protein composition of glial and nerve fibers. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **66**:285–289.
3. Brown, S., W. Levinson, and J. A. Spudich. 1976. Cytoskeletal elements of chick embryo fibroblasts revealed by detergent extraction. *J. Supramol. Struct.* **5**:119–130.
4. Brysk, M. M., R. H. Gray, and I. A. Bernstein. 1977. Tonofilament protein from newborn rat epidermis. *J. Biol. Chem.* **252**:2127–2133.
5. Cooke, P. 1976. A filamentous cytoskeleton in vertebrate smooth muscle fibers. *J. Cell Biol.* **68**:539–556.
6. Cooke, P. H., and R. H. Chase. 1971. Potassium chloride-insoluble myofilaments in vertebrate smooth muscle cells. *Exp. Cell Res.* **66**: 417–425.
7. Dahl, D., and A. Bignami. 1975. Glial fibrillary acidic protein from normal and gliosed human brain. *Biochim. Biophys. Acta.* **386**:41–51.
8. Davison, P. F., B-S. Hong, and P. Cooke. 1977. Classes of distinguishable 10 nm cytoplasmic filaments. *Exp. Cell Res.* **109**:471–474.
9. Day, W. A. 1977. Solubilization of neurofilaments from central nervous system myelinated nerve. *J. Ultrastruct. Res.* **60**:362–372.
10. Garrels, J. I., and W. Gibson. 1976. Identification and characterization of multiple forms of actin. *Cell.* **9**(part II):793–805.
11. Goldman, R. D., and D. M. Knipe. 1973. Functions of cytoplasmic fibers in non-muscle cell motility. *Cold Spring Harbor Symp. Quant. Biol.* **37**:523–534.
12. Hartwig, J. H., and T. P. Stossel. 1975. Isolation and properties of actin, myosin, and a new actin-binding protein in rabbit alveolar macrophages. *J. Biol. Chem.* **250**:5696–5705.
13. Hynes, R. O., and A. T. Destree. 1978. 10 nm filaments in normal and transformed cells. *Cell.* **13**:151–163.
14. Ishikawa, H., R. Bischoff, and H. Holtzer. 1968. Mitosis and intermediate-sized filaments in developing skeletal muscle. *J. Cell Biol.* **38**:538–555.
15. Izant, J. G., and E. Lazarides. 1977. Invariance and heterogeneity in the major structural and regulatory proteins of chick muscle cells revealed by two-dimensional gel electrophoresis. *Proc. Natl. Acad. Sci. U. S. A.* **74**:1450–1545.
16. Kane, R. E. 1975. Preparation and purification of polymerized actin from sea urchin egg extracts. *J. Cell Biol.* **66**:305–315.
17. Kane, R. E. 1976. Actin polymerization and interaction with other proteins in temperature-induced gelation of sea urchin egg extracts. *J. Cell Biol.* **71**:704–714.
18. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* **227**:680–685.
19. Lazarides, E. 1976. Actin, α-actinin, and tropomyosin interaction in the structural organization of actin filaments in non-muscle cells. *J. Cell Biol.* **68**:202–219.
20. Lazarides, E. 1978. The distribution of desmin in primary cultures of embryonic chick cardiac cells. *Exp. Cell Res.* **112**:265–273.
21. Lazarides, E., and D. R. Balzer, Jr. 1978. Specificity of desmin to avian and mammalian muscle cells. *Cell.* **14**:429–438.
22. Lazarides, E., and B. D. Hubbard. 1976. Immunological characterization of the subunit of the 100 Å filaments from muscle cells. *Proc. Natl. Acad. Sci. U. S. A.* **73**:4344–4348.
23. Maruta, H., and E. D. Korn. 1977. Purification from *Acanthamoeba castellanii* of proteins that induce gelation and syneresis of F-actin. *J. Biol. Chem.* **252**:399–402.
24. O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250**:4007–4021.
25. Pollard, T. D. 1976. The role of actin in the temperature-dependent gelation and contraction of extracts of *Acanthamoeba*. *J. Cell Biol.* **68**: 579–601.
26. Pollard, T. D., and R. R. Weihing. 1974. Cytoplasmic actin and myosin and cell movement. *CRC Crit. Rev. Biochem.* **2**:1–65.
27. Rubenstein, P. A., and J. A. Spudich. 1977. Actin microheterogeneity in chick embryo fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* **74**:120–123.
28. Schachner, M., E. T. Hedley-Whyte, D. W. Hsu, G. Schoonmaker, and A. Bignami. 1977. Ultrastructural localization of glial fibrillary acidic protein in mouse cerebellum by immunoperoxidase labeling. *J. Cell Biol.* **75**:67–73.
29. Schacterle, G. R., and R. L. Pollack. 1973. A simplified method for the quantitative assay of small amounts of protein in biologic material. *Anal. Biochem.* **51**:654–655.
30. Schlaepfer, W. W. 1977. Immunological and ultrastructural studies of neurofilaments isolated from rat peripheral nerve. *J. Cell Biol.* **74**: 226–240.
31. Shelanski, M. L., and F. Feit. 1972. Filaments and tubules in the nervous system. *In* The Structure and Function of Nervous Tissue, Vol. 6. G. H. Bourne, editor, Academic Press, Inc., New York. 47–80.
32. Slayter, E. M. 1970. Interactions of light waves in the imaging process. *In* Optical Methods in Biology. John Wiley and Sons, New York. 242–246.
33. Small, J. V., and A. Sobieszek. 1977. Studies on the function and composition of the 10-nm (100 Å) filaments of vertebrate smooth muscle. *J. Cell Sci.* **23**:243–268.
34. Starger, J. M., and R. D. Goldman. 1977. Isolation and preliminary characterization of 10-nm filaments from baby hamster kidney (BHK-21) cells. *Proc. Natl. Acad. Sci. U. S. A.* **74**:2422–2426.
35. Steinert, P., W. W. Idler, and S. B. Zimmerman. 1976. Self-assembly of bovine epidermal keratin filaments *in vitro*. *J. Mol. Biol.* **108**:547–567.
36. Storti, R. V., and A. Rich. 1976. Chick cytoplasmic actin and muscle actin have different structural genes. *Proc. Natl. Acad. Sci. U. S. A.* **75**:2346–2350.
37. Stossel, T. P., and J. H. Hartwig. 1976. Interactions of actin, myosin, and a new actin-binding protein of rabbit pulmonary macrophages. II. Role in cytoplasmic movement and phagocytosis. *J. Cell Biol.* **68**:602–619.
38. Taylor, D. L. 1977. Dynamics of cytoplasmic structure and contractility. *In* International Cell Biology 1976–1977. B. R. Brinkley and K. R. Porter, editors. Rockefeller University Press. 367–377.
39. Uehara, Y., G. R. Campbell, and G. Burnstock. 1971. Cytoplasmic filaments in developing and adult vertebrate smooth muscle. *J. Cell Biol.* **50**:484–497.
40. Wang, K., J. F. Ash, and S. J. Singer. 1975. Filamin, a new high-molecular-weight protein found in smooth muscle and non-muscle cells. *Proc. Natl. Acad. Sci. U. S. A.* **72**:4483–4486.
41. Wang, K., and S. J. Singer. 1977. Interaction of filamin with F-actin in solution. *Proc. Natl. Acad. Sci. U. S. A.* **74**:2021–2025.
42. Whalen, R. G., G. S. Butler-Browne, and F. Gros. 1976. Protein synthesis and actin heterogeneity in calf muscle cells in culture. *Proc. Natl. Acad. Sci. U. S. A.* **73**:2018–2022.
43. Wuerker, R. B. 1970. Neurofilaments and glial filaments. *Tissue Cell.* **2**:1–9.