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In vitro assembly of homopolymer and copolymer filaments from intermediate filament subunits of muscle and fibroblastic cells

(10-nm filaments/desmin/skeletin/decamin/vimentin)

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ABSTRACT This paper presents evidence that the intermediate filament (IF) subunits of muscle cells (skeletin or desmin) and fibroblastic cells (decamin or vimentin) separately form homopolymer IF *in vitro* and, when mixed, prefer to form copolymer IF *in vitro*. Because they coexist in cells, they may also form copolymers *in vivo*. The IFs of baby hamster kidney fibroblasts (BHK-21) consist of a major subunit, decamin, and two minor subunits which, on the basis of two-dimensional gel and peptide mapping criteria, are identical to the α and β subunits of smooth muscle desmin. The subunits differ only in their degrees of phosphorylation: α desmin contained 2 mol/mol of *O*-phosphoserine whereas β desmin contained none. The decamin and desmin subunits assembled into homopolymer IF *in vitro* in high yield from purified denatured subunits under identical conditions of pH and ionic strength. However, homopolymer decamin IF disassembled into soluble protofilaments in solutions of ionic strenth <0.05 mol/liter whereas homopolymer desmin IF disassembled at ionic strength <0.02 mol/liter. When decamin and desmin were mixed together as denatured subunits or as soluble protofilaments, the IF assembled *in vitro* had solubility properties intermediate between those of the homopolymer IFs, indicating that the two subunits had formed copolymer IF. The stoichiometry of copolymerization as determined in mixtures in which one subunit was present in excess was suggestive of the formation of three-chain units. The possibility of nonspecific aggregation was eliminated by isolation of stable three-chain α-helix-enriched particles from such IF. When tracer amounts of [^{35}S]methionine-labeled decamin were mixed with desmin, labeled IFs were obtained under conditions in which homopolymer decamin IFs were soluble. These *in vitro* findings may be of physiological significance because native BHK-21 IF also had solubility properties similar to those of the copolymer IF.

Of the three cytoskeletal filament systems in eukaryotic cells, intermediate filaments (IF) represent the most diverse group. Unlike the relative simplicity of the subunits of microtubules (tubulin) and microfilaments (actin), IF exhibit an almost bewildering complexity in their immunological and solubility properties and in the composition, size, and heterogeneity of their constituent subunits (1–3). For example, five subgroups of IF have been delineated largely on the basis of immunofluorescence data: those of mesenchymal cells (vimentin or decamin), muscle (skeletin or desmin), glial cells (glial fibrillary acidic protein), epithelial cells (keratin), and neuronal cells (neurofilaments) (1–3).||

Even this classification system, however, is inadequate because within each immunological subgroup there are distinct variations. The keratins of hair do not crossreact with those of the epidermis, inner root sheath, and other epithelial tissues, and vice versa (11, 12). In addition, IF subunits of the subgroups display varying degrees of heterogeneity. There are at least 10 different keratin IF subunits in bovine epidermis (13, 14), not one of which is apparently identical to the equally numerous IF subunits of human epidermis (15). Epidermal keratin subunits form obligate copolymer** IF *in vitro* because at least two different subunits are required for assembly (17). Similarly, isolated neurofilaments and those repolymerized *in vitro* are heterogeneous with respect to their subunit compositions (18, 19), and muscle desmin from several sources consists of two subunits (20, 21) which together can form native-type IF *in vitro* (21). These *in vitro* findings suggest that even within one type of cell there is an enormous variety in the possible types of IF. However, subunit complexity is not a universal characteristic of IF. The IF subunit components of Chinese hamster ovary and 3T3 cells (decamin/vimentin) (7, 22) and of glial cells (23) consist of a single protein that can form homopolymer IF *in vitro*.

Despite these differences, all IF are morphologically and structurally similar. Comparative studies of the IF of epidermal keratinocytes and BHK-21cells repolymerized *in vitro* have indicated they are structurally analogous α-type fibrous proteins that are composed of a basic three-chain structural building block, and their subunits consist of similar peptide domains (5, 24, 25). Other studies on avian muscle desmin (21), glial IF (23), and neurofilaments (26) have provided confirmatory data.

A further complication in the understanding of IF arises from the demonstration of the coexistence, in developing muscle cells (27, 28) and in BHK-21 fibroblasts (20, 29), of the muscle (desmin) and mesenchymal (decamin) types of IF subunits.

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Abbreviation: IF, intermediate filament(s).

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|| The term "vimentin" was introduced to define a protein, isolated from mouse 3T3 cells, that localizes by indirect immunofluorescence techniques in a pattern typical of the IF of mesenchymal cells (4). Decamin is the principal subunit of BHK-21 fibroblasts that can participate in the assembly of native-type IF *in vitro* (5, 6). Recently, we found that this protein is chemically indistinguishable from the single IF subunit of Chinese hamster ovary cells (7), antibodies to which localize at the ultrastructural level to the IF of 3T3 cells (8). The terms "skeletin" (9) and "desmin" (10) both define the IF subunits of muscle. Here, we shall use the term "desmin."

** The following definitions are used: protofilament, a three-chain structural unit or building block of IF [to be consistent with the earlier nomenclature system (16)]; homopolymer, a three-chain unit or an IF that contains only one subunit; copolymer, either a three-chain unit that contains two or three different subunits or an IF assembled from two or more different homopolymer three-chain units.

Also, transformed keratinocytes (30) and epithelial cells (31, 32) contain decamin and the keratin type IF subunits. A logical conclusion from these observations is that some cells can modulate their IF content during differentiation, in response to transformation (30, 33) or even to chemical assault (34). Implicit in this concept is the question of whether (*i*) the structurally similar but chemically and immunologically distinct IF subunit types within the cell function by forming separate IF characteristic of their type or (*ii*) they form copolymer IF: for example, do BHK-21 and developing muscle cells contain distinct decamin and desmin IF, or copolymer IF of the two, or some combination of these? Indirect immunofluorescence studies using antisera against decamin or desmin in developing muscle showed similar cytoplasmic arrays of IF (28), but the resolving power of this technique is not sufficient to demonstrate copolymerization.

In view of the ease with which epidermal keratin IF can be polymerized *in vitro* from purified denatured subunits (17), it seemed probable that this issue could be resolved by examining the capacity of decamin and desmin, separately and in mixtures, to form native-type IF *in vitro*. We demonstrate here that decamin and desmin readily form homopolymer IF *in vitro* from denatured subunits. Based on differences in their solubility properties, we also show that, when mixed *in vitro*, decamin and desmin prefer to copolymerize. The solubility of IF from BHK-21 cells which contain both types of subunits suggests that copolymerization also occurs *in vivo*.

MATERIALS AND METHODS

Isolation of IF Subunits. The IF of BHK-21 cells were isolated as intact filament caps and purified by two cycles of assembly/disassembly *in vitro* (5, 6). The three subunits (decamin of M_r ≈55,000 as the major component, and two minor desmin components) (see Fig. 1*A*) were separated by preparative NaDodSO$_4$/polyacrylamide gel electrophoresis using maximal unstacking parameters optimized for the M_r 50,000–60,000 zone (35) and their homogeneity was confirmed on two-dimensional gels. To prepare [^{35}S]decamin, Chinese hamster ovary cells were labeled in culture with [^{35}S]methionine and the decamin was purified as described (7, 22). The purified IF subunits could be stored indefinitely frozen (−70°C) in NaDodSO$_4$ solution containing 0.2 mM phenylmethylsulfonyl fluoride.

Isolation of Smooth Muscle Desmin. Desmin was isolated from Golden Syrian hamster stomach muscle as described by Tuszynski *et al.* (29) and purified by chromatography on DEAE-Sepharose (21). The product was resolved into two spots on two-dimensional gels which were separated by preparative NaDodSO$_4$ gel electrophoresis as above.

Analytical Procedures. Protein was estimated by the method of Bramhall *et al.* (36). All isoelectric focusing was done on 4% acrylamide gels using Bio-Lytes (Bio-Rad) in the pH range 5–7 (37). Histidine (50 mM) was used as the cathode electrolyte to enhance the separation of the IF subunits of pI 5–6 (38). Separation in the second dimension of two-dimensional gels was on 1.5-mm-thick 10–18% acrylamide gradient gels by the method of Douglas *et al.* (39). For quantitative studies, 4-mm-thick isoelectric-focused gels were stained with xylene brilliant cyanin G (ICN) and scanned at 610 nm with an ISCO model 659 gel scanner (17). The *O*-phosphoserine content of performic acid-oxidized subunits was estimated by reaction with methylamine (40). The protofilamentous forms of IF were subjected to limited tryptic digestion, and α-helix-enriched particles were separated for characterization as before (24, 25).

Peptide Mapping of IF Subunits. Purified IF subunits (0.1–0.2 mg/ml) were equilibrated in 0.1 M NH$_4$HCO$_3$, pH 8.3/0.1% NaDodSO$_4$/0.1 mM dithiothreitol and labeled with iodo[^{14}C]acetic acid (New England Nuclear) (41). After removal of the NaDodSO$_4$ (see below), the labeled subunits were digested with trypsin (Sigma, type XI). The peptides were resolved by two-dimensional chromatography and electrophoresis and characterized by fluorography (42).

IF Assembly *in Vitro* from Separated Subunits. Assembly into IF in high yields by subunits stored in NaDodSO$_4$ solution was critically dependent upon the complete removal of protein-bound NaDodSO$_4$. This was accomplished by using an ion-pair extraction procedure (43). The protein/NaDodSO$_4$ complex was precipitated from solution at 0°C with 0.2 vol of 2 M KCl, pelleted at 2000 × *g*, and extracted at 0°C with 20 vol of acetone/triethylamine/glacial acetic acid, 86:7:7 (vol/vol). The protein precipitate was reextracted twice with the same solvent made 5% (vol/vol) with water. By use of tracer [^{35}S]NaDodSO$_4$ we found that this method decreased the amount of NaDodSO$_4$ bound to proteins from ≈200 mol/mol to <0.01 mol/mol and thus was superior to procedures used earlier (17). Finally, the protein was redissolved (0.5–0.7 mg/ml) in 8 M urea/0.1 M Tris·HCl, pH 7.4/0.1 M 2-mercaptoethanol and dialyzed against 1000 vol of 5 mM Tris·HCl, pH 7.4/170 mM NaCl/1 mM dithiothreitol/0.5 mM phenylmethylsulfonyl fluoride/0.5 mM EDTA (IF assembly buffer) for 18 hr at 23°C.

IF Solubility Studies. The disassembly of IF in salt solutions of low ionic strength was done by dialysis of small volumes (0.5–1.0 ml) of preformed IF against IF assembly buffer containing 0–80 mM NaCl. The solutions were then centrifuged at 45,000 × *g* for 2 hr and the protein concentration was measured spectrophotometrically. The purified decamin and desmin subunits all had $E^{1\%}_{278\ nm}$ = 5.6. Intact IF of BHK-21 cells were resuspended by homogenization in IF assembly buffer, pelleted, and rewashed to remove lipid, soluble protein, and nucleic acid. Their disassembly was monitored by protein assay (36).

IF Copolymerization Studies. Samples of purified decamin and desmin (≈300 μg total protein) in NaDodSO$_4$ solution were mixed in relative amounts varying from 0.1% to 90%. After removal of NaDodSO$_4$, the protein was assembled into IF and then dialyzed for 18 hr against IF assembly buffer modified to contain only 40 mM NaCl. The IF still intact after this dialysis were sedimented at 45,000 × *g* for 2 hr and the subunit composition of the pellet was determined either by scanning of isoelectric-focused gels or by measurement of radioactivity.

RESULTS AND DISCUSSION

Characterization of IF Subunits. IF reassembled *in vitro* from isolated filament caps of BHK-21 cells consisted of three spots when resolved on two-dimensional gels (Fig. 1*A*). The major spot, decamin (M_r ≈55,000; pI ≈5.5), comigrated exactly with the only IF subunit of Chinese hamster ovary cells (7). The minor spots, each M_r ≈54,000, had pI values of ≈5.6 and 5.7 and have been termed the α and β subunits of muscle desmin (28, 29). Authentic desmin isolated from hamster stomach mus-

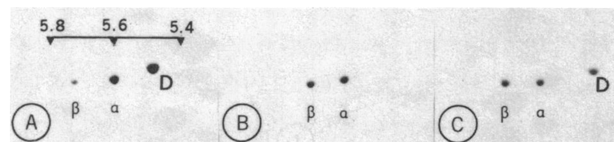

FIG. 1. Two-dimensional gel electrophoresis of IF preparations. (*A*) IF repolymerized twice from filament caps of BHK-21 cells; (*B*) desmin IF isolated and purified from hamster smooth muscle; (*C*) mixture of smooth muscle desmin and BHK-21 IF. α and β, the two desmin subunits; D, decamin. Horizontal dimension: isoelectric focusing; the approximate pH values are indicated. Vertical dimension: NaDodSO$_4$ gel electrophoresis.

cle was resolved into two spots on two-dimensional gels (Fig. 1*B*), which exactly comigrated with the minor IF subunits of BHK-21 cells (Fig. 1*C*). The tryptic peptide maps of these IF subunits were compared after labeling with iodo[^{14}C]acetic acid (Fig. 2). The α and β desmin subunits from both muscle and BHK-21 cells yielded identical maps, but the desmin subunits were clearly different from those of decamin (and see ref. 7). The identities of the α and β desmin maps indicate that significant portions of their amino acid sequences are the same. The *O*-phosphoserine contents of these subunits were: decamin, 0.5–0.8 residue/mol; α desmin, 1.7–2.2 residues/mol; and β desmin, <0.2 residue/mol. These differences in phosphate content are sufficient to account for the observed differences in pI. Thus, we tentatively conclude that the two desmin subunits differ from one another only in their degrees of phosphorylation.

Homopolymer IF Assembly *in Vitro*. The decamin of BHK-21 cells and the α and β desmin subunits of BHK-21 cells or of smooth muscle were observed to polymerize into IF *in vitro*. The optimal conditions for IF assembly were the same for all subunits and were: protein concentration, $\approx$0.5 mg/ml; 0.10 < ionic strength (mol/liter), < 0.20; and 7.0 < pH < 8.0. Under these conditions, about 90% of the protein assembled into IF, provided that a reducing environment was maintained. After negative staining, all IF appeared as long rods (>10 μm) and were uniformly 7–9 nm wide (Fig. 3). In addition, the IF examined yielded typical α-type x-ray diffraction patterns (data not shown). These morphological and ultrastructural features are identical to those described for IF reassembled *in vitro* from isolated filament caps of BHK-21 cells (5, 6), the decamin of Chinese hamster ovary cells (7), epidermal keratin (17), and avian desmin (21). Interestingly, the degree of phosphorylation of the desmin subunits did not appear to affect their facility for assembly *in vitro*. A similar phenomenon has been observed with other types of IF subunits as well (unpublished data).

These findings indicate that decamin and the two desmin subunits can form native-type homopolymer IF *in vitro*. Reassembly was effected from purified denatured subunits, which is a useful procedure first developed in the study of epidermal keratin IF assembly (17). This approach affords a more quantitative means of studying the factors involved in IF assembly *in vitro* and, by extrapolation, *in vivo* as well. To date, decamin, desmin, and glial fibrillary acidic protein (23) have been shown to be capable of homopolymer IF assembly *in vitro*. In contrast, epidermal keratin IF are obligate copolymers (17). Because both forms of desmin and decamin coexist within BHK-21 cells (20, 29) and developing muscle cells (27, 28), we looked for evidence of copolymerization of these subunits.

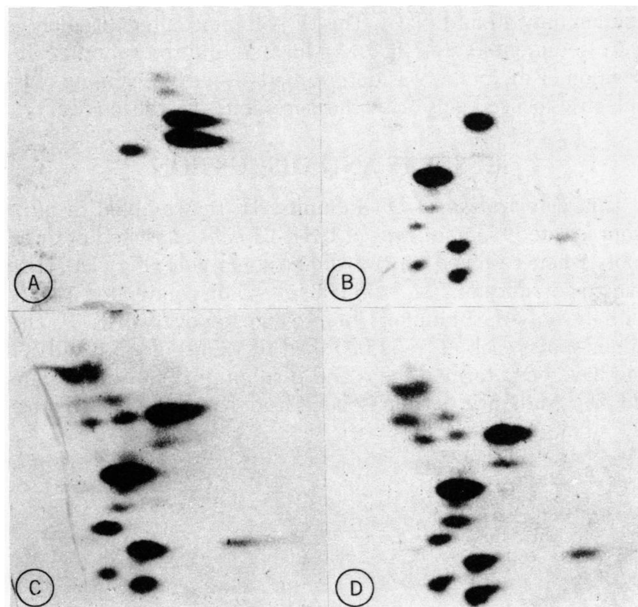

FIG. 2. Two-dimensional tryptic peptide maps of iodo[^{14}C]acetic acid-labeled IF subunits. (*A*) Decamin of BHK-21 cells; (*B*) α-desmin of BHK-21 cells; (*C*) β-desmin of smooth muscle; (*D*) mixture of α- and β-desmins of BHK-21 cells and smooth muscle. Horizontal dimension: electrophoresis; anode at left. Vertical dimension: ascending chromatography.

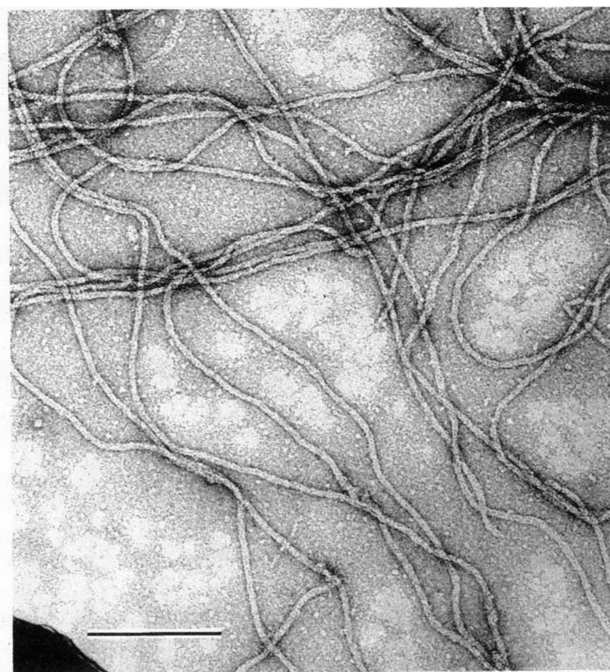

FIG. 3. Structure of homopolymer BHK-21 α-desmin IF. (Negative stain with 0.7% uranyl acetate; ×87,500; bar, 0.2 μm.)

Solubility Properties of IF. Whereas the ionic strength requirements for assembly of decamin and desmin were the same, the stabilities of the IF in solutions of decreased ionic strength were different (Fig. 4). At pH 6.8–7.4, homopolymer decamin IF began to disassemble at ionic strength 0.06 mol/liter and were completely disassembled into soluble nonsedimentable protofilaments by 0.04 mol/liter. The solubility characteristics of homopolymer IF of either α- or β-desmin were the same, but they were distinctly less soluble than decamin: disassembly did not begin until ionic strength 0.03 mol/liter and was complete at $\approx$0.01 mol/liter. This difference in solubility was pH dependent because at pH 8.5 the desmin IF became as soluble as decamin IF (Fig. 4*A Inset*). When preformed homopolymer decamin and desmin IF were mixed, a biphasic disassembly curve was obtained, which indicated the presence of two components of different solubility properties.

Mixtures of decamin and desmin made prior to the removal of $NaDodSO_4$ also readily polymerized *in vitro* into native-type IF in yields in excess of 90%. The solubility properties of such IF varied with the relative amounts of the two components (Fig. 4*B*). In mixtures containing no less than about 40% desmin, the IF disassembled at ionic strength 0.02–0.04 mol/liter; that is, they were about as soluble as, or somewhat more soluble than, homopolymer desmin IF but clearly less soluble than homopolymer decamin IF. When decamin was present in large excess in the mixtures, biphasic curves were obtained, suggestive of the presence of components of different solubility properties.

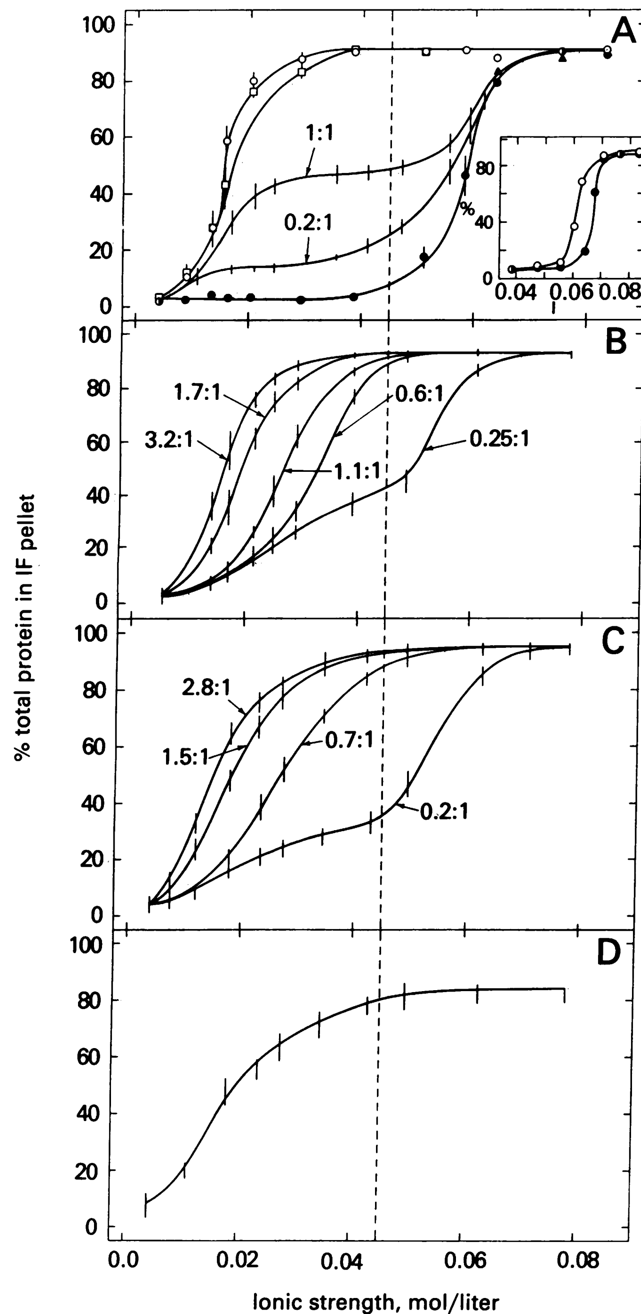

FIG. 4. Disassembly of IF. Vertical bars, ranges of protein concentration determined in duplicate or triplicate; ratios, relative amount of desmin to decamin in mixtures; broken vertical line, ionic strength = 0.045 mol/liter. (*A*) Homopolymer IF at pH 7.4; ○, BHK-21 α-desmin IF; □, β-desmin of smooth muscle; ●, decamin of BHK-21 cells. The central curves show mixtures of preformed desmin and decamin IF. (*Inset*) Disassembly of homopolymer IF in salt solutions at pH 8.5. (*B*) Purified BHK-21 α-desmin and decamin subunits mixed before removal of $NaDodSO_4$. (*C*) Mixtures of homopolymer smooth muscle α-desmin and decamin protofilaments (protein soluble at ionic strength = 0.01 mol/liter). (*D*) Native IF of BHK-21 cells of isolated filament caps that had been dispersed by homogenization and washed.

The most likely explanation for these observations is that decamin and desmin formed copolymer IF in intermediate mixtures or formed homopolymer IF when one component was present in excess.

The soluble protofilaments (ionic strength <0.01 mol/liter) of homopolymer decamin and desmin readily assembled into native-type IF either separately or when mixed together within 2 hr when the NaCl concentration was increased to 0.17 M (5, 6). The solubility characteristics of IF formed from such mixtures were examined (Fig. 4*C*); the disassembly curves were suggestive of copolymerization.

Based on studies of the kinetics of assembly of keratin IF *in vitro* from purified subunits soluble in urea or $NaDodSO_4$, the assembly mechanism proceeds in three steps (44): (*i*) the formation of protofilaments which may be a pair of three-chain units; (*ii*) lateral and longitudinal assembly of protofilaments into short IF; and (*iii*) elongation of IF. Reassembly of BHK-21 IF (6) and squid neurofilaments (19) *in vitro* by cycling of salt concentrations proceeds from step *ii*. Thus, it is possible that, in Fig. 4*B*, copolymer protofilaments were formed; in Fig. 4*C*, copolymer IF may have formed from homopolymer protofilaments.

To determine whether these *in vitro* assembly experiments were of physiological significance, the disassembly properties of native IF of BHK-21 cells were examined. Isolated filaments contain about 50% decamin, 30% desmin, and minor amounts of other non-IF proteins (Fig. 1*A*; ref. 6). The data of Fig. 4*D* indicate that these IF likewise had solubility characteristics suggestive of copolymer IF.

Stoichiometry of Copolymer IF. From the disassembly curves of Fig. 4 it is apparent that at ionic strength 0.045 mol/liter, decamin IF are soluble but copolymer IF and homopolymer desmin IF remain intact and can be pelleted. Because about 90% of the total protein in all decamin and desmin mixtures assembled into IF *in vitro*, this observation offered a means of quantitating the apparent stoichiometric relationship between decamin and desmin suggested from the disassembly curves of Fig. 4 *B* and *C*. Fig. 5 shows the result of an experiment in which increasing relative amounts of decamin were polymerized with desmin and the amounts of each in the IF pelleted at ionic strength 0.045 mol/liter were determined. At high concentrations of decamin, the amount of intact IF was small because the excess decamin formed homopolymer IF that were soluble at ionic strength 0.045 mol/liter. However, in mixtures containing high percentages of decamin, the intact copolymer IF always contained a minimum of one-third desmin and about two-thirds decamin. These data suggest the formation

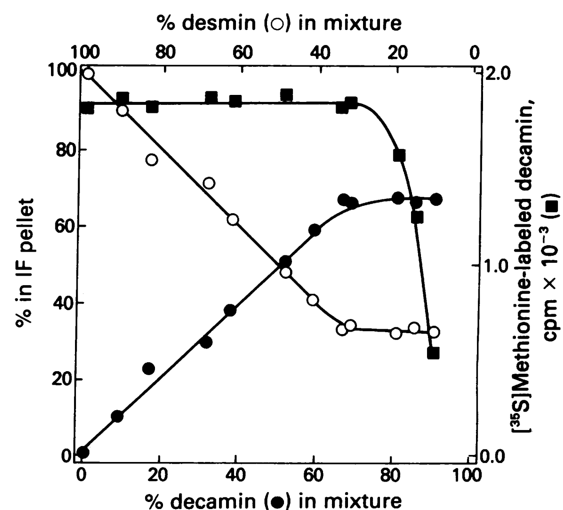

FIG. 5. Stoichiometry of copolymer IF. Aliquots of [^{35}S]methionine-labeled decamin (about 1.05 μg; 1950 cpm) were added to each mixture of decamin and BHK-21 α-desmin prior to removal of $NaDodSO_4$. The pellets of IF insoluble at ionic strength 0.045 mol/liter were redissolved in 0.5 ml of urea buffer; one-fifth was assayed for radioactivity and the remainder was used for quantitation of the desmin and decamin contents.

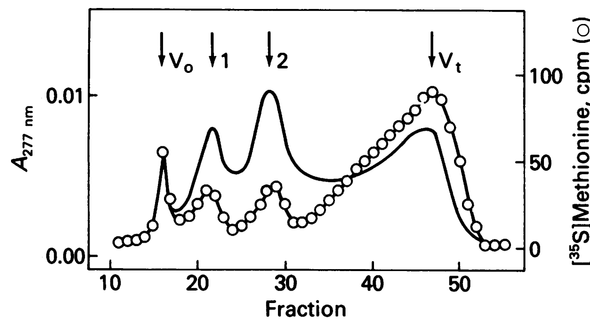

FIG. 6. Isolation of α-helix-enriched particles from copolymer IF. The IF were assembled from 350 μg of BHK-21 α-desmin and 1.8 μg (3350 cpm) of [^{35}S]methionine-labeled decamin. The copolymer IF insoluble at ionic strength 0.045 mol/liter were pelleted, resuspended, and digested with trypsin, and the products were chromatographed on Sepharose 6B as described (24, 25). Samples of fractions 22 (particle 1) and 28 (particle 2) were assayed by circular dichroism; their α-helix contents were 73% and 90%, respectively. Their chromatographic properties and α-helix contents thereby indicate they are identical to the particles isolated and characterized in detail from BHK-21 IF (25).

of a three-chain structure as found with epidermal keratin IF (17).

This experiment was also done with tracer amounts of [^{35}S]methionine-labeled decamin included in the mixtures (Fig. 5). In mixtures of high relative percentages of desmin, virtually all of the decamin was incorporated into copolymer IF that were not disassembled and were pelleted at ionic strength 0.045 mol/liter. With increasing percentages of decamin, the amount of label in the pellet diminished due to its assembly into soluble homopolymer decamin IF. This observation also strongly supports the idea of copolymerization of the two components and it favors the view that whenever decamin and desmin are mixed *in vitro* they tend to copolymerize.

Ultrastructure of Copolymer IF. IF intact at ionic strength 0.045 mol/liter that were assembled from excess BHK-21 α-desmin and [^{35}S]methionine-labeled decamin were subjected to limited tryptic digestion (Fig. 6). This procedure digests the non-α-helical regions of the IF, specifically releasing the more-resistant coiled-coil α-helical segments (24, 25). Because almost all of the protein was desmin, the recovery of [^{35}S]methionine from the decamin in the α-helical particles indicated that the decamin was associated with the desmin as a component of the basic α-helical structure of the copolymer IF rather than bound nonspecifically. In addition, this finding confirms our earlier view of the similarity of the ultrastructures of the decamin, desmin, epidermal keratin, and related IF (5, 25).

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1. Goldman, R. D., Milsted, A., Schloss, J. A., Starger, J. M. & Yerna, M.-J. (1979) *Annu. Rev. Physiol.* **41**, 703–722.
2. Lazarides, E. (1980) *Nature (London)* **283**, 249–256.
3. Zackroff, R. V., Steinert, P. M., Anyardi-Whitman, M. & Goldman, R. D. (1980) in *Cell Surface Reviews*, ed. Hartshorne, D. (Elsevier/North-Holland, Amsterdam), Vol. 7, in press.
4. Franke, W. W., Schmid, E., Osborn, M. & Weber, K. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5034–5038.
5. Steinert, P. M., Zimmerman, S. B., Starger, J. M. & Goldman, R. D. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 6098–6101.
6. Zackroff, R. V. & Goldman, R. D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6226–6230.
7. Cabral, F., Gottesman, M. M., Zimmerman, S. B. & Steinert, P. M. (1981) *J. Biol. Chem.* **256**, 1428–1431.
8. Cabral, F., Willingham, M. C. & Gottesman, M. M. (1980) *J. Histochem. Cytochem.* **28**, 653–662.
9. Small, J. V. & Sobieszek, A. (1977) *J. Cell Sci.* **23**, 243–268.
10. Lazarides, E. & Hubbard, B. D. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4344–4348.
11. Kemp, D. J. & Rogers, G. E. (1970) *J. Cell Sci.* **7**, 273–285.
12. Sun, T.-T., Shih, C. & Green, H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2813–2817.
13. Steinert, P. M. & Idler, W. W. (1975) *Biochem. J.* **151**, 603–614.
14. Steinert, P. M., Idler, W. W. & Wantz, M. L. (1980) *Biochem. J.* **187**, 913–916.
15. Fuchs, E. & Green, H. (1978) *Cell* **15**, 887–897.
16. Mercer, E. H., Rogers, G. E., Munger, B. L. & Roth, S. I. (1964) *Nature (London)* **201**, 367–368.
17. Steinert, P. M., Idler, W. W. & Zimmerman, S. B. (1976) *J. Mol. Biol.* **108**, 547–567.
18. Gilbert, D. S., Newby, B. J. & Anderton, B. H. (1975) *Nature (London)* **256**, 586–589.
19. Zackroff, R. V. & Goldman, R. D. (1980) *Science* **208**, 1152–1155.
20. Gard, D. L., Bell, P. B. & Lazarides, E. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3894–3898.
21. Huiatt, T. W., Robson, R. M., Arakawa, N. & Stromer, M. H. (1980) *J. Biol. Chem.* **255**, 6981–6989.
22. Cabral, F. & Gottesman, M. M. (1979) *J. Biol. Chem.* **254**, 6203–6206.
23. Rueger, D. C., Huston, J. S., Dahl, D. & Bignami, A. (1979) *J. Mol. Biol.* **135**, 53–68.
24. Steinert, P. M. (1978) *J. Mol. Biol.* **123**, 49–70.
25. Steinert, P. M., Idler, W. W. & Goldman, R. D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4534–4538.
26. Day, W. A. & Gilbert, D. S. (1972) *Biochim. Biophys. Acta* **285**, 503–506.
27. Bennett, G. S., Fellini, S. A., Totama, Y. & Holtzer, H. (1979) *J. Cell Biol.* **82**, 577–584.
28. Gard, D. L. & Lazarides, E. (1980) *Cell* **19**, 263–275.
29. Tuszynski, G. P., Frank, E. D., Damsky, C. H., Buck, C. A. & Warren, L. (1979) *J. Biol. Chem.* **254**, 6138–6143.
30. Franke, W. W., Schmid, E., Weber, K., Osborn, M. & Fusenig, N. (1979) *Differentiation* **14**, 35–50.
31. Franke, W. W., Schmid, E., Weber, K. & Osborn, M. (1979) *Exp. Cell Res.* **118**, 95–109.
32. Osborn, M., Franke, W. W. & Weber, K. (1980) *Exp. Cell Res.* **125**, 37–46.
33. Hynes, R. O. & Destree, A. T. (1978) *Cell* **13**, 151–163.
34. Borenfreund, E., Schmid, E., Bendich, A. & Franke, W. W. (1980) *Exp. Cell Res.* **127**, 215–235.
35. Jovin, T. M., Dante, M. L. & Chrambach, A. (1970) *Multiphasic Buffer Systems Output* (Natl. Tech. Serv., Springfield, VA), Publ. nos. 196085–196091; 203016; 259309–259312.
36. Bramhall, S., Noack, N., Wu, M. & Lowenberg, J. R. (1969) *Anal. Biochem.* **31**, 146–149.
37. Hearing, V. J., Nicholson, J. M., Montague, P. M., Ekel, T. M. & Tomecki, K. J. (1978) *Biochim. Biophys. Acta* **522**, 327–339.
38. Nguyen, N. Y. & Chrambach, A. (1980) *Electrophoresis* **1**, 14–22.
39. Douglas, M., Finkelstein, D. & Butow, R. (1979) *Methods Enzymol.* **56**, 58–66.
40. Kolesnikova, V. Y., Sklyankia, V. A., Baratova, L. A., Nazarova, T. I. & Avaeva, S. M. (1974) *Biokhimiya* **39**, 235–240.
41. Steinert, P. M. (1975) *Biochem. J.* **149**, 39–48.
42. Shih, T. Y., Williams, D. R., Weeks, M. O., Maryak, J. M., Vass, N. C. & Scolnick, E. M. (1978) *J. Virol.* **27**, 45–55.
43. Henderson, L. E., Oroszlan, S. & Konigsberg, W. (1979) *Anal. Biochem.* **93**, 153–157.
44. Steinert, P. M. (1977) in *Biochemistry of Cutaneous Epidermal Differentiation*, eds. Seiji, M. & Bernstein, I. A., (Tokyo Univ. Press, Tokyo), pp. 444–464.