Purification of the Intermediate Filament Protein Vimentin from Ehrlich Ascites Tumor Cells*

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Two procedures have been used for the purification of the intermediate filament protein vimentin from Ehrlich ascites tumor (EAT) cells grown in vitro. In one procedure, vimentin was first incorporated into residual cell structures by extracting EAT cells with Triton X-100 in the presence of Mg2+ and then solubilized with low ionic strength buffer in the absence of Mg2+. In the second procedure, which led to the purification of both vimentin and the Ca2+-activated proteinase specific for this intermediate filament protein, both components were immediately solubilized by extracting EAT cells with hypotonic buffer in the absence of Triton X-100. In both procedures, a vimentin-enriched fraction was obtained by precipitating vimentin with (NH4)2SO4 at 23% saturation. Following lyophilization and delipidation with chloroform/methanol, vimentin was purified by column chromatography on DEAE-Sepharose Cl 6B in 6 M urea. The first procedure resulted in a 33-fold purification of vimentin in a yield of 39%. The same method was also used to prepare [3H]vimentin from EAT cells labeled with [3H]-amino acids and resulted in a specific activity of 8 × 10^6 cpm/mg purified vimentin. By two-dimensional polyacrylamide gel electrophoresis only one protein species was seen following staining with silver. The purified protein had a subunit molecular weight of 58,000 and consisted of several isoelectric variants (pI ~ 5.3). In the absence of urea and at low salt concentration the purified vimentin was highly aggregated and had a sedimentation coefficient of 8 s.

The cells of most, if not all, higher vertebrates contain three classes of cytoplasmic filaments: microtubules, microfilaments, and intermediate filaments (1-3), which together probably form the microtrabecular lattice described by Wolosewick and Porter (4). Whereas a great deal is already known about the biochemistry, structure, assembly-disassembly, and function of microtubules and microfilaments, (see Refs. 5-9), intermediate filaments remain the least understood of these protein fibrils. However, results are accumulating on the distribution, subunit composition, and the assembly-disassembly of intermediate filaments (for review, see Refs. 1, 10). Furthermore, recent evidence indicates that they may be involved in cell movement and spreading and in the positioning of the nucleus and organelles in the cell (1, 10).

In order to understand in more detail the organization of intermediate filaments, their biochemistry and post-translational modification (phosphorylation), a simple procedure is required for their purification. Although the availability of various other intermediate filament proteins from tissues in situ has been reported (11-15, see Refs. 1, 10), vimentin, which is found in cells of mesenchymal origin and in cells grown in vitro (16, 17), has been more difficult to obtain. Denatured vimentin has been purified in small amounts using preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis (16-18). In addition, intermediate filaments have been isolated from birefringent caps of baby hamster kidney (BHK-21) cells (19) which contain not only vimentin but also desmin and several other contaminating proteins. Recently, Geisler and Weber (20) published a procedure for the purification of vimentin from porcine eye lens tissue which is very rich in vimentin. Although this material can be obtained from the slaughterhouse and is not as costly as cell culture, most studies on vimentin are carried out with in vitro grown cells and thus, it is important to have a method for the purification of vimentin from these cells. Furthermore, the procedure described here allows the preparation of large amounts of purified and radioactively labeled vimentin which can then be used in many in vitro studies currently being conducted in this laboratory.

The purification procedures described here have been developed from the results of earlier studies on the optimum ionic conditions for the incorporation of vimentin-containing intermediate filaments into residual cell structures upon treatment of EAT cells with Triton X-100 (21). The purified vimentin has been used initially as a substrate for the Ca2+-activated proteinase presented in the accompanying paper (22).

MATERIALS AND METHODS

Materials—Reagent grade chemicals and biochemicals were obtained from Merck AG (Darmstadt, FRG). [3H]-amino acid mixture (37 MBq/ml, Code No. TRK 440) was obtained from Amersham Buchler (Amersham, England). All molecular weight marker proteins and EGTA were purchased from Sigma. DEAE-Sepharose Cl 6B was obtained from Pharmacia (Uppsala, Sweden). Harker/Yates was purchased from Polysciences Inc. (Warrington, PA) and horseradish peroxidase-conjugated IgGs from DAKO Immunoglobulins Ltd. (Copenhagen, Denmark). Nitrocellulose filters (Type HA; 0.45 pm) were obtained from Millipore (Bedford, MA).

Buffers—The following buffers were used: 10 mM Tris-acetate, pH 7.6, 1 mM EGTA, 4 mM MgCl2, 6 mM 2-mercaptoethanol, 0.5% (w/v) Triton X-100 (Buffer A); 10 mM Tris-acetate, pH 7.6, 1 mM EGTA, 6 mM 2-mercaptoethanol (Buffer B); 6 M urea, 10 mM Tris-acetate, pH 7.6, 1 mM EGTA, 6 mM 2-mercaptoethanol (Buffer C).

Cell Culture—EAT cells were grown in suspension culture using MEM supplemented with 5% fetal calf serum as described previously (23).

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The abbreviations used are: EAT, Ehrlich ascites tumor; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; SDS, sodium dodecyl sulfate; MEM, minimum essential medium; BHK, baby hamster kidney.
Cells were harvested at a density of 1.5 × 10^6 cells/ml for the purification of only vimentin from Triton X-100-resistant residual cell structures (see Fig. 1), and at a density of 1 × 10^5 cells/ml for the purification of vimentin and the Ca^{2+}-activated proteinase (see Fig. 2 and Ref. 22). The cells were washed with 10 mM Tris-HCl, pH 7.5, 0.15 M NaCl (Tris-saline) and pelleted by centrifugation at 600 g for 5 min at 2 °C. Cells used for the purification of only vimentin were frozen in liquid nitrogen and stored at -80 °C.

The cells were resuspended in 450 ml of the original medium (containing all amino acids and 5% fetal calf serum), which had been kept sterile and warm at 37 °C, and incubated for a further 3 h at 37 °C. The cells (density: 1.5 × 10^6 cells/ml) were harvested and washed in Tris-saline as described above. The cells were then resuspended in 850 ml of Buffer A and extracted four times in a hypotonic buffer and the nuclei, membranes, and ribosomes removed by centrifugation at 4000 g for 10 min. The supernatants were then resuspended in 2.0 ml of Buffer B in a total volume similar to that above for the Triton X-100 extraction. 10 strokes were applied each time in Dounce homogenizers with 10 strokes every 5 min. The resulting supernatant was then centrifuged at 50,000 g for 5 min. The Ca^{2+}-activated proteinase was precipitated between 23 and 50% saturated (NH_4)_2SO_4 and stirred for 1 h at 0 °C. The precipitated vimentin was collected by centrifugation at 50,000 g for 10 min. The precipitate was redissolved in 30 ml of Buffer B by sonication (Branson Sonifier, Branson Sonic Power Co., Danbury, CT) operated at 240 watts and vimentin was reprecipitated as described above. Finally the precipitate was dissolved in 30 ml of Buffer B by sonication, dialysed overnight, and lyophilized.

**Extraction of Cells without Triton X-100 (Fig. 2)—EAT cells from a 20-liter culture (1 × 10^6 cells/ml) were harvested and washed in Tris-saline as described above. The cells were extracted in a hypotonic buffer and the nuclei, membranes, and ribosomes removed by centrifugation as described in an accompanying paper (22). Under these conditions both the vimentin and the Ca^{2+}-activated proteinase were recovered in the postribosomal supernatant. The vimentin was precipitated with (NH_4)_2SO_4 at 23% saturation by stirring for 1 h at 0 °C. Following centrifugation at 50,000 g for 10 min, the vimentin-enriched fraction was dissolved in Buffer B by sonication and precipitated with 23% saturated (NH_4)_2SO_4 by stirring for 1 h at 0 °C. Following centrifugation at 50,000 g for 10 min, the vimentin proteinase was precipitated between 23 and 50% saturated (NH_4)_2SO_4 and purified by a series of column chromatography steps (22).**

**Purification of Vimentin—** Unless otherwise stated all procedures were performed at 2 °C. The vimentin-enriched fractions from EAT cells extracted in the presence or absence of Triton X-100 were homogenized identically. The lyophilized powder was extracted five times with 30-ml portions of chloroform/methanol (2:1) by sonication (30 watt) at 2 °C for 5 min (24). Between extractions the vimentin was pelleted by centrifugation at 4000 g for 5 min. Remaining traces of chloroform/methanol mixture after the last extraction were removed under vacuum produced by an aspirator. The vimentin was dissolved in 20 ml of Buffer C by sonication and applied to a column (2 × 20 cm) of DEAE-Sepharose CI 6B, equilibrated in Buffer C, at a flow rate of 55 ml/h. The proteins were eluted with a linear (24 h) gradient of 0 to 500 mM NaCl in Buffer C produced by gradient mixer (LKB, Bromma, Sweden). The eluate was monitored with an Uvicord S UV monitor (LKB, Bromma, Sweden) at a wavelength of 280 nm and the NaCl concentration of each fraction determined with a Digitalmeter Digi 610 (Wissenschaftlich-Technische Werkstätten, Weilheim, FRG). The distribution of vimentin in the eluted fractions (6 ml) was determined by polyacrylamide gel electrophoresis in 6 µl urea, 6% acetic acid (see below). Peak vimentin fractions were combined, diluted 1-fold with Buffer C and applied to a column (1 × 10 cm) of DEAE-Sepharose CI 6B, equilibrated with Buffer C, at a flow rate of 50 ml/h. When all of the sample had been applied, the flow rate was reduced to 20 ml/h and the vimentin eluted with Buffer C containing 500 mM NaCl. The peak protein fractions (2 ml) were combined (total volume ~10 ml), dialysed overnight against Buffer C, divided into aliquots and frozen in liquid nitrogen. Purified [3H]vimentin was stored in liquid nitrogen and all other purified vimentin samples at ~80 °C. The final protein concentration was 1 to 2 mg/ml.

**Polyacrylamide Gel Electrophoresis—** Polyacrylamide gradient slab gel electrophoresis in 6 µl urea, 6% acetic acid or in the presence of SDS was performed as described previously (25). Gels were stained in Coomassie brilliant blue as described previously (29) or by the “quick stain” method (26). Two-dimensional polyacrylamide gels were also stained with silver by the method of Switzer et al. (27) as modified by Oakley et al. (28).

**Protein Determination—** All protein concentrations were determined with the Bio-Rad protein assay kit (Bio-Rad) using crystalline bovine serum albumin as the standard. The absolute amount of vimentin present in a protein sample was determined by polyacrylamide gel electrophoresis in 6 µl urea, 6% acetic acid (25). The Coomassie blue-stained gels were scanned in a Gilford 2400S spectrophotometer and the amount of vimentin determined by reference to a standard calibration curve of vimentin concentration plotted against Coomassie blue-staining intensity. In all cases, the amount of protein applied to the gel was directly proportional to the staining intensity of the protein bands (25).

**Fig. 1. Flow diagram of the protocol for the purification of vimentin from EAT cells extracted with Triton X-100. Details are under “Materials and Methods.”**
RESULTS

Extraction of Cells in the Presence of Triton X-100—Figs. 1 and 2 outline the two schemes used for the purification of vimentin from EAT cells. One of these (Fig. 1) has been based on our previous studies on the optimum conditions for the incorporation of vimentin into residual cell structures following extraction of EAT cells with Triton X-100 (21). The results of these studies showed that vimentin could be enriched in residual cell structures by extracting cells in a low ionic strength buffer containing 4 mM MgCl₂ and 0.5% Triton X-100. Table I shows that under these conditions approximately 12% of the total cellular vimentin was solubilized and that the remainder was retained in the residual cell structures. This resulted in a 4-fold purification of the vimentin.

As described in an accompanying paper (22), there is a Ca²⁺-activated proteinase present in the cell which has a high substrate specificity for vimentin. Therefore, it was also important to define the extraction conditions which solubilized the proteinase. Fig. 3 shows that the detergent extraction of EAT cells with buffer containing 4 mM MgCl₂ with or without KCl resulted in only a small amount of the proteinase being retained with the residual cell structures. However, when the extraction was performed in the absence of Ca²⁺-chelating agents (EGTA) or in the presence of additional 0.3 mM CaCl₂, substantial amounts of the proteinase were not solubilized. The removal of the proteinase from the vimentin fraction resulted in a higher recovery of vimentin and reduced the problem of removing degradation products in the later stages of purification.

![Flow diagram of the protocol for the purification of vimentin from EAT cells extracted under hypotonic buffer conditions. Details are under “Materials and Methods” (see also Ref. 22).](image)

![Table I: Purification of vimentin from 60 g of EAT cells extracted with Triton X-100 (see Fig. 1).](table)

<table>
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<tr>
<th>Protein Fraction</th>
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<th>Protein Concentration</th>
<th>Vimentin Concentration</th>
<th>Total Vimentin</th>
</tr>
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<td>Triton X-100 supernatant</td>
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<td>3.5 mg/ml</td>
<td>0.02 mg/ml</td>
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<tr>
<td>Residual cell structures</td>
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<td>6.0 mg/ml</td>
<td>0.67 mg/ml</td>
<td>80.4 mg</td>
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<tr>
<td>Nuclei</td>
<td>120 ml</td>
<td>2.5 mg/ml</td>
<td>0.05 mg/ml</td>
<td>6.0 mg</td>
</tr>
<tr>
<td>Postnuclear supernatant</td>
<td>350 ml</td>
<td>1.2 mg/ml</td>
<td>0.21 mg/ml</td>
<td>73.5 mg</td>
</tr>
<tr>
<td>Triton X-100 supernatant</td>
<td>30 ml</td>
<td>2.4 mg/ml</td>
<td>1.39 mg/ml</td>
<td>41.7 mg</td>
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</table>

In order to separate the vimentin from the nuclei, the residual cell structures were homogenized in a low ionic strength buffer in the absence of MgCl₂. Table I shows that under these conditions approximately 90% of the vimentin was recovered in the postnuclear supernatant. Furthermore, vimentin was the major protein species in the extract (Fig. 6A). This step resulted in a further 1.5-fold purification. The major contaminating protein had a molecular weight of 43,000 and was tentatively identified as actin. Most of the actin and many other contaminating proteins could be removed from the vimentin fraction by making the postnuclear supernatant 23% saturated with (NH₄)₂SO₄. Following centrifugation, the supernatant contained only 3% of the vimentin, the remainder was in the pellet. This step resulted in a further 3-fold purification.

The vimentin-enriched fraction was extracted by two cycles of dissolving the pellet in Buffer B by sonication and then...
Purification of the Intermediate Filament Protein Vimentin

reprecipitating vimentin with 23% saturated (NH₄)₂SO₄. Although vimentin was lost during this procedure (Table I), many of the minor protein contaminants were removed (Fig. 6A) as were the last traces of the Ca²⁺-activated proteinase (data not shown).

Following the final (NH₄)₂SO₄ precipitation step, the vimentin comprised 57% of the total amount of protein in solution (Table I).

**Extraction of Cells in the Absence of Triton X-100—**EAT cells from a 20-liter culture (~50 g) were disrupted under hypotonic buffer conditions whereby both the vimentin and the Ca²⁺-activated proteinase were solubilized. Details of the extraction procedure and the removal of the nuclei, membranes, and ribosomes by centrifugation (Fig. 2) are presented in an accompanying paper (see Ref. 22). Because of the many proteins and nucleic acids that were present in the samples (see Fig. 6B), it was not possible to determine the recovery of vimentin after the various centrifugation steps. However, a qualitative analysis of the SDS polyacrylamide profiles of samples of these various steps showed that some vimentin was lost during the centrifugation of the nuclei and the membranes (Fig. 6B).

To obtain a vimentin-enriched fraction that was free of the Ca²⁺-activated proteinase, the postnuclear supernatant was made 23% saturated with (NH₄)₂SO₄. The vimentin was quantitatively recovered from the precipitate (data not shown) and further purified by two cycles of dissolving the sample in Buffer B by sonication and reprecipitating it with 23% saturated (NH₄)₂SO₄ as described above. The final precipitation resulted in a vimentin-enriched fraction, in which the major contaminating proteins were a doublet with molecular weights of 54,000 and 52,000, tentatively identified as tubulin (Fig. 6B). The total amount of protein in this sample was 170 mg of which 23% (38.4 mg) was vimentin. The Ca²⁺-activated proteinase was further purified as described in an accompanying paper (22).

![Fig. 4](image_url)

**FIG. 4.** DEAE-Sepharose Cl 6B chromatography of the delipidated vimentin-enriched fraction from EAT cells extracted under hypotonic buffer conditions (Fig. 2). 170 mg of protein in 20 ml of Buffer C was loaded onto a column (2 x 20 cm) of DEAE-Sepharose Cl 6B equilibrated with Buffer C. Proteins were eluted with a linear gradient of 0 to 500 mM NaCl in Buffer C as described under "Materials and Methods." The determination of the distribution of vimentin in the eluted fractions and the concentration of protein was performed as described in the legend to Fig. 4.

**Column Chromatography—**Hereafter, the vimentin-enriched fractions from cells extracted in the presence or absence of Triton X-100 were treated identically (Figs. 1 and 2).

Following dialysis and lyophilization, the vimentin sample was delipidated by extracting the powder five times with 30-mI portions of chloroform:methanol (2:1) by sonication at 2°C. It was then dissolved in Buffer C and applied to a column of DEAE-Sepharose Cl 6B. All vimentin was bound to the matrix and could then be eluted as a sharp peak between 70 and 100 mM NaCl (Figs. 4 and 5). The distribution of vimentin in the eluted fractions was determined by polyacrylamide gel electrophoresis in 6 M urea, 6% acetic acid (Fig. 4). Peak vimentin fractions were combined, diluted 1-fold with Buffer C and applied to a small column of DEAE-Sepharose Cl 6B. The vimentin was eluted with 500 mM NaCl in Buffer C. Approximately 90 to 99% of the vimentin was recovered (data not shown). This method of protein concentration was preferred since the recovery of vimentin following concentration by (NH₄)₂SO₄ precipitation or ultrafiltration on an Amicon membrane was very low. Fig. 6A and B show that the resulting vimentin preparation contained only one protein species. The purified vimentin was also analysed by twodimensional polyacrylamide gel electrophoresis (Fig. 7) followed by staining with silver; only one major protein was found. Occasionally, it was just possible to identify two degradation products of vimentin with molecular weights of 56,000 and 54,000, respectively, however these proteins constituted less than 1% of the total amount of protein.

The final vimentin preparation, from EAT cells extracted with Triton X-100, was purified over 33-fold with a 39% yield compared to the sum of the vimentin in the Triton X-100 supernatant and residual cell structure fractions. From cells of a 20-liter EAT cell culture, extracted under hypotonic conditions, 25.6 mg of vimentin were purified, which was a slightly lower yield than obtained by the detergent-extraction method, assuming that there was a similar recovery of protein during the purification procedure.

**[³H]Vimentin—**The purification of [³H]vimentin was identical to the method described above for EAT cells extracted with Triton X-100. The specific activity was 8 x 10⁵ cpm/mg purified vimentin.

**Partial Characterization of the Purified Vimentin—**The subunit molecular weight of vimentin was 58,000 as deter-
determined by SDS polyacrylamide gel electrophoresis (Fig. 6, A and B). However, when the purified vimentin was centrifuged on a sucrose density gradient in the presence of EDTA and at low ionic strength, it sedimented as a single, sharp peak with an apparent sedimentation coefficient of approximately 8 s (Fig. 7). Furthermore, the purified vimentin was excluded from Sephacryl S-300 in solutions of high and low salt concentra-

tion and in the presence of glycerol (data not shown). These results show that the purified vimentin forms aggregates of a homogeneous size. Up until now, we have been unable to find conditions to solubilize the vimentin in a monomeric form, except in the presence of 6 M urea or SDS.

When analysed by two-dimensional polyacrylamide gel electrophoresis, the purified vimentin migrated as a series of up to 5 isoelectric variants with apparent PI between 5.3 and 5.4 (Fig. 8; for comparison see Ref. 20).

Fig. 9 shows that the purified vimentin is an intermediate filament protein by its cross-reactivity with the highly specific monoclonal antibody, aIFA. This antibody has been shown to cross-react only with intermediate filament proteins (29). The antibody does not cross-react with any other proteins in the Triton X-100-extracted residual cell structure except vimentin and vimentin-derived proteolytic degradation products. The latter were shown to have originated from vimentin since degradation of purified vimentin by the specific Ca²⁺-activated proteinase (22) produced an identical set of proteins. Antibodies raised in rabbits against purified vimentin also decorate a filamentous network in cells.²

The uv absorption spectrum of vimentin showed a maximum at 279 nm. The ratio of absorbance at 278 nm to that at

²S. Kühn and P. Traub, unpublished observations.
with the highly α-helical structure of proteins of the k-m-e-f-
strength buffer containing of vimentin from EAT cells was similar to that isolated from 260 nm was duees were present in relatively large quantities in agreement obtained when EAT cells were extracted in a low ionic framework of these conditions are not optimal for the quantitative recovery of vimentin in the residual cell structures (also called the detergent-resistant cytoskeleton (33) and the cytoskeletal framework (34)). The highest recovery of vimentin (87%) was obtained when EAT cells were extracted in a low ionic strength buffer containing 4 mM Mg²⁺ (21). Furthermore, these conditions were optimal for the solubilization of the vimentin-specific, Ca²⁺-activated proteinase. EAT cells were used because they can be easily obtained in large quantities and are relatively rich in vimentin (approximately 3% of the total cellular protein).

Previously, we used a low ionic strength buffer that contained, in addition to Triton X-100, 1.5 mM Mg²⁺ and 10 mM KCl for the preparation of partially purified vimentin (35). However, more recent results have shown that this buffer underwent a chemical change upon storage at 4 °C resulting in a solution that stabilized the intermediate filaments and led to the incorporation of substantial amounts of the Ca²⁺-activated proteinase into detergent-resistant residual cell structures. This buffer, when freshly prepared, solubilized most of the vimentin and virtually all proteinase (21). It is likely that

260 nm was 1.35. This result, together with the relatively low extinction coefficient, indicated that vimentin had a low content of aromatic amino acids. This was confirmed by the amino acid analysis (Table II). The amino acid composition of vimentin from EAT cells was similar to that isolated from porcine eye lens and from birefringent caps from BHK-21 cells (Table II). Glutamic acid, alanine, leucine, and arginine residues were present in relatively large quantities in agreement with the highly α-helical structure of proteins of the k-m-e-f-group (30).

**DISCUSSION**

The insolubility of intermediate filament proteins in buffers of high ionic strength that contain nonionic detergents (31, 32) has made possible their isolation and partial biochemical characterization. However, we have recently shown that many of these conditions are not optimal for the quantitative recovery of vimentin in the residual cell structures (also called the detergent-resistant cytoskeleton (33) and the cytoskeletal framework (34)). The highest recovery of vimentin (87%) was obtained when EAT cells were extracted in a low ionic strength buffer containing 4 mM Mg²⁺ (21). Furthermore, these conditions were optimal for the solubilization of the vimentin-specific, Ca²⁺-activated proteinase. EAT cells were used because they can be easily obtained in large quantities and are relatively rich in vimentin (approximately 3% of the total cellular protein).

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**Table II**

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**Fig. 8.** Two-dimensional polyacrylamide gel electrophoresis of the purified vimentin after DEAE-Sepharose Cl 6B chromatography. The first-dimension electrophoresis was performed in 100-μl capillary tubes containing 5% polyacrylamide and 2% ampholines (pH 4 to 7.5 (LKB)). Isoelectric-focusing was completed after 2 h at maximum settings of 0.1 watt/gel, 2500 V and 2 mA.  The electrophoresis in the second dimension was performed on 9 to 15% gradient polyacrylamide gels as described under "Materials and Methods," except that a stacking gel was not used. Gels were stained with silver by the method of Switzer et al. (27) as modified by Oakley et al. (28).

**Fig. 9.** Cross-reactivity of the monoclonal antibody, aIFA, with proteins extracted from residual cell structures (see also Fig. 6A, slot 4) and with purified vimentin. The protein samples and the polyacrylamide gel electrophoresis in the presence of SDS were performed as described in the legend to Fig. 6 and under "Materials and Methods" except that a homogeneous 9% acrylamide gel was used. The proteins were transferred onto a nitrocellulose filter using the electrophoretic blotting procedure (48). The nitrocellulose filter was incubated with aIFA in Tris-saline containing 0.5 g/100 ml bovine serum albumin. After washing with Tris-saline, the nitrocellulose filter was incubated with a horseradish peroxidase-conjugated IgG preparation (1:1000 dilution in Tris-saline containing 0.5 g/100 ml bovine serum albumin). The nitrocellulose filter was again washed in Tris-saline and then soaked in a freshly-prepared solution containing 100 mg Hanker/Yates, 0.3% (v/v) H2O2 in 100 ml of 200 mM Tris-HCl, pH 7.4, to visualize the protein bands labeled by the antibody. Slots 1 to 3; electrophoretic blotting from a polyacrylamide gel of proteins extracted from the residual cell structure (slot 1), purified vimentin (slot 2) and rabbit skeletal muscle actin (slot 3); slots 4 to 7, an identical polyacrylamide gel as used for the electrophoretic blotting was stained with Coomassie blue; proteins extracted from the residual cell structure (slot 4), purified vimentin (slot 5), rabbit skeletal muscle actin (slot 6) and molecular weight markers (slot 7; for details see legend to Fig. 6A, slot M). Note that only vimentin (V) and vimentin-derived degradation products (arrows) are labeled.
the oxidation of Triton X-100 or Tris during long exposures of the buffer to oxygen and fluorescent light (36, 37) is responsible for the filament stabilizing effect in solution of low ionic strength and Mg²⁺-concentration.

The vimentin incorporated into the residual cell structures was in a filamentous form (38) and could be solubilized, and thus separated from the nucleus, by treatment with a low ionic strength buffer in the absence of Mg²⁺ and Triton X-100. The vimentin was released into the supernatant with the remaining polysomes, actin, and the boundary lamina (38). To obtain a vimentin-enriched fraction, this postnuclear supernatant was made 23% saturated with (NH₄)₂SO₄.

A vimentin-enriched fraction could also be obtained, by using an identical method, from the postribosomal supernatant of EAT cells extracted under hypotonic buffer conditions in the absence of Triton X-100. This second extraction procedure was only used when the Ca²⁺-activated proteinase was purified, since the recovery of vimentin was lower than by the extraction procedure using Triton X-100.

The property of vimentin to precipitate from a 23% saturated (NH₄)₂SO₄ solution seems to be general for other intermediate filament protein species in this fraction (approximately 50 to 60% of the total protein from Triton X-100-extracted EAT cells), this method can also be used for the screening of various cell lines and tissues for intermediate filament proteins. Furthermore, the detection of vimentin can be improved by analysing the proteins by polyacrylamide gel electrophoresis in 6 M urea, 6% acetic acid as buffer system (25).

The major contaminating protein in the vimentin-enriched fraction from the detergent-treated EAT cells was actin. We have previously shown that actin is associated with the boundary lamina of the residual cell structures and does not sediment with vimentin in sucrose density gradients (38). Therefore, it is unlikely that actin interacts directly with vimentin. The vimentin-enriched fraction from EAT cells extracted under hypotonic conditions was contaminated with tubulin and only traces of actin. There is some morphological evidence that microtubules and intermediate filaments may be associated (39, 49). This tubulin-contaminated, vimentin-enriched fraction may, therefore, be a useful starting point for the partial purification of vimentin from EAT cells.

The purification of vimentin was achieved by a single chromatography step on DEAE-Sepharose Cl 6B in 6 M urea. This simple procedure was possible because of the relatively high purity of the vimentin following the final precipitation with 23% saturated (NH₄)₂SO₄. Furthermore, by extracting the lipids with chloroform/methanol (2:1), the vimentin eluted from the DEAE-Sepharose Cl 6B as a single sharp peak, well separated from other contaminating proteins. It was found that when a vimentin sample contaminated with lipids was applied to a DEAE-Sepharose Cl 6B column, the vimentin eluted as a broad band that contained many other proteins including actin. Furthermore, when a vimentin sample which contained lipids was centrifuged on a KBr-density gradient to equilibrium, approximately 50% of the filament protein remained on top of the gradient as a white film. Following delipidization with chloroform/methanol, all the vimentin entered the gradient (data not shown). An analysis of the lipids associated with vimentin and their effect on filament assembly will be presented elsewhere. Following the chromatography step, the vimentin was judged to be 98% pure and free of other proteins such as actin, high molecular weight proteins, and the 68,000-dalton protein noted in previous studies (19).

That the purified vimentin was readily degraded by the purified, vimentin-specific, Ca²⁺-activated proteinase with the production of identical degradation products, as observed in the case of the original native vimentin, demonstrates its complete renaturation after removal of urea.

The vimentin was purified over 33-fold with a yield from detergent-treated EAT cells of 39%. It is not possible to compare these results with any other vimentin purification scheme since the recovery of vimentin was not given in the only purification method published so far (20).

The purified vimentin was highly aggregated and was excluded from Sepharcl S-300. Analysis by sucrose density gradient centrifugation at low ionic strength and in the presence of EDTA showed that the vimentin had a sedimentation coefficient of 8 s. Furthermore, the solubilization of vimentin in a monomeric form was only possible in urea and SDS. Under these denaturing conditions, vimentin migrated as a monomer in the course of gel permeation chromatography, with an apparent molecular weight of 58,000 similar to that reported by others (16, 20, 41, 42). In two-dimensional polyacrylamide gel electrophoresis, the vimentin migrated as several isoelectric variants with the same molecular weight. Up to five variants were observed following staining with silver, and these variants were collected following staining with Coomassie brilliant blue. Previous studies have shown that these isoelectric variants of vimentin are due to different degrees of phosphorylation (43-46).

The purification procedures described here are relatively simple and result in a good recovery of vimentin from EAT cells. The first method, whereby cells are extracted with Triton X-100, is generally preferred since it results in a better recovery of vimentin. Furthermore, this method can be used for the screening of cells for the presence of vimentin and the simple preparation of large quantities of radioactively labeled vimentin. A second procedure has also been worked out for the parallel purification of the Ca²⁺-activated proteinase and vimentin. These results provide systems which will permit a full biochemical characterization of vimentin and a detailed examination of its interaction with other cellular constituents, such as nucleic acids, which is now under investigation and which will be published in the near future.

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Purification of the Intermediate Filament Protein Vimentin

Purification of the intermediate filament protein vimentin from Ehrlich ascites tumor cells.

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