A Synthetic Peptide Representing the Consensus Sequence Motif at the Carboxy-terminal End of the Rod Domain Inhibits Intermediate Filament Assembly and Disassembles Preformed Filaments

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Abstract. All intermediate filament (IF) proteins share a highly conserved sequence motif at the COOH-terminal end of their rod domains. We have studied the influence of a 20-residue peptide, representing the consensus motif on filament formation and stability. Addition of the peptide at a 10–20-fold molar excess over keratins K8 plus K18 had a severe effect on subsequent IF assembly. Filaments displayed a rough surface and variable diameters with a substantial amount present in unravelled form. At higher peptide concentration (50–100-fold molar excess), IF formation was completely inhibited and instead only loose aggregates of "globular" particles were formed. The peptide also influenced preformed keratin IF in a dose-dependent manner. While a three-fold molar excess was sufficient to cause partial fragmentation of IF, a 50-fold molar excess caused complete disassembly within 5 min. Loosely associated protofibrils, short needlelike IF fragments, and aggregates of globular particles were detected. The motif peptide also caused the disassem-

bly of filaments formed by desmin, a type III IF protein. Peptide concentrations and incubation times required for complete disassembly were somewhat higher than for the filaments containing K8 plus K18. A 50-fold molar excess was sufficient to cause complete disassembly within 1 h. Peptides unrelated in sequence to the motif did not interfere with filament formation or stability even when present for more than 12 h at a 100-fold molar excess.

The results suggest that the motif sequence normally binds to a specific acceptor site for which the motif peptide can successfully compete. Taken together with current models of IF structure the results indicate that normal binding of the motif sequence to its acceptor must play an essential role in IF formation, possibly by directing the proper alignment of neighboring tetramers or protofilaments. Finally we show that in vitro formed IF are much more sensitive and dynamic structures than previously thought.

INTERMEDIATE filaments (IF)[1] in addition to microfilaments and microtubules are major structures of the cytoskeleton. IF are composed of different but related structural proteins which show remarkable cell type–specific expression patterns (Moll et al., 1982; Osborn and Weber, 1983; Quinlan et al., 1985). Based on amino acid sequences, five different classes of vertebrate IF proteins have been defined: acidic keratins (type I), neutral to basic keratins (type II), desmin, vimentin, glial fibrillary acidic protein, and peripherin (type III), and neurofilament proteins NF-L, NF-M, and NF-H (type IV). The nuclear lamins are usually considered as type V (Steinert and Roop, 1988).

All IF proteins share a common structural principle: a central α-helical rod domain of ∼310 amino acids (∼350 for nuclear lamins and invertebrate cytoplasmic proteins), displaying long heptad repeat patterns, is flanked by non-α-helical head and tail domains which can vary strongly in size and sequence (Geisler and Weber, 1982; Steinert et al.,

1983, 1985; Hanukoglu and Fuchs, 1983; McKeon et al., 1986; Fisher et al., 1986; Weber et al., 1988). Despite their sequence divergence, all classes of IF proteins share two highly conserved consensus sequences. These are located at the NH₂-terminal and the COOH-terminal ends of the rod domain. The mechanisms leading to polymerization of IF proteins are complex and not yet fully understood. In the first step, IF polypeptides interact via their α-helical domains to form coiled coil dimers with the two chains arranged parallel and in register (Parry et al., 1985; Quinlan et al., 1986). In case of the obligatory hetero-polymeric keratins the coiled-coil is a heterodimer containing one type I and one type II keratin (Hatzfeld and Franke, 1985; Hatzfeld and Weber, 1990a; Coulombe and Fuchs 1990; Steinert, 1990). Association of two coiled coil molecules into tetramers requires only sequences of the rod domain. Several studies have suggested that the coiled coil molecules in the tetramer are arranged in an anti-parallel fashion and are staggered (Geisler and Weber, 1982; Quinlan et al., 1984; Ip et al., 1985; Parry et al., 1985; Geisler et al., 1985; Fraser et al., 1985; Stewart

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1. *Abbreviation used in this paper:* IF, intermediate filaments.

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et al., 1989). Tetramers assemble into a series of higher order structures, the protofilaments of 2–3 nm diameter, the 4.5-nm protofibrils and the 8–10-nm filaments (Aebi et al., 1983). These steps depend on the rods but involve an additional contribution of the non-α-helical head domains, since proteolytical removal (Kaufman et al., 1985) as well as cDNA deletion mutagenesis and transfection studies (Lu and Lane, 1990; Raats et al., 1990) indicate that IF proteins lacking the NH₂-terminal head domain are assembly incompetent. The non-α-helical tail domains seem not to play an obligatory role during in vitro filament assembly (Kaufman et al., 1985; Hatzfeld and Weber, 1990b) although they may have some specific function in vivo (Lu and Lane, 1990; Wong and Cleveland, 1990; Gill et al., 1990).

Efforts to characterize specific sequences of the rod domain essential in one of the polymerization steps have used mutagenesis of cDNA clones. Deletion mutagenesis and transfection studies indicate that the highly conserved sequences of the rod located at the NH₂-terminal end of coil 1a and the COOH-terminal end of coil 2 are essential for polymerization in vivo (Albers and Fuchs, 1987, 1989; Wong and Cleveland, 1990; Gill et al., 1990). Transfection of deletion mutants of keratins, desmin, and neurofilament proteins lacking parts of the conserved sequence motif at the COOH-terminal end of coil 2, in addition to the entire tail domain, result in the disruption of the endogenous IF networks (Albers and Fuchs, 1987; Wong and Cleveland, 1990; Gill et al., 1990). Moreover, similar deletion mutants of keratin 14 coassemble into IF with wild type K5 with a reduced efficiency and reveal a wider diameter and a strong tendency to aggregate in vitro (Coulombe et al., 1990). Point mutations in two of the most highly conserved positions of this consensus sequence lead to a modulation of the assembly properties of keratins 8 and 18 in vitro (Hatzfeld and Weber, 1991). A possible explanation for the assembly properties of these mutant keratins is that the invariant amino acid residues have a specific function in IF assembly. To test directly for a specific binding of the sequence motif to a hypothetical acceptor site, we have studied the influence of a synthetic peptide representing the consensus motif on filament formation and stability. We show that a 20-residue peptide comprising most of the consensus sequence at the COOH-terminal end of the rod inhibits in a concentration dependent manner the in vitro formation of IF from keratins 8 plus 18, typical for simple epithelia, or from desmin, a member of type III IF proteins. Moreover, this peptide leads within a few minutes to a complete disassembly of preformed keratin IF. This remarkable and unexpected sensitivity of the filaments is compatible with structural models of IF in which the consensus sequence binds to a specific acceptor site for which the synthetic peptide can compete. We propose that the normal binding of the consensus sequence to its acceptor is an important step in filament formation and integrity and very likely involves the correct alignment of neighboring tetramers.

Materials and Methods

Purification Procedures

Recombinant keratins K8 and K18 were purified from bacterial cultures as described (Hatzfeld and Weber, 1990a,b, 1991). Desmin was purified from

chicken gizzard (Geisler and Weber, 1980). Protein concentrations were determined according to the method of Bradford (1976).

Peptide synthesis based on F-Moc chemistry was performed on an automatic synthesizer (model 9050; Milligen, Burlington, MA) following the standard program of the manufacturer. After removal of the protecting groups and the resin, the synthetic peptides were purified by reverse phase HPLC using a C18 column. Purities were checked by gas phase sequencing of an aliquot of the C18 peak fractions. Peptide stock solutions were made ∼5 mM in H₂O. Absolute concentrations were calculated from the amino acid analysis of an aliquot.

Electron Microscopical Analysis of In Vitro Assembly and Disassembly

Keratins K8 and K18 were combined in equimolar amounts in 8.5 M urea buffer (8.5 M urea, 10 mM Tris-HCl, pH 7.5, 5 mM EDTA) and diluted to a concentration of 250 μg/ml (i.e., ∼5 μM). Samples were dialyzed overnight at room temperature to keratin filament buffer (50 mM Tris-HCl, pH 7.6, 5 mM EDTA, 1 mM 2-mercaptoethanol). For assembly studies in the presence of peptides a dialysis tubing with a reported molecular weight cut off of 1,000 or 2,000 was used (Spectra/Por; Spectrum Medical Industries, Inc., Los Angeles, CA) both for the control without peptide and the samples containing peptides. Peptides were added at a ∼10–100-fold molar excess over K8 plus K18 before dialysis.

Desmin was diluted to 250 μg/ml in 8.5 urea buffer (8.5 M urea, 10 mM Tris-HCl, pH 7.5, 5 mM EDTA, 1 mM 2-mercaptoethanol) and dialyzed to desmin filament buffer (10 mM Tris-HCl, pH 7.5, 170 mM NaCl, 1 mM 2-mercaptoethanol) overnight at 4°C. Peptides were added at a ∼10–100-fold molar excess and dialysis was carried out using membranes with a molecular weight cut off of 1,000 or 2,000.

Filaments reconstituted by overnight dialysis were used in disassembly studies. Aliquots were taken as control and processed for negative staining. To another set of aliquots peptide was added from stock solutions to give final concentrations between 12.5 and 500 μM (i.e., a 2.5–100-fold molar excess of peptide). Samples were incubated at room temperature and aliquots were taken after 5', 10', 15', 30', 60', 3h, 6h, and after more than 16 h.

Structures formed were analyzed after negative staining with 2% uranylacetate as described (Hatzfeld and Franke, 1985; Hatzfeld and Weber, 1990a,b). The peptide itself did not form any recognizable structures detectable after negative staining at concentrations up to 500 μM. To show that proteolysis did not occur during the prolonged treatments of filaments, protein samples were tested before and after the incubation by SDS-PAGE. No degradation products were detected.

Results

Design of Synthetic Peptides

All cytoplasmic IF proteins and nuclear lamins so far sequenced show at the COOH-terminal end of their rod domains the sequence

−18		−16		−12		−8			−4		−1 +1		+4
K	M	A	L D I	E I A T	Y	R K L	L	E G E	E S R	I			

or a very closely related sequence. Owing to irregularities at the very end of the rods in certain type I keratins, the consensus motif is best counted using the second to last E as position −1. The motif has at least four strictly invariant residues (K-18; E-12; Y-8; L-4) and three nearly invariant residues where only one highly homologous replacement is known (L-15; I-11; L-5). Except for some keratins I, positions −1,1, and 3 are also well conserved. In the various sequence presentations found in the literature, positions −18, −15, −11, −8, −4, and −1 are thought to be interior or a and d positions of the presumptive coiled-coil extending to the COOH-terminal end of the rod domain (for a recent review see Fig. 1 in Hatzfeld and Weber, 1991).

The synthetic peptide C L E L E I A T Y R K L L E G E E S R I (peptide 1) covers 20 residues of the motif including
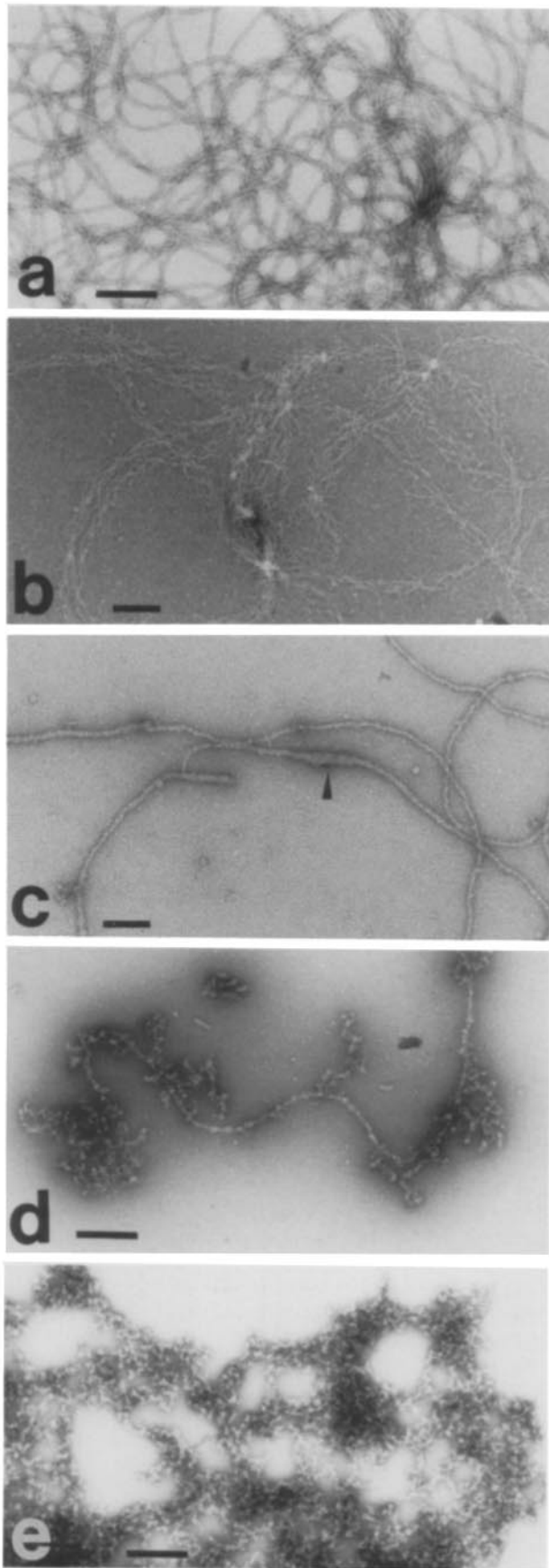
three strictly invariant and all nearly invariant residues. Except for positions −16, −14, and −13 which are highly conservative replacements, peptide 1 follows the sequence of hamster vimentin (Quax et al., 1983). The shorter synthetic dodecapeptide I A T Y R K L L E G E E (peptide 2) represents exactly the hamster vimentin sequence and covers three strictly invariant and two nearly invariant residues. The proline containing synthetic peptide C P E L E P A T P R K L P E G E E S R I (peptide 3) differs from consensus peptide 1 by the introduction of four prolines.

All synthetic peptides were purified by reverse phase HPLC and their purity (>95%) ascertained by gas phase sequencing. Additional control peptides were the 18 residue peptide S T V H E I L C K L S L E G D H S T (peptide 4) and the 20 residue C M K G A G T D E D V L I E I L A S R T (peptide 5). Both peptides are unrelated to IF proteins. Peptide 4 covers the NH_2-terminal residues of annexin II. Its first twelve residues form after acetylation of the NH_2-terminal serine the binding site for the annexin II ligand p11 (Becker et al., 1990). Peptide 5 is a consensus sequence from the second repeat segment of annexins (Gerke, 1990). In the crystal structure of annexin V such sequences start within helix *a* and extend to the COOH-terminal end of helix *b* (Huber et al., 1990).

Inhibition of Keratin Filament Formation in the Presence of the Consensus Peptide 1

Equimolar amounts of keratins K8 and K18 were mixed in 8.5 M urea and peptide 1 was added in a ∼10–100-fold molar excess (total keratin concentration 250 μg/ml). Mixtures were dialyzed using special dialysis tubing with a low molecular weight cut off (see Materials and Methods) against standard keratin IF buffer (50 mM Tris-HCl, pH 7.6 containing 1 mM 2-mercaptoethanol and 5 mM EDTA) at room temperature and the structures formed were analyzed by negative staining. Whereas K8 and K18 assembled into normal IF under these conditions (Fig. 1 *a*), assemblies in the presence of the peptide did not yield normal IF. Different structures were detected, depending on the concentration of the peptide present during polymerization: at rather low peptide concentrations (∼10-fold molar excess over the sum of K8 and K18) a few regular filaments were still formed but a considerable part of the sample consisted of filaments in an unraveled

Figure 1. Electron micrographs of structures assembled by equimolar amounts of keratins K8 plus K18 (total protein concentration 0.25 mg/ml) in the presence of increasing concentrations of the 20 residue consensus peptide 1. Samples dialyzed from 8.5 M urea into 50 mM Tris-HCl, pH 7.6, 1 mM 2-mercaptoethanol were analyzed by negative staining with 2% uranyl-acetate. (*a*) Typical keratin IF assembled from K8 plus K18 in the absence of peptide. (*b*) Unravelled filaments formed by K8 plus K18 in the presence of an ∼10-fold molar excess of peptide 1. (*c*) Filaments formed in the presence of an ∼20-fold molar excess of peptide 1. Such filaments have an uneven surface. Thickenings seem to occur at regular intervals. Filament diameters range from ∼10–30 nm. (*d*) Structures formed in the presence of an ∼50-fold molar excess of the peptide. (*e*) Loose aggregates of globular particles obtained in the presence of an ∼100-fold molar excess of peptide. Bars, 0.2 μm.
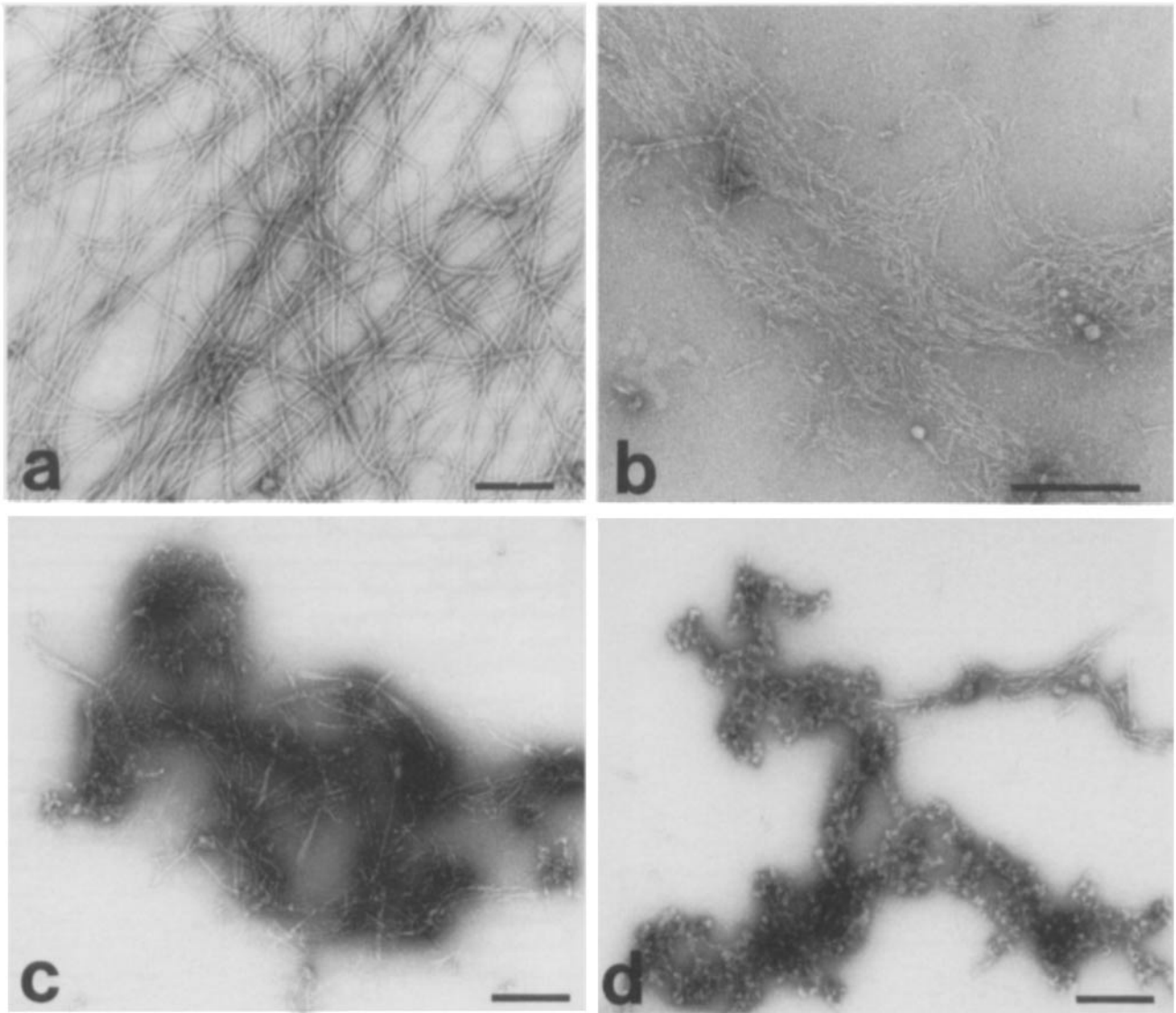
Figure 2. Disassembly of preformed keratin IF by the consensus peptide 1. (*a*) Filaments formed by overnight dialysis of keratins K8 plus K18 against standard keratin filament buffer. (*b–d*) Structures detected after a 5-min incubation of filaments with a ~10-fold molar excess of consensus peptide 1. Loosely associated protofibrillar material (*b*); short (0.1–0.3 μm) and stiff needlelike structures (*c*), and the aggregates of globular structures (*d*) are found. Bars, 0.2 μm.

form (Fig. 1 *b*) similar to the unraveled keratin filaments assembled in the presence of phosphate buffer (Aebi et al., 1983). At a 20-fold molar excess of peptide 1 the filaments that formed showed a drastically altered morphology. They had an uneven surface and very irregular diameters ranging from ~10–30 nm (Fig. 1 *c*). Unraveled filaments were also present (not shown). At a ~50-fold molar excess of peptide, small amounts of fibrous material characterized by an irregular surface was detected (Fig. 1 *d*). Most of the sample was present as small globular particles forming loose aggregates (Fig. 1 *d*). At a 100-fold molar excess of peptide no filaments were detected and instead only large loose aggregates of globular particles were seen (Fig. 1 *e*). These results show that the consensus peptide inhibits keratin filament formation in a concentration dependent manner.

Disassembly of Preformed Keratin Filaments by the Consensus Peptide 1

To analyze whether the peptide can also act on intact fila-

ments, we assembled K8 and K18 into filaments by dialysis and then added an excess of peptide 1. Structures present after different times of incubation at room temperature were monitored by negative staining. Fig. 2 shows an untreated keratin filament control (Fig. 2 *a*) and the result of a 5-min incubation with a ~100-fold molar excess of peptide (Fig. 2, *b–d*). Several aberrant structures were prominently displayed under these conditions. A rather small part of the keratin sample was present as protofilaments and protofibrils resembling unraveled filaments (Fig. 2 *b*). However, the number of protofibrils aligned side by side was much higher than in normal unravelled filaments, for which a number of three to four has been reported (see Aebi et al., 1983; and Fig. 1 *b*). In addition, short and stiff rods with a diameter of 7–10-nm or somewhat less were very clearly displayed. These needlelike structures had a strong tendency to aggregate (Fig. 2 *c*). Finally, loose globular aggregates similar to those obtained during assembly of keratins in the presence of peptide (see Fig. 1 *e*) were the major structures detected.

After incubation times of 15 min or more, only the needle-like structures and the globular aggregates were detected. After 1 h or more, the globular aggregates predominated. Similar structures were not detected when the peptide itself was diluted in keratin filament buffer and analyzed by negative staining.

Filament disassembly was also observed at lower concentrations of the peptide. At a threefold molar excess of peptide 1, filament disassembly was already striking, but not completed within 1 h. Filaments with irregular surface and diameter, most of them present as short fragments (~0.2–0.6 μm length), were readily detected (Fig. 3 a). At a ~12-fold molar excess of peptide, the predominant structures were short stiff IF fragments of variable diameter (Fig. 3 b) resembling the needlelike structures of Fig. 2 c. At a ~50-fold molar excess of peptide, filament disassembly was complete and the loose globular aggregates were the major organizations in the samples (Fig. 3 c).

As a control for the specificity of the interaction between peptide 1 and the keratins we used two different peptides completely unrelated in sequence but similar in length (see above). Peptides 4 and 5 showed no influence on keratin filament formation even when present at a 100-fold molar excess. When preformed keratin filaments were incubated for 5 min to 18 h with a 100-fold molar excess, neither peptide 4 nor peptide 5 had any destructive effect. Electron micrographs documented only ultrastructurally normal keratin filaments (for peptide 5 see Fig. 4 a).

We have also studied the effect of two peptides related to peptide 1. The dodecapeptide 2 lacks the eight NH₂-terminal residues of peptide 1. Incubation of keratin filaments with a 30-fold molar excess resulted within 10 min in unraveling of a substantial part of the filaments (Fig. 4 b). No additional major changes were seen when the incubation time was increased to 1 h or more. At a 120-fold molar excess, the major effect of the peptide 2 was again unraveling of filaments and a modulation of filament diameter and surface. In addition, a few disassembled filaments as well as some regular filaments were detected. Thus peptide 2 is clearly less active than the longer consensus peptide 1. This is also the case for peptide 3, which has the same length as peptide 1 but contains four substitutions of conserved amino acids by proline. When keratin filaments were incubated with a 20-fold molar excess of peptide 3 the filamentous structures were retained after 5 min (Fig. 4 c) and after prolonged times such as 1 h or longer. However, these filaments differed from the control by an enlarged diameter (up to 20 nm instead of ~10 nm) and by an uneven surface. Fig. 4 d shows normal keratin IF at high magnification. Their very even and smooth surface differs distinctly from the uneven surface of filaments treated with peptide 3 (Fig. 4 e). The thickenings present on the latter filaments seemed to have a regular spacing of ~20 nm, suggesting that the peptide binds to the filaments at regular intervals. The increase in filament thickness is probably a indirect consequence of the bound peptide rather than reflecting the direct decoration. When used at 65-fold molar excess, peptide 3 induced disassembly after 1 h (Fig. 4 f). This effect is comparable to that exerted by peptide 1 at a 10-fold molar excess.

Desmin Assembly and Filament Stability in the Presence of the Consensus Peptide 1

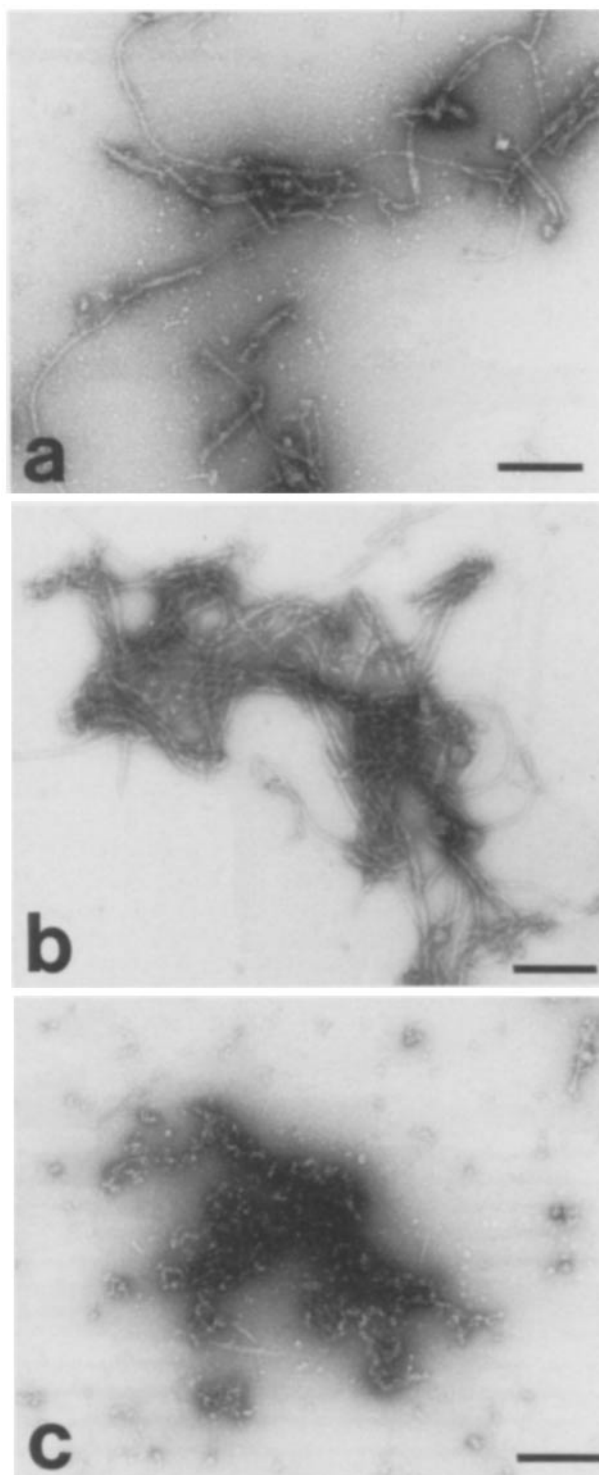To see whether filament disassembly by peptide 1 is a

Figure 3. Electron micrographs of representative structures obtained after incubation of keratin filaments with various concentrations of consensus peptide 1. Filaments were assembled by overnight dialysis of K8 plus K18 against standard filament buffer containing 2-mercaptoethanol. Aliquots of the sample were incubated at room temperature at different peptide concentrations. Structures formed were visualized by negative staining with 2% uranyl acetate. (a) Short and irregular filament fragments with variable diameter and a rough surface obtained after 15 min with approximately threefold molar excess of peptide 1. (b) Short rodlike structures and filament fragments detected after 15-min incubation with a ~12-fold molar excess of peptide. (c) Globular aggregates obtained after 15 min with a ~50-fold molar excess of peptide. Bars, 0.2 μm.
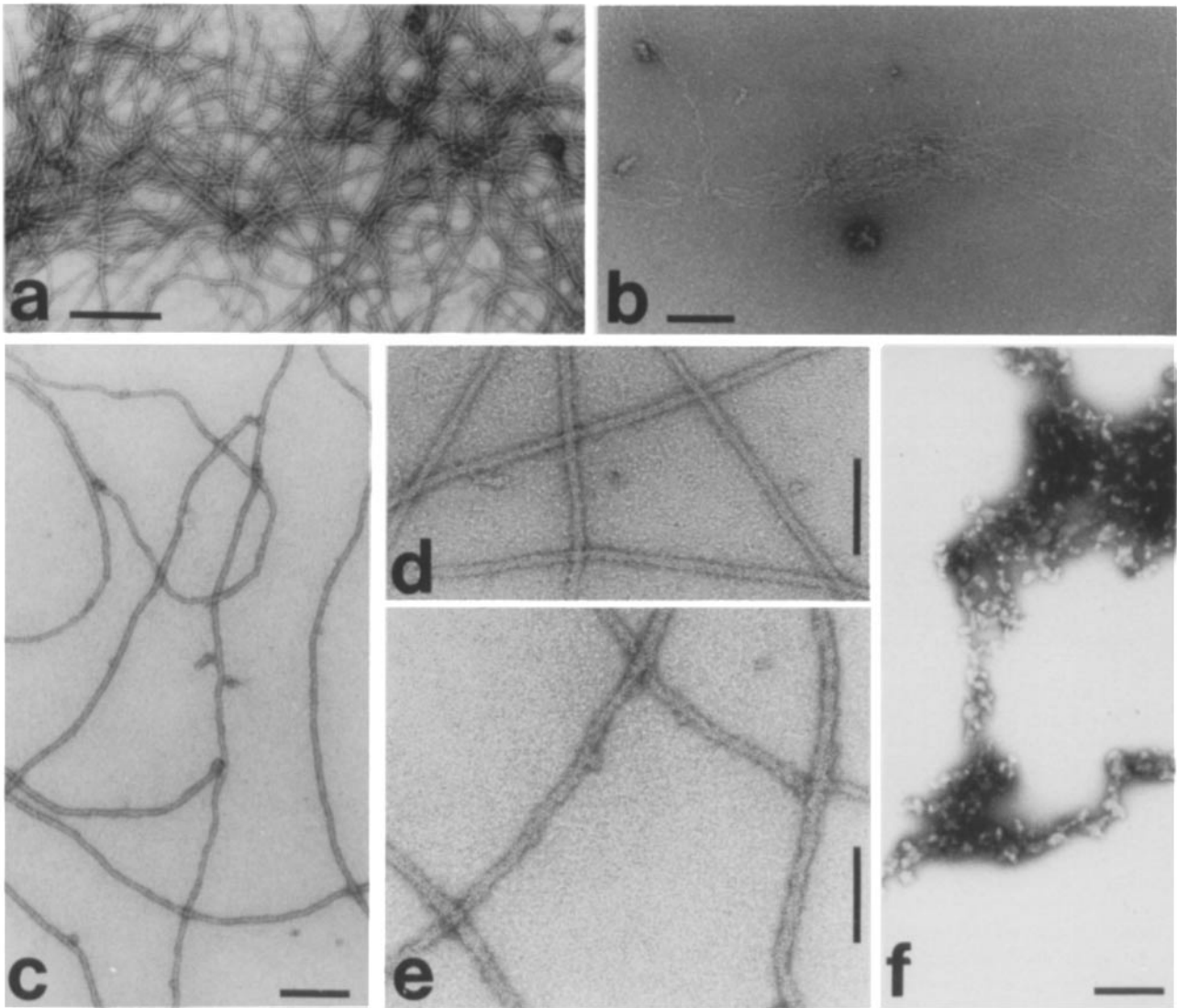
Figure 4. Structures formed after incubation of keratin filaments (0.25 mg/ml) with different peptides. (*a*) Regular IF detected after 1 h of incubation with peptide 5 (100-fold molar excess) which is unrelated to the motif sequence. (*b*) Unravelled keratin filaments detected after incubation with a ∼30-fold molar excess of the 12-residue consensus peptide 2 for 10 min. (*c* and *e*) Keratin filaments after incubation with a ∼20-fold molar excess of the proline containing consensus peptide 3. Filaments treated with peptide 3 have a rough surface and a larger diameter than regular untreated filaments (*d*). (*f*) Structures detected after incubation of keratin filaments with a ∼65-fold molar excess of peptide 3 for 1 h. Bars: (*a–c*, and *f*) 0.2 µm; (*d* and *e*) 0.1 µm.

general property of IF or restricted to filaments containing keratins 8 and 18, the keratins typically found in simple epithelia, we have also analyzed the assembly and stability of desmin IF. Purified chicken desmin was mixed with the 50-fold molar excess of peptide 1 and dialyzed at 4°C using special dialysis tubing from 8.5 M urea into 10 mM Tris-HCl, pH 7.5, 170 mM NaCl containing 1 mM 2-mercapto-ethanol. EM showed the extensive formation of globular aggregates as described above for keratins. In addition, some modulated filaments with a prominent beading pattern of around 20 nm were seen (Fig. 5), probably indicating binding of the peptide to desmin filaments at regular intervals.

Desmin filament stability was also studied. An aliquot of the filaments visualized by negative staining served as control (Fig. 6 *a*). Other aliquots were incubated with various concentrations of peptide 1 at room temperature. A ∼12-fold molar excess of peptide 1 caused the fragmentation of a sub-

stantial amount of the filaments within 15 min (Fig. 6 *b*). After 1 h most of the filaments were converted into the globular aggregates, although a few filaments were still detected (Fig. 6 *c*). A ∼50-fold molar excess of peptide 1 induced after short incubation times (∼5 min) predominantly the formation of needlelike structures (Fig. 6 *d*), which converted after longer incubation times into the globular aggregates (Fig. 6, *e* and *f*). Control experiments with peptides 4 and 5, which are unrelated in sequence to peptide 1 but have a similar length, showed no effect on desmin filament integrity, even when used at a 100-fold molar excess for 5 h (for peptide 4 see Fig. 6 *i*).

The shorter consensus peptide 2 had only a weak effect on desmin filaments. After incubation with a ∼30-fold molar excess for 1 h filaments appeared shorter and partially un-ravelled. They also appeared more irregular than the un-treated control but were clearly not disassembled (Fig. 6 *g*).
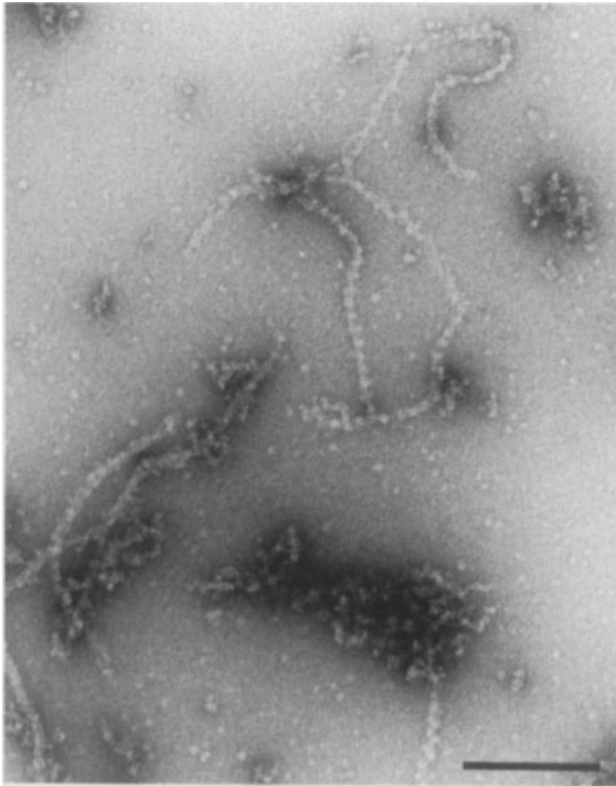
Figure 5. Electron micrograph of structures formed by desmin during assembly in the presence of peptide 1 at a ∼50-fold molar excess. In addition to globular aggregates short and irregular filaments are found. They have a prominent beading pattern at regular 21-nm intervals. Samples were analyzed by negative staining. Bar, 0.2 μm.

Similarly peptide 3, the proline containing homologue of peptide 1, had only a weak effect on desmin filaments. Thus a 65-fold molar excess of peptide 3 did not disassemble preformed filaments. However, the treated filaments seemed shorter in length and displayed in some places a larger diameter than the controls (Fig. 6 *h*).

Discussion

We have used a synthetic peptide of 20 residues which reflects the highly conserved sequence motif at the COOH-terminal end of the rod domains of the IF proteins. This consensus peptide (peptide 1) inhibits in a dose-dependent manner the in vitro assembly of the two IF types tested. It affects the obligatory heteropolymer system of keratins 8 and 18, typically found in simple epithelia, and the homopolymer system of desmin, a member of the type III IF proteins. Consensus peptide 1 acts in a dose-dependent manner on the keratin 8 plus 18 assembly as well as on the assembly of desmin. The full inhibition of filament assembly seen at higher peptide concentrations (50–100-fold molar excess) is specific since two similar sized peptides, which have no sequence homology, do not affect the assembly even when used at higher concentrations.

Although IF isolated by extraction from cells or tissues or formed in vitro are usually considered as rather stable structures unless partial disassembly is induced by very low ionic strength and/or increased pH, peptide 1 leads to a rapid and again dose-dependent disintegration of preformed filaments containing either keratin 8 plus 18 or desmin under standard filament conditions. As the same results are obtained with the type I plus type II keratin system and with the type III IF protein desmin the inhibition of IF formation and the disassembly of preformed filaments by the consensus peptide seems a general feature of IF.

The combined results are consistent with a model in which the consensus motif at the COOH-terminal end of the rod is recognized during filament formation by a specific acceptor site for which the added peptide can compete. Since keratin tetramer formation as assayed by cross-linking experiments in 4 M urea seems not affected by the peptide (results not shown), we tentatively assume that the peptide affects the assembly at a later stage of oligomer formation. There are several possible explanations for the rapid disassembly of filaments induced by the peptide (see the complete disassembly of keratin IF within 15 min or less by a 50-fold molar excess in Fig. 3 *c* and desmin disassembly after 5 min in Fig. 6 *c*). The peptide could act specifically on soluble subunits, e.g., tetramers by blocking their acceptor sites. If such blocked oligomers cannot be incorporated into the filaments, their removal from an equilibrium with the filaments could lead to progressive depolymerization. Alternatively the tetramer-bound peptide could enter the filaments by a rapid subunit exchange along the filaments or it could be directly incorporated into the filaments. A possibly similar observation has been made by Ngai et al. (1990), who followed the in vivo incorporation of newly synthesized vimentin driven by an inducible gene construct into the immunologically distinct endogenous vimentin filaments. They postulated that vimentin assembly can proceed by incorporation of subunits into preexisting filaments at discrete foci without a restriction to the perinuclear area. Their study and the work on mutated IF proteins in transfected cells (Albers and Fuchs, 1987, 1989; Raats et al., 1990; Gill et al., 1990; Wong and Cleveland, 1990) show that the intracellular IF network is more dynamic than originally believed. This point has also been raised by intracellular microinjection of biotinylated vimentin although in this case a vectorial assembly has been favored (Vikstrom et al., 1989). Our results strongly suggest that IF are also dynamic in vitro. Although the molecular events leading to filament disassembly by the added consensus peptide are not yet established, it is interesting to note that STEM shows polymorphism in mass per unit length measurements on various filament preparations (Steven et al., 1983; Eichner et al., 1985; Engel et al., 1985). If the number of protofibrils can vary along a filament the free ends of some protofibrils might represent regions of tetramer and/or peptide incorporation.

Although our results do not yet directly identify the postulated acceptor sites for the consensus motif along the IF protein sequence, this information may be obtained in the future by cross-linking experiments or overlay assays and related procedures on fragments of IF proteins. Current models of IF structure emphasize an antiparallel arrangement of the two dimers of the tetramer with the coil 2 domains protruding without binding partners (Fraser et al., 1985; Geisler et al., 1985; Potschka et al., 1990; Stewart et al., 1989). Upon polymerization into IF, tetramers align laterally and longitudinally. The contribution of specific domains required in this process are not yet defined. In the various models, the con-
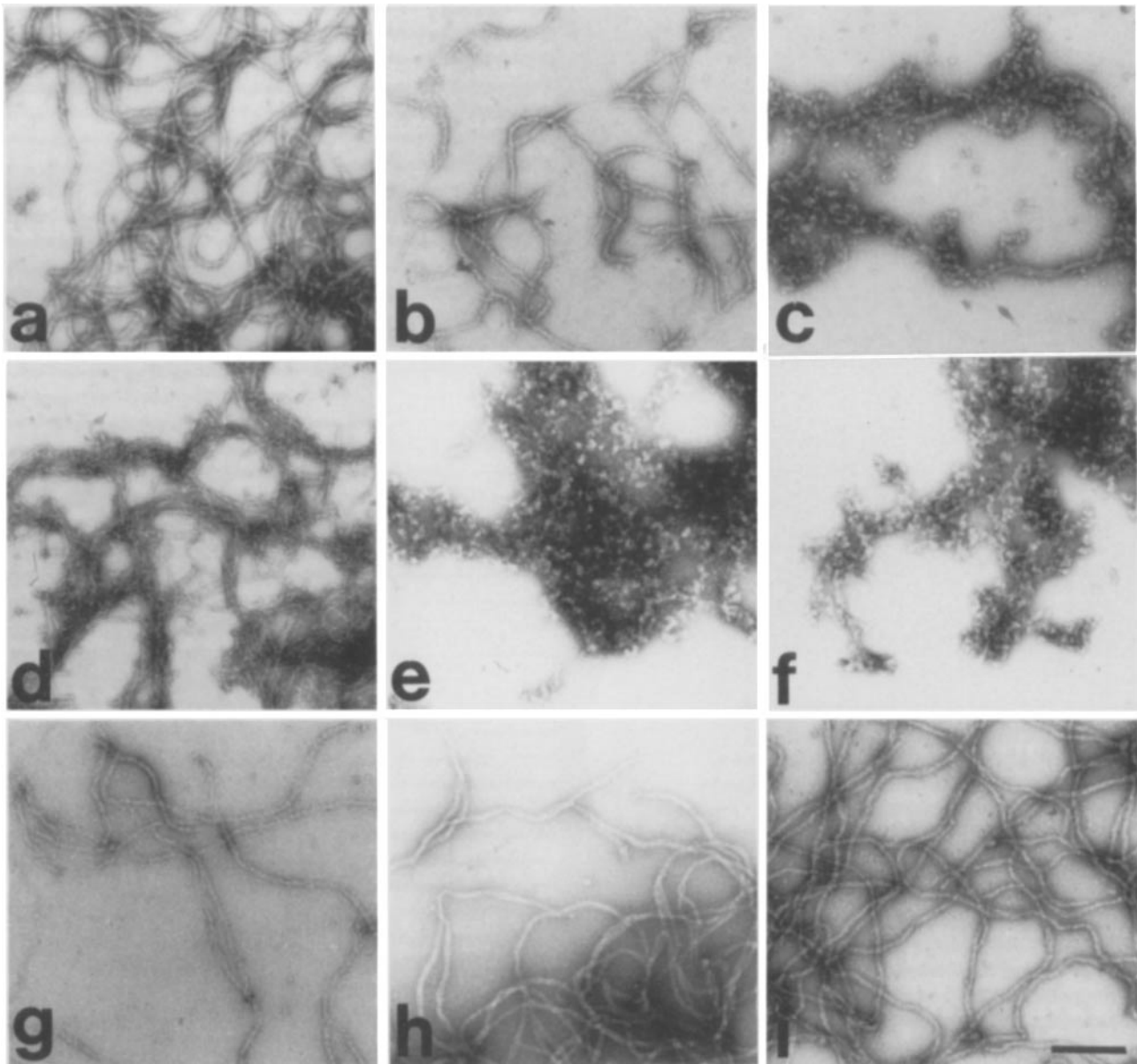
Figure 6. Electron micrographs of representative structures obtained after incubation of desmin filaments with the consensus peptide 1. Structures were visualized by negative staining with 2% uranyl acetate. (*a*) Regular filaments assembled from purified desmin (0.25 mg/ml) by dialysis from 8 M urea against 10 mM Tris-HCl, pH 7.5, 170 mM NaCl, 1 mM 2-mercaptoethanol at 4°C. (*b*) Filament fragments detected in the same sample after a treatment with a ∼12-fold molar excess of peptide 1 for 15 min. (*c*) Globular aggregates formed after an incubation for 1 h with ∼12-fold molar excess of peptide 1. (*d–f*). Structures detected after incubation of desmin filaments with a ∼50-fold molar excess of peptide 1 for different times. Samples for negative stain were taken after 5 min (*d*), 15 min (*e*), and 60 min (*f*). (*g*) Partially unravelled desmin filaments detected after treatment with a ∼30-fold molar excess of the 12-residue peptide 2 for 1 h. (*h*) Desmin filaments detected after incubation with a ∼30-fold molar excess of the proline containing peptide 3 for 1 h. (*i*) Desmin filaments detected after incubation with a ∼65-fold molar excess of the unrelated peptide 4 for 1 h. Bars, 0.2 μm.

sensus motif at the COOH-terminal end of the rod could have to face another well conserved sequence. In the model of Stewart et al. (1989) it would be the antiparallelly oriented motif itself which is in contact with the longitudinally and the laterally associated tetramers. In other models it could be the start of coil 2, i.e., the region referred to as coil 2a which is involved in correct lateral alignment (Fraser et al., 1985). In addition elongation could involve an association of the head domains and/or the conserved sequence motif of coil 1a with the consensus sequence. In either case the con-

sensus motif at the COOH-terminal end of the rod would be involved in the proper alignment of the tetramers during elongation and fibril formation. This view is consistent with the results of various mutational studies. Deletions removing parts of the consensus motif lead in transfection to the collapse of the corresponding endogenous network and point mutants introduced into the motif resulted in a modulated filament assembly in vitro. While earlier reports emphasized that a lack of or a change in the motif sequence has deleterious effects on assembly (Albers and Fuchs, 1987; Gill et al.,

1990; Wong and Cleveland, 1990; Hatzfeld and Weber, 1991), our study shows that also the presence of a molar excess of the motif, provided by the added peptide, interferes with filament assembly and stability.

Interestingly, desmin filaments need higher concentrations of the consensus peptide and longer incubation times to disassemble than do filaments containing keratins 8 and 18. This differential sensitivity is particularly pronounced when the filaments are treated with the two variants of the consensus motif, the shorter peptide 2 and the proline containing peptide 3 (see below). Several parameters could be responsible for a higher rate of subunit exchange or an increased accessibility of the acceptor sites in the filaments containing keratins 8 and 18. The higher ionic strength of the assembly buffer necessary for optimal desmin assembly is a variable which cannot be directly evaluated with keratin filaments because of their notorious clumping in the presence of salt. Slight ultrastructural differences between the two filament types are indicated by the observation that the 21-nm beading pattern of glycerol-sprayed and metal-shadowed type III IF seems only poorly displayed by keratin filament preparations including those containing keratins 8 and 18 (Hendersson et al., 1982). This difference, thought to reflect different folding patterns of the non-α-helix terminal domains (Birkenberger and Ip, 1990), could be directly related to the differential sensitivity against peptide 1. In all structural models of IF (see above), the terminal domains, which differ strikingly in type III and keratin IF, are close to the location of the ends of the rod domains. Given the difference in the terminal domains of keratins from stratified and simple epithelia it seems also not surprising that filaments containing epidermal keratins from bovine snout are less sensitive to peptide 1 than the filaments of keratins 8 and 18, which are typical for simple epithelia (data not shown). Alternatively, the heightened sensitivity of keratin 8 plus 18 filaments might be caused by the irregularities in the consensus sequence of certain type I keratins such as keratin 18 (reviewed by Hatzfeld and Weber, 1991), which might destabilize the secondary structure of this domain and facilitate incorporation of the peptide.

We have used two variants of the consensus peptide 1: the proline containing homologous peptide 3, and the shorter peptide 2. At the concentrations used, both peptides have only subtle effects on desmin filaments. They alter the filament morphology but do not cause disassembly. In contrast peptide 3 is still able to disassemble IF containing keratins 8 plus 18 but a tenfold higher concentration is needed than in the case of peptide 1. Since peptides unrelated to the motif sequence have no influence on desmin or keratin filament assembly and stability (see above) the effect of the proline containing peptide 3 on keratin IF opens the question whether the COOH-terminal end of the rod domain is really a true coiled-coil or adopts a less-restricted conformation. A more detailed analysis of the consensus sequence by systematically changed synthetic peptides should answer this question and identify those residues in the motif which are crucial for the binding to the acceptor. The results obtained with peptide 1 invite similar studies with synthetic peptides corresponding to other conserved sequences, such as, for instance, sequences from helix 1a, or sequences conserved only in IF subtypes. Use of such peptides should allow to map some of the interaction sites involved in filament stability.

Note Added in Proof. We have learned from Drs. P. Kouklis and S. Georgatos, EMBL, Heidelberg, Germany, that they have found independently that a peptide representing the consensus motif at the COOH-terminal end of the rod domain inhibits the assembly of vimentin and disassembles preformed vimentin filaments.

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