Molecular Assembly, Secretion, and Matrix Deposition of Type VI Collagen

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Abstract. Monoclonal antibodies reactive with the tissue form of type VI collagen were used to isolate the type VI collagen polypeptides from cultured fibroblasts and muscle cells. Two [^{35}S]methionine-labeled polypeptides of 260 and 140 kD were found intracellularly, in the medium, and in the extracellular matrix of metabolically labeled cells. These polypeptides were disulfide cross-linked into very large complexes. The 260- and 140-kD polypeptides were intimately associated and could not be separated from each other by reduction without denaturation. In the absence of ascorbic acid, both polypeptides accumulated inside the cell, and their amounts in the medium and in the matrix were decreased. These results suggest that both the 260- and the 140-kD polypeptides are integral parts of the type VI collagen molecule. Examination of type VI collagen isolated from the intracellular pool by electron microscopy after rotary shadowing revealed structures corresponding to different stages of assembly of type VI collagen. Based on these images, a sequence for the intracellular assembly of type VI collagen could be discerned. Type VI collagen monomers are ~125 nm long and are composed of two globules separated by a thin strand. The monomers assemble into dimers and tetramers by lateral association. Only tetramers were present in culture media, whereas both tetramers and multimers were found in extracellular matrix extracts. The multimers appeared to have assembled from tetramers by end-to-end association into filaments that had prominent knobs and a periodicity of ~110 nm. These results show that, unlike other collagens, type VI collagen is assembled into tetramers before it is secreted from the cells, and they also suggest an extracellular aggregation mechanism that appears to be unique to this collagen.

TYPE VI collagen is widely distributed throughout connective tissues (13, 24). It has been isolated from aorta and placenta in the form of high molecular weight, pepsin-resistant fragments composed of 50–80-kD polypeptides (3, 8, 9, 19, 13, 14, 16, 19, 21). It has also been isolated from these tissues by guanidine hydrochloride extraction as a protein composed of polypeptides ranging from 140 kD (10, 15) up to 190 kD (21). One outstanding feature of both the pepsin fragments and the larger forms of type VI collagen is the extensive interchain disulfide cross-linking. It appears from chemical and morphological analyses of type VI collagen that it contains relatively short triple-helical domains, contributing one-third or less to the total mass of the protein (7, 10, 13, 15, 19).

The molecular architecture (2, 7, 13, 15), the large amount of globular structure, and the extensive disulfide cross-linking in type VI collagen suggest that it is unique among collagens, but little is known about its biosynthesis, processing, and biological function. Type VI collagen is synthesized by fibroblasts and muscle cells in culture and is deposited into their extracellular matrix (11, 13, 22, 24). The type VI collagen immunoreactivity in cell cultures has been attributed to polypeptides of either 140 (2, 4, 24) or 240 kD (21) or both (13).

Because relatively little is known about type VI collagen and because of the conflicting reports in the literature as to its precursor form, we wanted to study its biosynthesis in cell culture. Specifically, we wanted to investigate (*a*) if different cell lines make type VI collagen of different compositions, (*b*) if the 140- and 240-kD polypeptides are monomer and dimer of the same polypeptide or structurally unrelated to each other, and (*c*) if the two chains are part of the same molecule or if the two chains associate into a complex outside the cell. For this purpose we have used monoclonal antibodies in combination with indirect immunofluorescence, SDS PAGE, and electron microscopy after rotary shadowing. Our data confirm our previous observation (13) that type VI collagen molecules are composed of both 140- and 260-[1] kD polypeptides and suggest two unique mechanisms of assembly of type VI collagen: lateral assembly into tetramers before secretion and longitudinal assembly into polymers in the extracellular matrix.

Materials and Methods

Cell Culture

The following cell lines were obtained from the American Type Culture Collection (Rockville, MD) and used in this study: MRC-5 (human lung

[1] Because the fibronectin subunit is now known to be ~250 kD, the longer polypeptide of type VI collagen, previously estimated to be 240 kD, has been re-estimated to be 260 kD based on their respective mobilities in SDS PAGE.

fibroblast), MG63 (osteogenic sarcoma), SK LMS-1 (leiomyosarcoma), RD (rhabdomyosarcoma), and L6 (rat myoblast). Stock cultures of cells were propagated in Eagle's minimum essential medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. In most experiments, the cells were cultured in the presence of 50 μg/ml ascorbic acid (J. T. Baker Chemical Co., Phillipsburg, NJ); in other experiments, the ascorbic acid was omitted or 80 μg/ml α,α-dipyridyl (Sigma Chemical Co., St. Louis, MO) were added.

Antibodies

Several monoclonal antibodies to type VI collagen have been partially characterized and described previously (13). An additional monoclonal antibody to type VI collagen, 3C4, was obtained through another project (our unpublished work). Rabbit antiserum was produced by immunization with the pepsin-resistant domains of type VI collagen purified from placenta using chromatography on monoclonal antibody (13). The antiserum was adsorbed with human serum proteins and mouse IgG coupled to Sepharose. Some of the characteristics of the antibodies are summarized in Table I.

Indirect Immunofluorescence

Cells were dissociated with trypsin (100 μg/ml, Sigma type III) and EDTA (20 mM) in phosphate-buffered saline (PBS), pH 7.2, and plated onto glass coverslips. Cells were cultured on the coverslips for 24–48 h in Eagle's minimum essential medium supplemented with 10% fetal bovine serum and antibiotics in the presence or absence of ascorbic acid or α,α-dipyridyl, washed with PBS, and fixed in acetone for 10 min. The cells were washed again in PBS and incubated with antibodies for 1–2 h at room temperature. Monoclonal antibodies were used in the form of hybridoma culture medium, and polyclonal antibodies were used in the form of rabbit antiserum diluted 1:50 in PBS. Bound antibodies were detected by incubating the cells with fluorescein- or rhodamine-labeled antibodies to mouse or rabbit IgG (Cappel Laboratories, Cochranville, PA). The coverslips were mounted on glass slides with 90% glycerol in PBS, and the cells were examined under a Zeiss microscope equipped with epifluorescent optics.

Metabolic Labeling

Cells were dissociated, plated in plastic tissue culture flasks, and cultured in Eagle's minimum essential medium with 10% fetal bovine serum for <24 h. Subconfluent cultures were then incubated in methionine-free Eagle's minimum essential medium with 0.5% serum and 50 μCi/ml [^{35}S]methionine (New England Nuclear, Boston, MA) in the presence or absence of ascorbic acid or α,α-dipyridyl. After 24 h, the medium was collected and to it the following substances were added (final concentrations in parentheses): Tris-HCl, pH 7 (0.05 M), NaCl (0.5 M), Triton X-100 (0.1%), phenylmethylsulfonyl fluoride (0.2 mM), and EDTA (20 mM). The cell layer was then extracted with 0.5% Triton X-100 in 0.05 M Tris-HCl, pH 7, 0.5 M NaCl, 0.2 mM phenylmethylsulfonyl fluoride, and 20 mM EDTA (cell extract). Extraction with Triton X-100 did not affect type VI collagen in the extracellular matrix as evidenced by indirect immunofluorescence. In some experiments, the cells were first detached by trypsin-EDTA, washed with 1 mg/ml soy bean trypsin inhibitor in PBS, and then extracted with Triton X-100 as above. The extracellular matrix remaining after extraction of cell monolayers with Triton X-100 was solubilized with 8 M urea, 0.5 M NaCl, 0.05 M Tris, pH 7, 0.5% Triton X-100, 0.2 mM phenylmethylsulfonyl fluoride. The extract was dialyzed against 0.05 M Tris, pH 7, 0.5 M NaCl, 0.5% Triton X-100, and centrifuged. In some experiments, 10 mM *N*-ethyl maleimide was added to all samples to block free sulfhydryl groups and as an additional protease inhibitor.

Table I. Characteristics of Antibodies to Type VI Collagen

Antibody	Reactivity		
	With pepsin-resistant fragment (16)	With hydroxylated 240 + 140 kD complex	With unhydroxylated 240 + 140 kD complex
2C6	−	+	−
3C4	−	+	+
4B10	+	+	−
5C6	+	+	−
Rabbit antiserum	+	+	±

Reduction and Alkylation

Culture media and cell extracts containing type VI collagen were reduced for 4 h at room temperature in 10 mM dithiothreitol or 100 mM 2-mercaptoethanol at pH 8 with or without 6 M urea. The samples were then alkylated in 100 mM iodoacetic acid at pH 8.5 for 2 h. Reduced and alkylated samples were dialyzed against 0.05 M Tris, 0.5 M NaCl, 0.5% Triton X-100.

Immunoprecipitation

Isolation of type VI collagen from radioactive medium and extracted cells and matrices was done using monoclonal anti-type VI collagen and protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ), saturated with rabbit anti-mouse IgG, as described previously (13) or using rabbit anti-type VI collagen and protein A-Sepharose. SDS PAGE was performed with a discontinuous buffer system (17) and extended electrophoresis on 5% acrylamide gels.

Electron Microscopy

Media, cell extracts, and dialyzed matrix extracts were prepared as described above for the metabolically labeled samples, except on a larger scale. The type VI collagen in these samples was then isolated using monoclonal antibody coupled to Sepharose. After incubation of the sample with the immunoadsorbent for 2 h at room temperature, the immunoadsorbent was packed in a small column, washed with 0.15 M ammonium acetate containing 0.05% octyl-β-glucoside, and eluted with 1 M acetic acid containing 0.05% octyl-β-glucoside. Peak fractions were processed for rotary shadowing essentially as described (13) except that pure tungsten was used for the shadowing. Film was ~2–3 nm thick. Samples were examined in a Hitachi H-600 electron microscope.

Results

Effect of Ascorbic Acid on Secretion and Matrix Deposition of Type VI Collagen

When MG63 cells were cultured in the presence of ascorbic acid, which is an essential co-factor for the prolyl and lysyl hydroxylases (20), the cell-associated type VI collagen was localized predominantly in the extracellular matrix as detected by indirect immunofluorescence (Fig. 1*a*). In contrast, if ascorbic acid was omitted from the culture medium, type VI collagen appeared to accumulate intracellularly as evidenced by intracellular staining using monoclonal or polyclonal antibodies (Fig. 1*b*).

When α,α-dipyridyl, an inhibitor of prolyl and lysyl hydroxylases (20), was added to the cultures, six out of seven monoclonal antibodies as well as the rabbit antiserum (Table I) failed to detect any intracellular type VI collagen and only detected trace amounts of extracellular type VI collagen. Only one monoclonal antibody, 3C4, did give intracellular staining of the α,α-dipyridyl-treated MG63 cells (not shown). Thus, type VI collagen behaves like other collagens in that its intracellular assembly and secretion can be modulated by agents that affect hydroxylation (20). Furthermore, the antibody 3C4 reacts with an epitope of type VI collagen that is formed early in the protein folding process and that is independent of hydroxylation and triple helix formation (Table I).

Biosynthesis and Secretion of Type VI Collagen

To study the synthesis of type VI collagen, MRC-5 cells cultured under different conditions were labeled with [^{35}S]-methionine, and type VI collagen was isolated from media, detergent extracts of cells, and urea extracts of extracellular matrices using antibodies. As reported before, the biosynthetic form of type VI collagen present in culture medium comprised two classes of polypeptides, of 140 and 260 kD, disulfide-bonded into a high molecular weight aggregate (13). Analysis

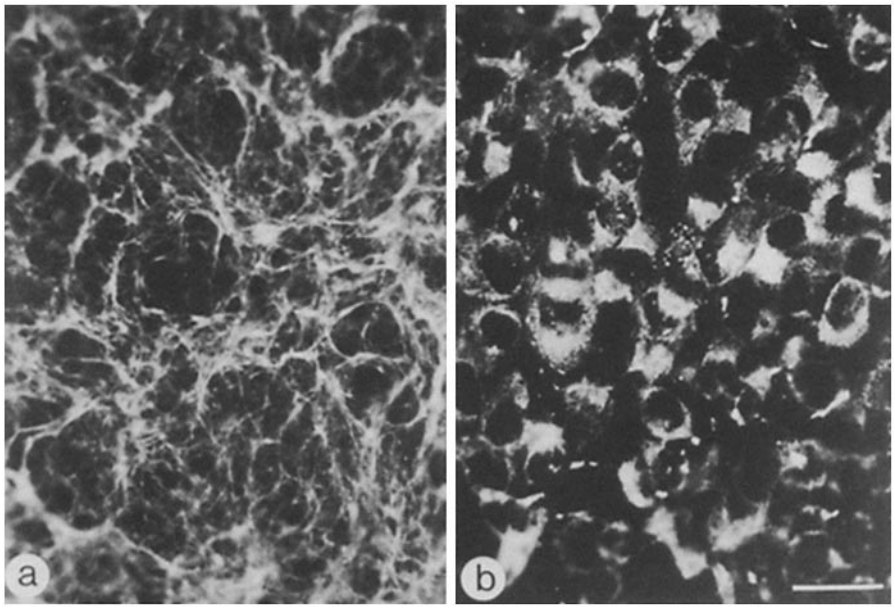

Figure 1. Indirect immunofluorescence on MG63 osteosarcoma cells using monoclonal antibody 5C6. Cells in *a* had been cultured for 24 h with 50 μg/ml ascorbic acid, in *b* without ascorbic acid. Bar, 50 μm.

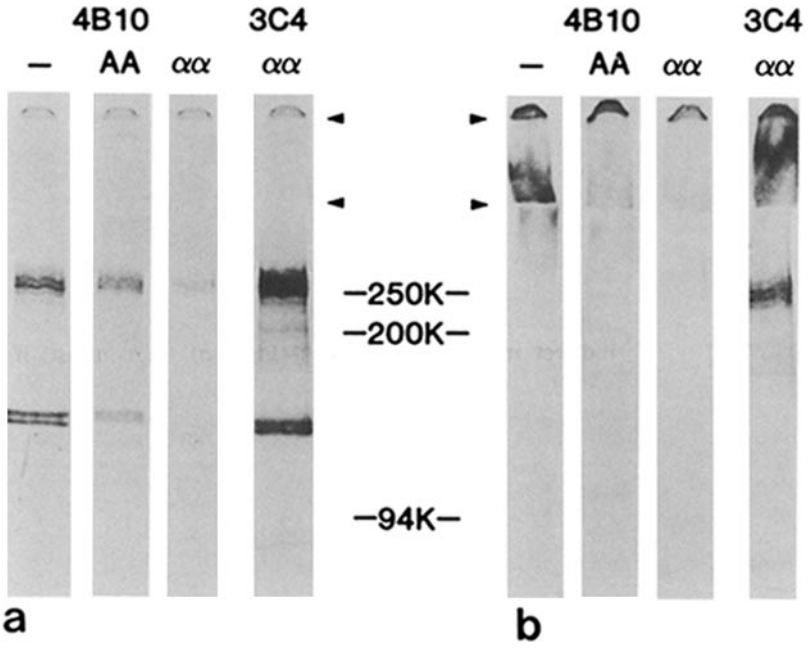

Figure 2. SDS PAGE and autoradiography of immunoprecipitates from Triton X-100 extracts of MRC-5 cells. (*a*) Reduced; (*b*) unreduced. MRC-5 cells were cultured for 24 h in medium containing [^{35}S]methionine and no addition (–), 50 μg/ml ascorbic acid (*AA*), or 80 μg/ml α,α-dipyridyl (α,α). Labeled proteins were isolated using monoclonal anti-type VI collagen (4B10 or 3C4) and electrophoresed on a gel of 5% acrylamide with a 4% stacking gel. Top of the stacking and separating gels (arrowheads) and the mobility of standard proteins (*250K*, fibronectin; *200K*, myosin; *94K*, phosphorylase b) are indicated. In *a* the proteins were electrophoresed after reduction with 1% 2-mercaptoethanol. In *b* the proteins were analyzed unreduced.

of detergent extracts of cells showed that aggregates of the 140- and 260-kD polypeptides were also present intracellularly (Fig. 2 *a*). Furthermore, larger amounts of both polypeptides could be extracted with Triton X-100 from cells cultured without ascorbic acid than from cells cultured in the presence of ascorbic acid (Fig. 2 *a*). This is in agreement with the immunofluorescence results which indicated intracellular accumulation of type VI collagen under conditions of ascorbic acid deprivation. Analysis of cell culture media showed that more of the 140- and 260-kD polypeptides accumulated in the medium of cells cultured with ascorbic acid than without (not shown).

When the metabolic labeling was done in the presence of α,α-dipyridyl, very little radioactive material could be isolated with the 4B10 antibody (Fig. 2). This antibody also failed to stain α,α-dipyridyl-treated cells in immunofluorescence. The 3C4 antibody, which did stain treated cells, immunoprecipitated material similar to that isolated from cells cultured without α,α-dipyridyl (Fig. 2 *a*). However, the lower molecular weight component of this material moved significantly faster than the 140-kD component from untreated cells. The altered mobility of the 140-kD component is probably due to a lack of hydroxylation of the proline and lysine residues. The mobility of the 260-kD component was essentially unchanged (Fig. 2 *a*).

When the different type VI collagen preparations were electrophoresed without reduction (Fig. 2 *b*), most of the protein was found on top of the 4% stacking gel. This indicates that the 140- and 260-kD polypeptides were present in disulfide cross-linked complexes of very large sizes. Collagens I–V assemble intracellularly into triple-helical molecules (collagen monomers) containing three disulfide cross-linked polypeptide chains (1, 5). However, the lack of penetration into the gel in SDS PAGE of most of the unreduced type VI collagen suggests that type VI collagen molecules may be composed of more than three disulfide-linked polypeptides. Material iso-

lated from cells cultured without ascorbic acid or with α,α-dipyridyl partially entered the stacking gel, indicating somewhat smaller disulfide cross-linked complexes. Regardless of the culture conditions used, the unreduced material isolated using the 3C4 antibody contained a band at 260 kD. This probably represents an excess of unlinked 260-kD component of type VI collagen present intracellularly, since this unlinked 260-kD polypeptide was not found in media or in extracellular matrix preparations (not shown).

Nature of the Association of 140- and 260-kD Polypeptides

In an attempt to investigate the nature of the association of the 140- and 260-kD polypeptides, culture media and cell extracts were reduced and alkylated before incubation with antibody. When the samples were reduced under nondenaturing conditions, both monoclonal and polyclonal antibodies immunoprecipitated material containing both 260- and 140-kD polypeptides (Fig. 3 *a*). This shows that the 260- and 140-kD polypeptides are intimately associated via noncovalent bonds in addition to disulfide bonds. When the reduction and alkylation were done under denaturing conditions, only the 3C4 antibody recognized antigenic material in the sample, immunoprecipitating the 260-kD polypeptide only (Fig. 3 *b*).

Lack of Proteolytic Processing of Type VI Collagen in Cell Culture

Type VI collagen isolated from media or urea-extracted extracellular matrices had polypeptides of similar sizes (Fig. 4), suggesting that no major proteolytic cleavage of the secreted type VI collagen is necessary for it to become deposited in the extracellular matrix.

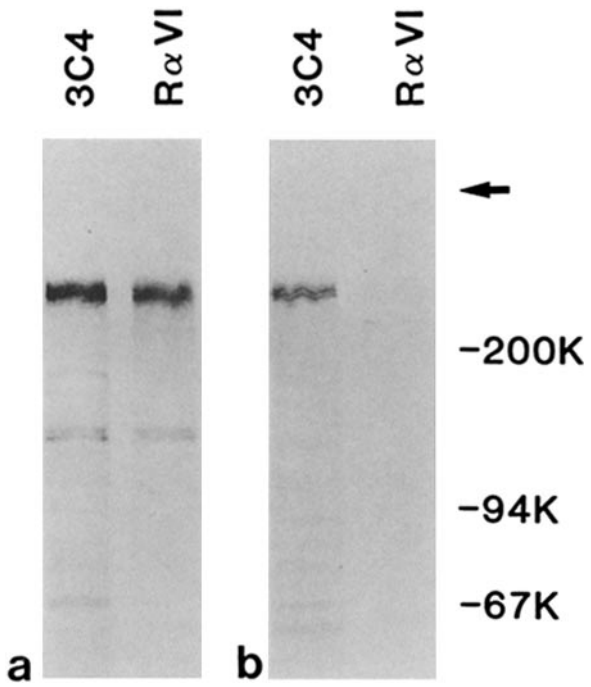

Figure 3. Immunoprecipitation of reduced and alkylated type VI collagen. Metabolically labeled culture medium from MRC-5 fibroblasts was reduced and alkylated, reacted with monoclonal (3C4) or polyclonal (RαVI) antibody to type VI collagen, and material that bound to the antibody was analyzed by SDS PAGE and autoradiography. In *a*, reduction and alkylation was done without denaturation of the protein. In *b*, the reduction and alkylation were done in the presence of 6 M urea. Samples were not treated with mercaptoethanol before electrophoresis.

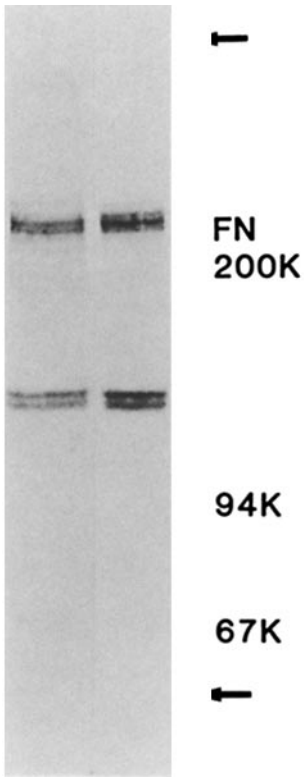

Figure 4. SDS PAGE of type VI collagen isolated from 8 M urea-extracts (left lane) and from the medium (right lane) of fibroblasts.

Similarity of Type VI Collagen from Different Cell Types

Immunofluorescence and metabolic labeling experiments were performed with the MRC-5 fibroblast, the MG63 sarcoma, the SK LMS-1 leiomyosarcoma, the RD rhabdomyosarcoma, and the L6 myoblast cell lines. In all experiments, type VI collagen behaved in the same way, and there was no evidence for any cell type specific variants of type VI collagen (not shown).

Ultrastructure of Type VI Collagen

Preparative amounts of unlabeled type VI collagen were isolated from large-scale cultures of cells by affinity chromatography on monoclonal antibody. The type VI collagen that eluted from the immunoadsorbent with 1 M acetic acid was examined by electron microscopy after rotary shadowing. Type VI collagen from medium of cells cultured in the presence of ascorbic acid (Fig. 5 *a*) resembled images of the tissue forms of type VI collagen (15, 24). Most of the structures were ~150 nm long and had a central, 60-nm-long rodlike region with two closely associated globules at each end. Extending from each of these globules was a thin strand with another globule at its outer end. In a small number of the structures, the inner rod was split into two thinner rods, separating the structure symmetrically at one end.

Type VI collagen isolated from the extracellular matrix extracts was more heterogeneous and contained what appeared to be oligomers of the structures obtained from media (Fig. 5, *b–g*). These appeared to have formed by end-to-end associations of the media form with some overlap at the ends.

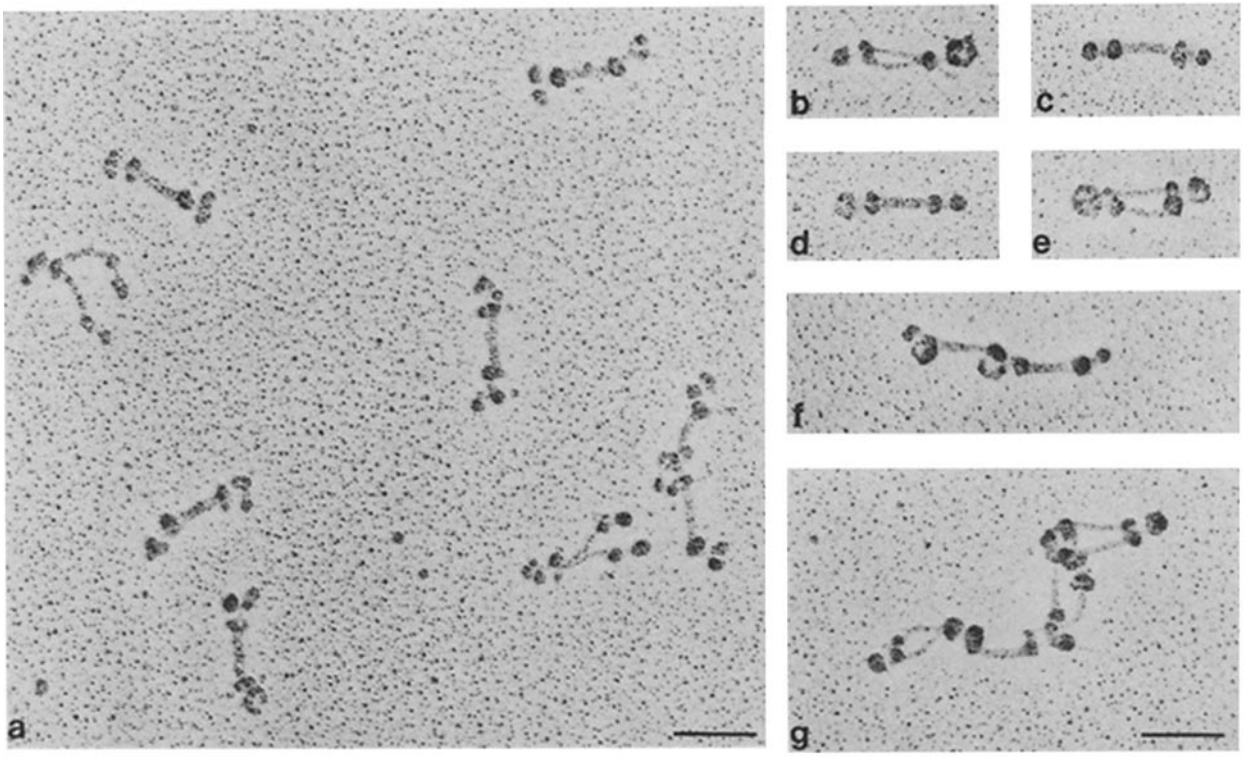

Figure 5. Electron microscopic images after rotary shadowing of type VI collagen isolated from medium or extracellular matrix extracts of MRC-5 fibroblasts. *a* shows a field of representative molecules isolated from medium. *b–g* show selected molecules seen in preparations of type VI collagen isolated from matrix extracts. Bars, 100 nm.

In most structures, the inner rod was split, and some of the globular structures appeared to have fused with one another. Some of the variability seen in these structures may have been caused by the dissociating conditions used to prepare the extracellular matrix extracts.

Because relatively large amounts of type VI collagen accumulated intracellularly when no ascorbic acid was added to cell culture, it was also possible to isolate the intracellular collagen in amounts adequate for ultrastructural studies. Fig. 6 shows a few of the fields seen in the electron microscope after rotary shadowing of such preparations. The most abundant structures were individual globules ranging from 5 to 40 nm in diameter. Some of the smaller of these were also present in control preparations and were probably aggregates of cellular proteins bound nonspecifically to IgG-Sepharose. Other globules, especially the larger ones, were not present in control preparations and may represent type VI collagen. The next most abundant structures seen were about 125 nm long and had two globules connected by a strand. We interpret these to be dimeric structures because we also found, less frequently, similar structures with thinner strands (Fig. 6, *b* and *c*). These may represent monomers of type VI collagen, the thin strand supposedly being the triple-helical domain. In some apparent dimers the thin strands of the monomers were partly intertwined (Fig. 6, *b* and *c*). A small number of apparent dimers were found in which the monomers were aligned with a stagger of ~90 nm (Table II).

Structures resembling those seen in the preparations from media were also seen relatively frequently in cell extracts (Fig. 6, *a* and *c*). They appeared to be tetramers with a total length equal to that of the staggered dimers. The tetramers were the largest structures present in four preparations of type VI collagen purified from detergent extracts of trypsinized cells. The relative abundance of the different structures seen in representative samples are summarized in Table II.

In one preparation of type VI collagen from a detergent extract of cell monolayers, a single filamentous structure was found (Fig. 7). This appeared to be a tetradecamer of type VI collagen tetramers and resembled some of the structures seen in preparations of type VI collagen isolated from extracellular matrix (Fig. 5*g*) but was longer. This structure probably originated from the extracellular matrix of the cells and was incidentally present as a contaminant in this cell extract preparation.

Discussion

We used a panel of monoclonal antibodies (13) to study type VI collagen from different types of cultured cells. The monoclonal antibodies were made against the native tissue form of type VI collagen, and their reactivities, therefore, should accurately reflect the in vivo structure of type VI collagen. Consistent with this, most of these antibodies reacted with conformational antigenic determinants in the type VI collagen molecule. One exceptional antibody reacted with a sequential determinant and proved useful in the analysis of the synthesis and early stages of assembly of type VI collagen.

Type VI collagen appears to be composed of 140- and 260-kD polypeptides. Like the secretion and extracellular matrix deposition of other collagens, those of type VI collagen appear to be dependent on proper hydroxylation of prolines and lysines in its collagenous domains (20). We therefore used agents that affect the hydroxylating enzymes to see whether

the synthesis, secretion, and matrix deposition of the 140- and 260-kD polypeptides would occur coordinately under different conditions.

Indeed, both the 140- and 260-kD polypeptides accumulated intracellularly in ascorbic acid deficient cultures and both were coordinately secreted and deposited in the extracellular matrix in ascorbic acid supplemented cultures. Furthermore, there was no apparent difference in the composition of type VI collagen from different cell types.

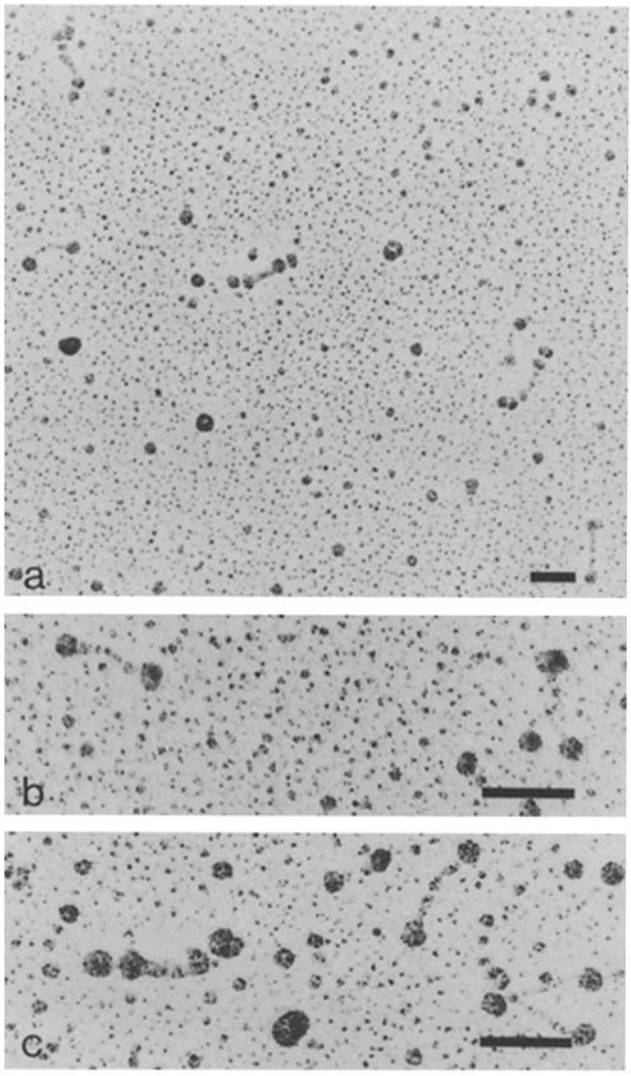

Figure 6. Electron microscopic images after rotary shadowing of type VI collagen isolated from detergent extracts of fibroblasts. *a–c* show fields with representative structures. Bars, 100 nm.

The 140- and 260-kD polypeptides appeared to be intimately associated in the type VI collagen molecules by both disulfide and noncovalent bonds since the polypeptides could only be separated from each other by reduction under denaturing conditions. The complex between the two chains even appears to form at an early stage in the synthesis process before appreciable secondary or tertiary structure has formed. This was suggested by the fact that the antibody specific for a sequential determinant in the 260-kD component could react with disulfide cross-linked aggregates containing both 260- and 140-kD polypeptides in α,α-dipyridyl-treated cells. The other antibodies also reacted with aggregates containing both components, but they did not react with type VI collagen in cells in which prolyl and lysyl hydroxylation was inhibited. Most of the antibodies thus appeared to react with epitopes formed after hydroxylation. Most of our antibodies probably would not react with the triple-helical domains of type VI collagen, however, but they probably react with domains whose structures are indirectly dependent on triple-helix formation.

Several laboratories have reported that the peptides typical of the pepsin-resistant portions of type VI collagen can be generated from type VI collagen preparations containing mainly 140-kD polypeptides. The 140-kD component has therefore been thought to be the genuine type VI collagen (11, 12, 15, 24). However, a larger polypeptide has also been noted in some type VI collagen preparations, and Trueb and Bornstein (21) presented evidence for a type VI procollagen chain of 250-kD. Our results indicate that both types of polypeptides are present in type VI collagen. The reasons for some of these divergent results are probably technical. Most laboratories have identified type VI collagen components using immunoblotting of denatured material, whereas we and others (13, 22) have used immunoprecipitation of native protein to identify the intact type VI collagen. It is also possible that the varying and minor amounts of high molecular weight polypeptides in some types of preparations, especially from tissue sources, is due to selective protease sensitivity of the 260-kD polypeptide.

Our results agree with earlier studies (2) that reported that the 140- and 260-kD polypeptides are immunologically (2) and chemically (22) distinct and not monomer and dimer of the same polypeptide. One of the present monoclonal anti-

Table II. Percentage of Different Type VI Collagen Structures Observed in Randomly Selected Fields of Collagen Isolated from Cell Extracts and Culture Medium

	Structure observed					
			Dimers			
Origin of type VI collagen	Globules*	Monomers	Without overlap	With overlap	Tetramers	No. of structures classified
Cells treated with α,α-dipyridyl‡	98	0	0	0	1.5	133
Untreated cells‡	72	5	12	1	10	207
Untreated cells§	69	0	7	0	24	29
Medium with ascorbic acid§	0	0	0	0	100	130

* Only larger globular structures that could be clearly distinguished from smaller globules in control preparations were included.

‡ Isolated using antibody 3C4.

§ Isolated using antibody 2C6.

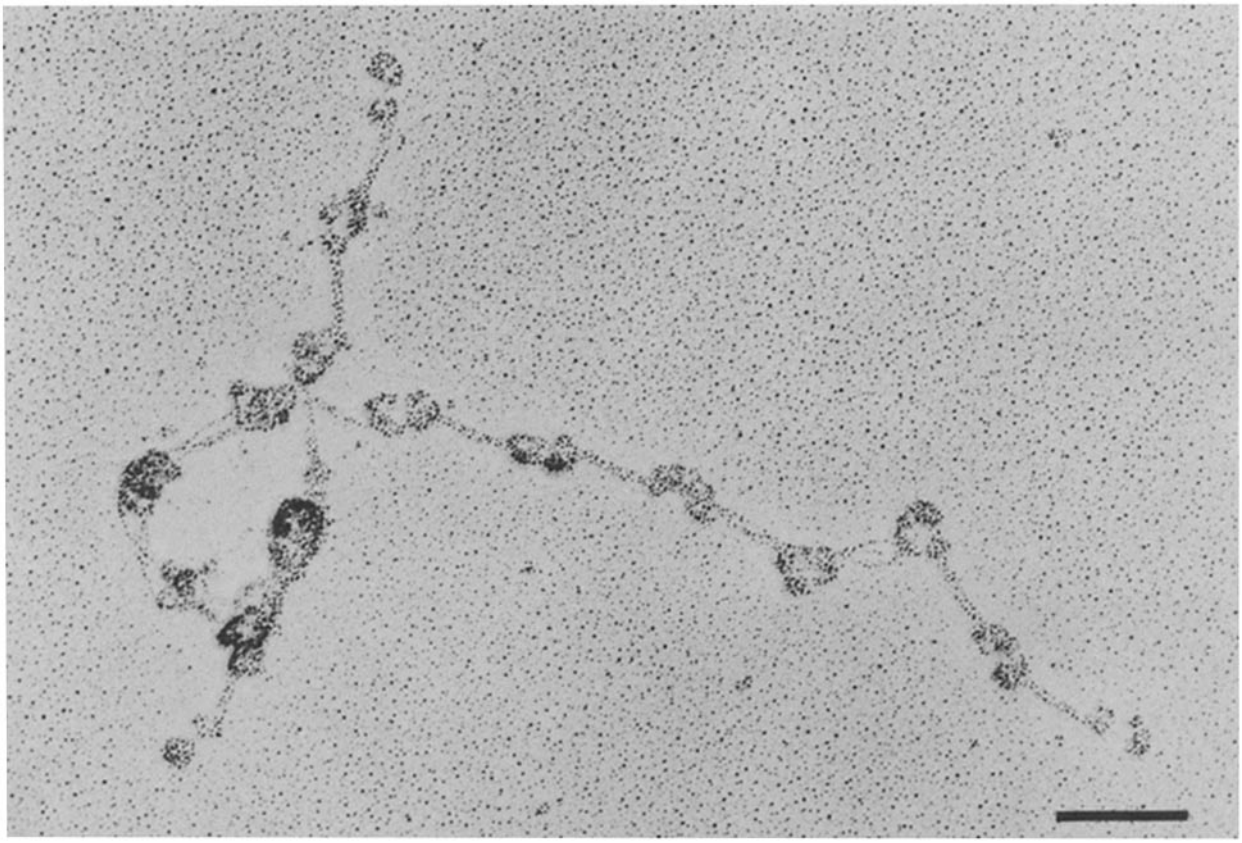

Figure 7. Electron microscopic image of a polymer found in a particular preparation of type VI collagen from MRC-5 cells. Bar, 100 nm.

bodies, 3C4, reacted with denatured free 260-kD polypeptide but not with free 140-kD polypeptide, indicating that the 3C4 epitope is present on one of the chains only. Furthermore, the 140-kD component of the presumably underhydroxylated type VI collagen from α,α-dipyridyl-treated cells were smaller than normal in SDS PAGE (Fig. 2; reference 22), whereas the size of the 260-kD component was unaffected by this treatment. Thus, the 140-kD component seems to contain a larger proportion of hydroxylated proline residues than the 260-kD component. In fact, there are no data to show that the 260-kD polypeptide is collagenous at all. It is insensitive to digestion with bacterial collagenase (2; our unpublished observations), whereas the 140-kD polypeptide can be digested with collagenase. Even if the 260-kD polypeptide is noncollagenous, all of our experiments show that the 260-kD polypeptide is part of the type VI collagen molecule and becomes linked to the 140-kD polypeptide via disulfide and noncovalent bonds early in the intracellular assembly process. As new extracellular matrix molecules are discovered, it appears that the classification of these proteins into collagens, globular proteins, and proteoglycans may become unsatisfactory. For example, it has been shown recently that type IX collagen has chondroitin sulfate side chains and thus is a hybrid between a collagen and a proteoglycan (18, 23). Type VI collagen could also be a hybrid molecule in having a noncollagenous subunit.

Both the 260- and 140-kD polypeptides show heterogeneity on SDS PAGE. The 140-kD polypeptide separated into two distinct bands which may represent two different polypeptides. The heterogeneity in the 260-kD region appeared different. Three or more closely spaced bands were seen on most gels. These polypeptides were clearly related since the 3C4 antibody reacted with all of them. The reason for the heterogeneity of the 260-kD polypeptide is not known, but it could conceivably be due to factors such as differences in glycosylation or other post-translational modifications, proteolysis, or alternative splicing of mRNA.

Unlike other collagens, type VI collagen appears to assemble into multimers within cells. SDS PAGE analysis of type VI collagen isolated from detergent-extracted cells revealed disulfide cross-linked aggregates that were larger than expected of triple-helical monomers. Other collagens are secreted as monomeric molecules composed of three collagenous polypeptides, and their assembly into multimers occurs extracellularly (1, 6).

Electron microscopy after rotary shadowing of type VI collagen isolated from different cell culture compartments provided further evidence for the unique oligomeric assembly of type VI collagen molecules. Cell culture medium contained only the aggregates thought to be made up of four triple-helical type VI collagen monomers, indicating that this is the secreted form of type VI collagen. Cell extracts contained, in addition, smaller units, the structures of which suggested that they were monomers and dimers. However, the putative tetramers were also found in the cell extracts, suggesting that the type VI collagen assembly progresses to the tetramer stage before its secretion. Alternatively, the intracellular precursors could contain all of the information necessary for assembly and form tetramers during the isolation procedure. The structure of the tetramers agrees well with the observations of

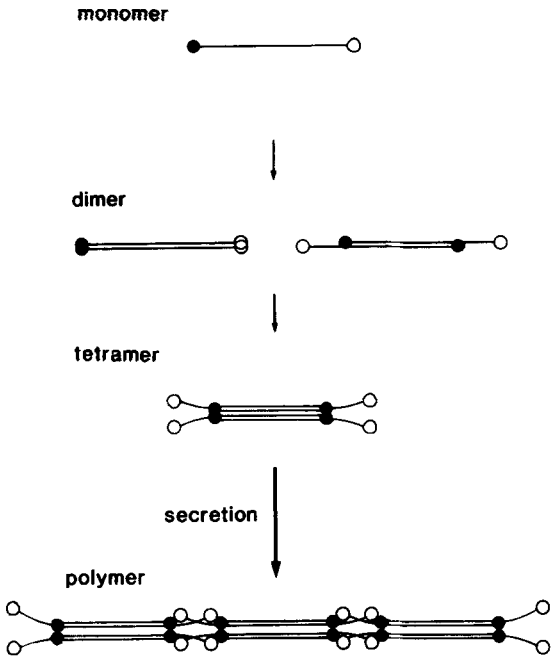

Figure 8. Schematic model for the assembly of type VI collagen.

Furthmayr et al. (7) on pepsin fragments of type VI collagen, except that the globular domains of our images are larger. This is probably due to the larger sizes of the polypeptides in our cell culture-derived material.

It appears that the tetramers of type VI collagen can form polymers in the extracellular matrix without prior proteolytic processing. The assembly occurs by an overlapping, end-to-end association of the tetramers and results in a thin fiber with prominent knobs at a periodicity of ~110 nm.

Based on these results and results from other laboratories (7, 15, 24), we propose the model shown in Fig. 8 for the intracellular assembly of type VI collagen into tetramers and the extracellular association of the tetramers into fibrils. The specific sites of interaction involved in this assembly, as well as the structures of the polypeptides in the molecule, may be important areas of research in future studies on structure and function of type VI collagen.

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