Isolation and Characterization of Type IV Procollagen, Laminin, and Heparan Sulfate Proteoglycan from the EHS Sarcoma†

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ABSTRACT: We have studied the extractability of type IV collagen, laminin, and heparan sulfate proteoglycan from EHS tumor tissue grown in normal and lathyritic animals. Laminin and heparan sulfate proteoglycan were readily extracted with chaotropic solvents from both normal and lathyritic tissue. The collagenous component was only solubilized from lathyritic tissue in the presence of a reducing agent. These results indicate that lysine-derived cross-links and disulfide bonds stabilize the collagenous component in the matrix but not the laminin or the heparan sulfate proteoglycan. The majority of the collagen present in the extracts had a native triple helix based upon the pattern of peptides resistant to pepsin digestion and visualization in the electron microscope by the rotary shadow technique. This protein was composed of chains (M_r 185 000 and 170 000) identical in migration to the chains of newly synthesized type IV procollagen. This finding confirms earlier work that indicates that the biosynthetic form, type IV procollagen, is incorporated as such in the basement membrane matrix. Material with smaller chains (M_r 160 000 and 140 000) appeared on storage in acetic acid solutions. These results indicate that the lower molecular weight collagen in acid extracts of basement membrane arises artifactually due to an endogenous acid-active protease.

Basement membranes are thin extracellular matrices (Kefalides, 1973; Vracko, 1974; Timpl & Martin, 1982) composed of certain unique macromolecules, including type IV collagen (Kefalides, 1973), laminin (Timpl et al., 1979b; Chung et al., 1979), a heparan sulfate proteoglycan (Hassell et al., 1980), and entactin (Carlin et al., 1981). Type IV collagen is believed to be the primary structural element of the basement membrane. Laminin is a glycoprotein which binds to type IV collagen and may serve, in addition to other roles, as an attachment factor for epithelial and endothelial cells (Terranova et al., 1980). It is likely that the heparan sulfate proteoglycan creates a permeability barrier of anionic nature with the basement membrane that blocks the passage of negatively charged macromolecules (Kanwar et al., 1980). The roles of entactin and other components are less well delineated.

Some progress has been made in defining the molecular arrangement of components in basement membrane. Previous studies (Tanzer & Kefalides, 1973; Robey & Martin, 1981) have shown that the collagen molecules are linked by covalent cross-links derived from lysines as found with other collagens. In the basement membrane, the collagen appears as a fine meshwork lacking the banded fibrillar structure observed with other collagens. Recent studies suggest that this protein is arranged with like ends of the molecules in apposition (Kühn et al., 1981; Timpl et al., 1981). Each molecule has a globular region at one end and a disulfide-rich region at the other. Portions of four molecules interact at the disulfide-rich region through disulfide bonds. This structure resists digestion with bacterial collagenase and is termed 7S (Timpl et al., 1979a). The central portion of these molecules are probably not in alignment.

The exact size of the collagen molecules in the basement membrane is not well established. Some studies suggest that the biosynthetic form, type IV procollagen, is converted to a smaller molecule, type IV collagen, which is incorporated into the matrix (Grant et al., 1972). Other studies suggest that processing of type IV procollagen does not occur and that the biosynthetic form itself is incorporated into the matrix (Minor et al., 1976; Heathcote et al., 1978; Dehm & Kefalides, 1978; Crouch & Bornstein, 1979; Karakashian et al., 1982; Leivo et al., 1982). Material with procollagen-like chains (M_r 185 000 and 170 000) and material with smaller chains (M_r 160 000 and 140 000) have been extacted from basement membranes (Veis & Schwartz, 1981). However, recent studies by Karakashian et al. (1982) suggest that the larger form of type IV collagen is the major form of the protein in situ. Little of the collagen in basement membrane can be extracted even in tissue from lathyritic animals where the formation of lysine-derived cross-links is blocked but type IV collagen does dissolve after reduction of disulfide bonds (Tanzer & Kefalides, 1973).

We have also measured the extractability of basement membrane collagen, laminin, and heparan sulfate from the EHS tumor grown in normal and lathyritic mice. The EHS tumor is a convenient model for studying basement membrane components since its cells produce only basement membrane as their matrix (Orkin et al., 1976; Timpl et al., 1978, 1979a). We find that most of the laminin and heparan sulfate proteoglycan in the tissue can be extracted from normal as well as lathyritic animals. The collagenous component is released by reducing agents from lathyritic but not from control tissues and resembles the biosynthetic protein in the size of its component chains. A model is proposed for the arrangement and the interactions of type IV procollagen, laminin and the heparan sulfate proteoglycan in the basement membrane.

Experimental Procedures

Preparation of Type IV Collagen. The EHS tumor was grown in C57/Bl mice made lathyritic by the inclusion of β-aminoproprionitrile (BAPN)[1] in their diet as previously described (Orkin et al., 1976). Some tumor tissue was also

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[1] Abbreviations: BAPN, β-aminoproprionitrile; PBS, phosphate-buffered saline; DTT, dithiothreitol; ELISA, enzyme-linked immunosorbent assay; DEAE-cellulose, diethylaminoethylcellulose.

grown in animals maintained on a normal diet. All extraction procedures were carried out at 4 °C in the presence of protease inhibitors: 0.002 M *N*-ethylmaleimide and 8.0 mM ethylenediaminetetraacetic acid. Harvested tumor tissue was homogenized in 3.4 M NaCl–0.05 M Tris-HCl, pH 7.4, plus the protease inhibitors. The tumor tissue was then divided into equal parts and extracted in parallel or portions were extracted sequentially with one solvent after another. For parallel extractions, the following buffers were used: (1) 0.5 M NaCl–0.05 M Tris-HCl, pH 7.4, (2) 2.0 M Gdn·HCl–0.05M Tris-HCl, pH 7.4, (3) 2.0 M Gdn·HCl–0.05 M Tris-HCl, pH 7.4, containing 2.0 mM dithiothreitol (DTT), and (4) 4.0 M Gdn·HCl–0.05 M Tris-HCl, pH 7.4, containing 2.0 mM DTT. In one experiment, the tissue was divided into several equal parts after a 0.5 M NaCl–0.05 M Tris-HCl, pH 7.4, wash and extracted with varying concentrations of DTT in either 0.02 M sodium phosphate, pH 7.4, containing 0.15 M NaCl (PBS) or 2.0 M Gdn·HCl–0.05 M Tris-HCl, pH 7.4, or 2.0 M urea–0.05 M Tris-HCl, pH 7.4. For sequential extractions, the following buffers were used in the order given: (1) 0.5 M NaCl–0.05 M Tris-HCl, pH 7.4, (2) 2.0 M Gdn·HCl–0.05 M Tris-HCl, pH 7.4, (3) 2.0 M Gdn·HCl–0.05 M Tris-HCl, pH 7.4, containing 2.0 mM DTT, and (4) 4.0 M Gdn·HCl–0.05 M Tris-HCl, pH 7.4, containing 2.0 mM DTT. As shown below, the highest yield of collagen was obtained after extraction of the tissue with 2.0 M gdn·HCl–0.05 M Tris-HCl, pH 7.4, containing 2.0 mM DTT. The material isolated from the tissue following this sequence of extractions was generally >95% pure as judged by the pattern of proteins apparent after gel electrophoresis on 5% polyacrylamide gels (Laemmli, 1970). However, the collagen was also purified by DEAE-cellulose column chromatography. In this procedure, the guanidine–DTT extract was dialyzed against 4 M urea–0.05 M Tris-HCl, pH 7.4, containing 0.25 M NaCl and 2.0 mM DTT and chromatographed on a DEAE-cellulose column equilibrated in the same buffer. The unbound material from the DEAE-cellulose column was dialyzed against 4 M urea–0.05 M Tris-HCl, pH 7.4, containing 2.0 mM DTT and applied to a second DEAE-cellulose column equilibrated in the same buffer. The unbound material was stored as such or dialyzed into 0.5 M acetic acid. The native type IV collagen was not as soluble as interstitial collagens. DTT was required to maintain full solubility. Removal of the DTT resulted in precipitation of some type IV collagen although most remained in solution. If dialyzed against water and lyophilized, type IV was only poorly resolubilized. When DTT or mercaptoethanol was added before lyophilization, the collagen was readily redissolved.

Acid-extracted type IV collagen was prepared as previously described (Robey & Martin, 1981). Labeled type IV procollagen was prepared from an organ culture of minced tumor tissue as previously described (Tryggvason et al., 1980).

Electron Microscopy. The rotary shadow technique was performed as described by Engel et al. (1981). DEAE-cellulose column purified type IV procollagen (30 μg/mL) in 60% glycerol plus 0.2 M ammonium acetate, pH 7.4, was sprayed onto freshly cleaved mica disks by using a nebulizer (Ladd catalog no. 26500). The samples were shadowed with platinum/palladium followed by carbon at angles of 8:1 and 8:13, resspectively, by using the rotation speed control at medium setting in an evacuated chamber (Denton DV-502, 5×10^{-4} torr). The replicas were floated onto distilled water, picked up on 150-mesh grids, and viewed in a Phillips electron microscope 201 or 400 at 60 kV. The length of 50 individual molecules per sample was measured with a Hewlett-Packard 9874A digitizer (Corvallis, OR).

Table I: Parallel Extraction of Type IV Collagen from Lathyritic Tumor Tissue

extraction conditions[c]	μg of type IV collagen/g of tissue wet wt[a]	
	no additions	+DTT[b]
0.5 M HOAc	62	ND[d]
0.15 M NaCl	ND	263
0.5 M NaCl	66	ND
1.0 M Gdn·HCl	78	744
2.0 M Gdn·HCl	101	2975
4.0 M Gdn·HCl	1850	2975
1.0 M urea	100	462
2.0 M urea	238	2338
4.0 M urea	525	1975

[a] All data were obtained by ELISA using purified type IV collagen as a standard. [b] 2.0 mM DTT was used. [c] All buffers except 0.5 M HOAc contained Tris-HCl, pH 7.4. [d] ND denotes not determined.

Other Procedures. $NaDodSO_4$–polyacrylamide gel electrophoresis was carried out by using a 5% acrylamide separating gel as previously described (Laemmli, 1970). Amino acid analysis of type IV procollagen purified on a DEAE-cellulose column was carried out on a Durrum amino acid analyzer after hydrolysis under N_2 in 6 N HCl at 105 °C for 24 h. Antibodies against the guanidine–DTT- and acid-extracted type IV collagen were prepared in rabbits by standard procedures (Yaoita et al., 1978) and tested in the enzyme-linked immunosorbent assay (ELISA) (Rennard et al., 1980). Quantitation of the amounts of type IV collagen, laminin, and proteoglycan were made by ELISA assay using antibodies specific for each protein. The extraction experiments were done at least twice, and all extracts were assayed by ELISA in duplicate with eight different concentrations of each tested. The data are expressed as the average of duplicates that did not differ by more than 15%. The amounts of type IV procollagen and heparan sulfate proteoglycan in the standards were determined by dry weight, while the amount of laminin was determined by the Lowry procedure (Lowry et al., 1951). Pepsin digestion of purified type IV collagen was carried out at 15 °C at an enzyme to substrate ratio of 1 to 10 as described previously (Tryggvason et al., 1980). Cell attachment assays were carried out as previously described with bacteriological plastic plates (35-mm diameter) coated with 10 μg of collagen (Kleinman et al., 1979). The adhesion of Chinese hamster ovary cells to type IV collagen in the presence of either laminin or fibronectin was tested (Terranova et al., 1980).

Results

Little type IV collagen was present in extracts of tumor tissue grown in nonlathyritic mice by using any of the solvents as measured by immunoassay or by electrophoresis of the extracted proteins. However, we could extract the majority of the type IV collagen in tissues from lathyritic animals with 2.0 M guanidine plus DTT (Table I). Type IV collagen was extracted at relatively low levels of DTT in the presence of 2 M guanidine or 2 M urea while very little was extracted with PBS plus DTT (Table I and Figure 1). The combination of 2.0 M guanidine plus DTT extracted 48 times as much type IV collagen (2975 μg/g wet weight) than 0.5 N acetic acid (62 μg/g wet weight) as determined by ELISA. Essentially all of the type IV collagen was extracted from the tissue by this procedure since the tissue residues were very small and amino acid analyses of the tissue residue revealed little or no hydroxyproline. These results confirm that lysine-derived cross-links occur in basement membrane collagen, since the

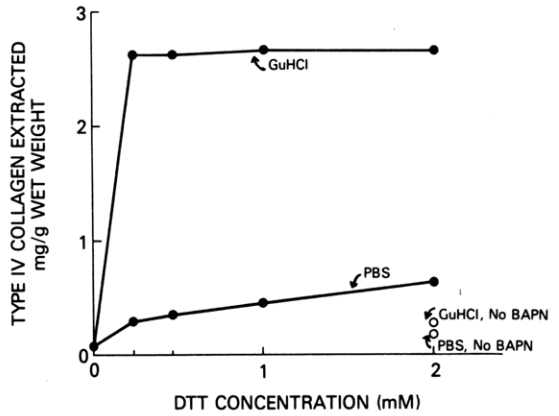

FIGURE 1: Effect of varying concentrations of DTT on the extractability of basement membrane collagen. Equal amounts of tissue were extracted in parallel with either PBS, 2.0 M urea, or 2.0 M guanidine buffers in the presence of varying amounts of DTT. The amount of type IV collagen in the extracts was determined by ELISA. Unless indicated, the tissues used were from animals made lathyritic with BAPN.

protein is not extractable from tissue grown in nonlathyritic animals. In addition, disulfide bonds between collagen molecules and possibly other components must also stabilize this collagen, since a reducing agent is necessary for maximal extraction.

Examination of the proteins in the various extracts by $NaDodSO_4$ gel electrophoresis showed the characteristic chains of laminin (R_r 200 000 and 400 000) in the 0.5 M NaCl and 2.0 M guanidine extracts as well as in the 2.0 M guanidine plus DTT extract (Figure 2A, all lanes). Using 2.0 M guanidine and DTT (Figure 2A, lane 3) or 2 M urea and DTT as extractants (not shown), two collagenous chains (M_r 185 000 and 170 000) were apparent. These chains migrated in a fashion identical with that of the chains of biosynthetically labeled collagen type IV and were destroyed by incubation with purified bacterial collagenase (not shown). Entactin (M_r 158 000) was also present in all the extracts of the tumor.

Some laminin as well as the bulk of the collagen was present in the 2.0 M guanidine plus DTT extract of the lathyritic tumor tissue (Figure 2A, lane 3). However, when the tissue was extracted sequentially with 2.0 M guanidine followed by guanidine containing DTT, guanidine (2.0 M) alone effectively extracted laminin but not type IV collagen (Figure 2B, lane 2, and Figure 3). In addition, usually all of the tissue laminin was removed by 2.0 M guanidine since limited pepsin digestion (under conditions that are used to prepare laminin fragments) of the residue after guanidine–DTT extraction failed to release any laminin fragments. Subsequent extraction of the guanidine residue with 2.0 M guanidine plus DTT solubilized the type IV collagen, yielding a preparation essentially free of laminin and other contaminating proteins (Figure 2B, lane 3, and Figure 3). The type IV collagen extracted with 2.0 M guanidine plus DTT had an amino acid composition characteristic of type IV collagen (Table II) and was composed of two chains (M_r 185 000 and 170 000). These data demonstrate that laminin and type IV collagen are not linked to each other in the tissue by disulfide bonds.

The extractability of heparan sulfate proteoglycan from tumor tissue grown in normal and lathyritic animals was estimated from uronic acid assays (data not shown) and ELISA assays (Figure 3) on various tissue extracts. The extractability of the proteoglycan was similar from normal and lathyritic tissues (Figure 3). The majority of the heparan sulfate proteoglycan was removed by extraction with guanidine and did not require DTT. These studies suggest that the heparan

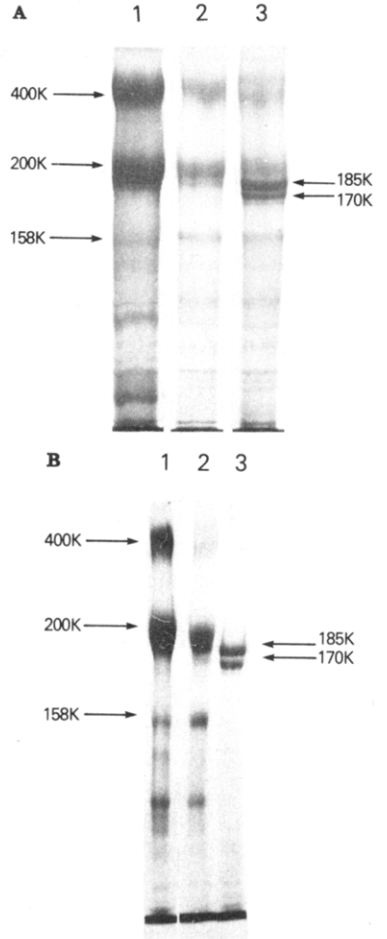

FIGURE 2: $NaDodSO_4$ gels of parallel and sequential extracts of tumor tissue. (A) Parallel extraction of tissue. Equal aliquots of tumor tissue were extracted with either 0.5 M NaCl buffer (lane 1), 2.0 M Gdn·HCl buffer (lane 2), or 2.0 M Gdn·HCl buffer containing 2.0 mM DTT (lane 3). The arrows in lane 1 designate the 400K and 200K chains of laminin and the 158K chain of entactin. The arrows in lane 3 designate the 185K and 170K chains of type IV procollagen. (B) Sequential extraction of tissue. Tumor tissue was extracted sequentially with 0.5 M NaCl buffer (lane 1), 2.0 M Gdn·HCl buffer (lane 2), and 2.0 M Gdn·HCl buffer containing 2.0 mM DTT (lane 3).

Table II: Amino Acid Analyses[a]

	2 M Gdn·HCl + DTT extract	lens capsule[b]		2 M Gdn·HCl + DTT extract	lens capsule[b]
3-Hyp	3	15	Met	16	8
4-Hyp	70	85	Ile	33	29
Asp	62	55	Leu	63	58
Thr	61	29	Tyr	15	10
Ser	38	42	Phe	34	32
Glu	102	93	Hyl	31	35
Pro	64	68	His	17	10
Gly	234	275	Lys	26	13
Ala	54	43	Arg	37	43
Val	46	30			

[a] Residues per 1000. [b] From Kefalides (1973).

sulfate proteoglycan is present with other components in a rather stable complex but that the proteoglycan is not held in the matrix by disulfide bonds.

The extracted collagen appeared to be related to the acid-solubilized collagen. Antibodies prepared against the guanidine–DTT-extracted collagen cross-reacted in ELISA with acid-soluble collagen and antibodies against acid-soluble collagen cross-reacted with the extracted collagen (data not

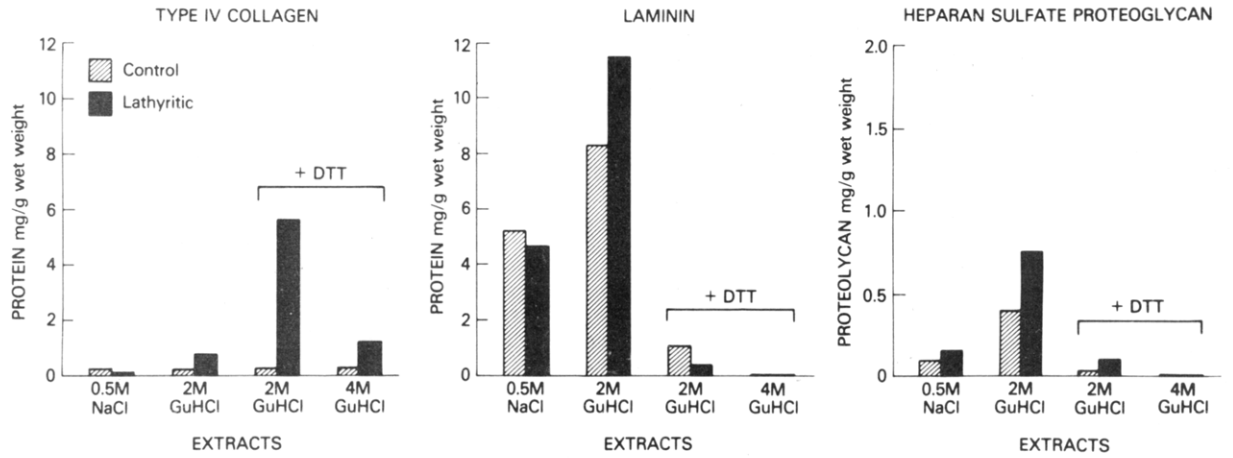

FIGURE 3: Quantitation of basement membrane components extracted with various buffers from control and lathyritic tumor tissue. All extracts were carried out sequentially, and the amounts of type IV procollagen, laminin, and heparan sulfate proteoglycan present in the extracts were determined by specific ELISA assays.

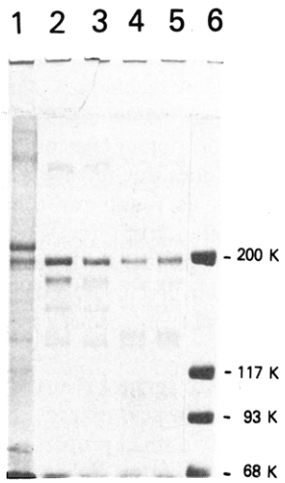

FIGURE 4: Pepsin digestion of type IV procollagen. Type IV procollagen extracted with guanidine and DTT (lane 1) was digested at 15 °C in an enzyme to substrate ratio of 1 to 10 for 2 h (lane 2), 6 h (lane 3), 12 h (lane 4), and 24 h (lane 5). The assays were stopped by freezing the samples followed by lyophilization. The samples were analyzed by $NaDodSO_4$ gel electrophoresis. Lane 6 contains the indicated molecular markers, which are grobular proteins. Collagen migrates anomalously in $NaDodSO_4$ gels, and direct comparison with globular standards cannot be made.

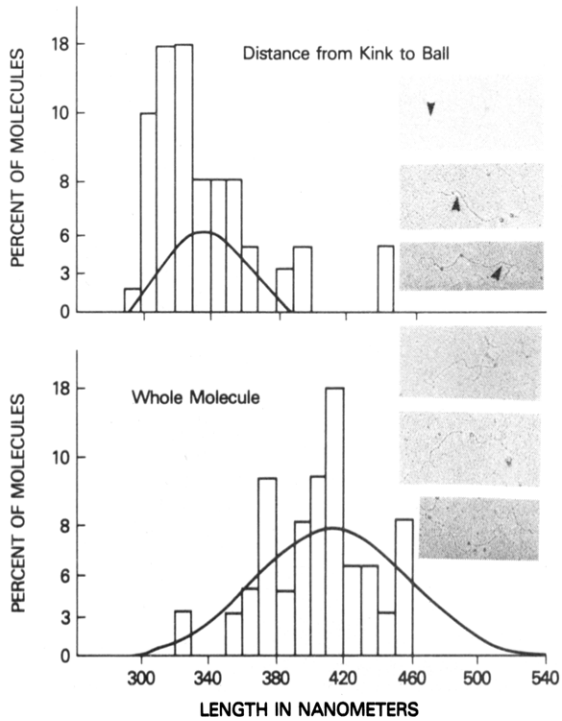

FIGURE 5: Examination of type IV procollagen molecules by electron microscopy after rotary shadowing. Shown on the right part of the figure are type IV procollagen molecules extracted from the EHS tumor with guanidine and DTT and purified on a DEAE-cellulose column. The bar graphs show the lengths obtained by measuring a minimum of 50 molecules in the various preparations. The length of the entire molecule (lower panel) was measured. In addition, the distance from the kink (designated by an arrowhead in the upper panel) to the ball end was measured. The latter distance corresponds to the length of the acid-solubilized type IV collagen (Timpl et al., 1981). The lengths of the molecules were analyzed by computer, and the closest fit normal distribution was determined and is shown as solid lines in the bar graphs. The midpoint or maximum was used to determine the final length of the molecule.

shown). This confirms that the two molecules were antigenically related. In addition, the guanidine–DTT-extracted collagen, when used in cell attachment assays with laminin, promoted cell adhesion in a manner identical with that observed with the acid-soluble collagen (data not shown).

Three lines of evidence indicate that the type IV collagen in the 2.0 M guanidine plus DTT extracts contained a native collagenous helix. Incubation with pepsin at 15 °C caused cleavages of the protein with high yields of large fragments similar in size to those reported for procollagen type IV exposed to pepsin (Figure 4) (Tryggvason et al., 1980). In contrast, collagen samples heated to 45 °C for 10 min prior to incubation with pepsin were totally degraded by pepsin to dialyzable peptides. When the 2.0 M guanidine plus DTT extracted protein was examined in the electron microsocope after rotary shadowing, it appeared as a 400 nm long particle similar to that of type IV procollagen (L. A. Liotta, unpublished experiments) (Figure 5). Many dimers joined at the globular ends as well as some single molecule were seen. Large aggregates (the spider forms) formed by the interaction at the 7S end of the molecule were not observed most likely due to the use of DTT in the isolation procedure. The type IV collagen extracted with guanidine plus DTT was longer (400 nm) than the acid-extracted type IV collagen observed by us (330 nm) and others (320 nm) (Timpl et al., 1981). Both forms of the protein contained the globular region, while the acid-extracted material terminated at a position corresponding to a bend toward the end of the larger molecule. On the basis of current models of type IV procollagen, it would appear that the acid-extracted form of type IV collagen lacks the 7S region.

The collagenous protein freshly extracted with guanidine plus DTT contained two chains that showed identical migration

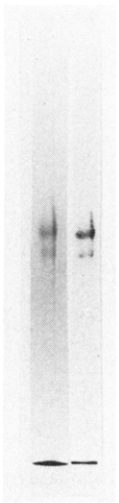

FIGURE 6: Comigration of DTT-extracted type IV collagen with radiolabeled type IV procollagen. Radiolabeled type IV procollagen and the DTT-extracted type IV collagen were electrophoresed together in the same lane of a 5% acrylamide gel. The gel was stained with Coomassie blue (left lane) to visualize the extracted material and then processed for fluorography (right lane).

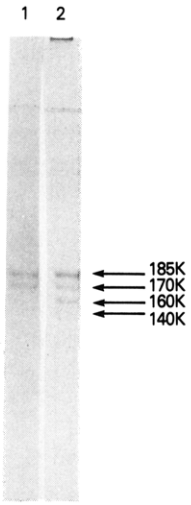

FIGURE 7: Degradation of type IV procollagen after storage at 4 °C in 0.5 M HOAc for 1 week. Bands of 160K and 140K (lane 2) appear in the sample.

in $NaDodSO_4$ gels with biosynthetically labeled chains (Figure 6). However, when stored in dilute acetic acid in the refrigerator, two smaller chains appeared with time in the preparation (Figure 7). Storage of the samples at −20 °C greatly reduced the amount of cleavage. The migration of these chains was identical with that of the chains of acid extracted type IV collagen (M_r 160 000 and 140 000). These results suggest that the majority of the collagenous protein in the tissue is composed of chains of M_r 185 000 and 170 000 and that there is no major change in the size of the collagenous protein deposited in the matrix after synthesis as found in interstitial collagens.

Discussion

We have examined the extractability of various basement membrane components, including type IV collagen, laminin, and the heparan sulfate proteoglycan from the matrix of the EHS tumor. Previous studies have shown that basement membrane contains lysine-derived cross-links of the type known to stabilize and insolubilize other collagens (Tanzer & Kefalides, 1973; Robey & Martin, 1981). Only a small amount of type IV collagen can be extracted with 0.5 N acetic acid from tissue grown in lathyritic animals. However, this material is not soluble in neutral solvents and contains chains smaller than those present in the biosynthetic precursors. As discussed below, it seems likely that a proteolytic enzyme active at acid pH is removing a portion of the protein that maintains the molecule in an insoluble form. This cleavage likely occurs at the bend in the molecule, and this would be expected since the bend probably indicates a nonhelical region.

Karakashian et al. (1982) have shown that reducing and alkylating disulfide bonds greatly increase the extractability of collagenous protein from lathyritic tumor tissue. In their studies, some 25% of the collagen in the tumor was extracted with 0.5 M NaCl and DTT. We have confirmed their studies and found that most of the collagen in lathyritic tissue is removed by 2 M guanidine plus DTT. In both studies, the protein contains chains estimated as 185 000 and 170 000 that are similar or identical in size to the chains of the biosynthetic form. It is likely that the DTT dissociated interchain disulfide bonds but that the helical portion of the structure must have been preserved since the denatured molecules would not be seen by the rotary shadow technique. These studies indicate as suggested previously (Minor et al., 1976; Clark & Kefalides, 1978) that the incorporation of the collagenous protein into basement membrane does not involve any substantial proteolytic modifications as found for other collagen types. Presumably, the precipitation of type IV collagen in the matrix involves different mechanisms than those that occur with other collagens where a soluble precursor, procollagen, is converted to a less soluble form that spontaneously assembles into ordered fibers.

Three lines of evidence suggest that the shortened form of type IV collagen found in acid extracts of the tumor arises artifactually. First, only a small proportion of the extractable collagen is of the shortened form. Second, storage of the intact molecule in acid allows the generation of the smaller material. Third, the smaller molecule lacks the 7S region of type IV collagen but is otherwise similar to the large component. It seems likely that an endogenous protease active at acid pH cleaves the 7S region from the intact molecule. During the extraction of tissue in acid, the enzyme must cleave the region of the molecule containing disulfide bonds and liberates the truncated form in spite of the presence of certain protease inhibitors.

The extractability of laminin and heparan sulfate proteoglycan was also measured. Both molecules were readily extracted from both control and lathyritic tissue and in similar proportions. There did not appear to be any significant effect on the extraction of these molecules attributable to the reduction of disulfide bonds. These studies indicated that laminin and heparan sulfate proteoglycan, in contrast to type IV collagen, are not held in the tissue by covalent bonds. Indeed, after exhaustive extraction of the tissue without DTT, all the laminin could be removed and essentially pure type IV procollagen was obtained. Whereas laminin binds to type IV collagen (Terranova et al., 1980), disulfide bonds do not stablize this interaction.

These studies offer new information on the assembly and interaction of type IV collagen, laminin, and heparan sulfate proteoglycan in the basement membrane. Type IV procollagen molecules cross-link to one another and possibly to other, as yet unidentified, components by disulfide bonds located in the 7S region. The interaction of molecules through the globular end presumably involves noncovalent bonds that may be stabilized by lysine-derived crosslinks. Laminin is bound to type

IV collagen by noncovalent bonds. However, it is likely that the binding is specific and reasonably strong since 2 M guanidine is required to solubilize all of the laminin. Various studies show that heparan sulfate proteoglycan binds to laminin (Sakashita et al., 1980). The interaction of these components may occur during the formation of the basement membrane rather than having the type IV procollagen form the initial structure to which laminin and heparan sulfate proteoglycan are added.

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