Type IV collagen was isolated from human placenta by selective salt precipitation at acidic and neutral pH. The native protein was resistant to human skin collagenase but was cleaved by a rat mast cell protease. Molecular sieve chromatography of the reduced and alkylated material separated relatively homogeneous components of molecular weight 140,000 (140K) and 100,000 (100K) and a third component of molecular weight 70,000 which was further fractionated on CM-cellulose into 70K-I and 70K-II components.

Amino acid compositions and peptide maps produced by digestion of the denatured chains with cyanogen bromide and mast cell protease indicated that the 100K and 70K-I fragments were derived from the larger 140K fragment, but that the more basic 70K-II fragment represented a different sequence which was most probably derived from a related but distinct collagen chain. The data are consistent with the presence of two genetically distinct type IV-like collagen chains in placenta.

The existence of a pepsin-resistant collagenous fragment that contains approximately one-third glycine and has a molecular weight of 140,000 by the criteria of molecular sieve chromatography and acrylamide gel electrophoresis, and 145,000 by sedimentation equilibrium, argues for the presence of a triple-helical region in type IV collagen that is longer than an α chain of type I collagen, even when the hydroxylysine-linked carbohydrate content of this chain is considered.

Structural Studies on Human Type IV Collagen

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Type IV collagen was isolated from limited pepsin digests of human placenta by selective salt precipitation at acidic and neutral pH. The native protein was resistant to human skin collagenase but was cleaved by a rat mast cell protease. Molecular sieve chromatography of the reduced and alkylated material separated relatively homogeneous components of molecular weight 140,000 (140K) and 100,000 (100K) and a third component of molecular weight 70,000 which was further fractionated on CM-cellulose into 70K-I and 70K-II components.

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In the last several years, studies on the "basement membrane" or type IV collagens have been concerned not only with structural questions but with aspects of type IV secretion, processing, deposition, and binding to cells and cell-surface-associated proteins. It is becoming increasingly evident that this collagen type belongs to a unique subfamily of collagens which is both structurally and functionally distinct. For example, recent experiments by Kleinman et al. (1) and Murray et al. (2) indicate that epidermal cells preferentially attach to and differentiate on type IV collagen, in contrast to other cell types which adhere equally well to types I-IV.

Structural studies on type IV collagen have been carried out largely by Kefalides and co-workers (for review, see Ref. 3) who have presented evidence for the chain composition α1(IV), for basement membrane collagen isolated by pepsin digestion (4). Other investigators have reported a considerable (5) or more limited (6, 7) number of apparently heterogeneous collagenous molecules from pepsin-treated basement membranes. Although major discrepancies exist concerning the size distribution and molecular organization of these molecules, most laboratories agree that the pepsin-resistant collagen chains have disulfide bonds and an amino acid composition which is distinct from types I, II, III, and AB.

Resolution of some of these difficulties is likely to emerge from a biosynthetic approach. Studies on Reichert's membrane and lens capsule have demonstrated the synthesis of a single precursor of M2, 160,000 or 180,000 (8, 9). On the other hand, studies with a murine basement membrane tumor and an amniotic fluid cell culture system, which have utilized the increased resolving power of SDS-polyacrylamide gel electrophoresis, have suggested the existence of two closely spaced chains of an average M2 = 150,000 and 170,000, respectively (10, 11).

This report deals with structural studies on type IV collagen and, more specifically, with the characterization of four polypeptide fragments which comprise a significant proportion of the type IV collagen that can be isolated from human placenta. Two objectives of this study were: 1) an explanation of the apparent heterogeneity which has been reported for type IV collagen, and 2) a comparison of the structure of type IV with that of other collagen types.

MATERIALS AND METHODS

Isolation of Collagen—Human term placentas, dissected free of amnion and chorion, were put through a meat grinder and extracted at 4°C with four changes each of water (2 days), 1 M NaCl, 50 mM Tris-HCl, pH 7.5 (4 days), and 0.5 M acetic acid (3 days), essentially as described by Epstein et al. (12). All subsequent procedures were also performed at 4°C. After the final extraction, the tissue was filtered through a collander and cheesecloth and was suspended in 0.5 M acetic acid (1 liter/200 g of tissue (wet weight)). Pepsin (pepsin A, Worthington) was then added in an enzyme/substrate weight ratio of 1:400 and the reaction mixture stirred for 24 h (13). The digest was then centrifuged for 1 h at 7100 × g and the resulting supernatant was used in subsequent steps.

The procedure used for isolation of type IV collagen is outlined in Fig. 1. To the clarified pepsin digest was added solid NaCl to a concentration of 1 M. The solution was stirred for 24 h and the resulting precipitate, which contained principally types I and III collagens, was removed by centrifugation. The NaCl concentration was then adjusted to 1.8 M and, after 24 h, the precipitate was recovered by centrifugation. This material, consisting of types IV and AB and some residual types I and III collagens, was dissolved in 1 M NaCl, 50 mM Tris-HCl, pH 7.5. At this point, the pepsin has been inactivated. The solution was centrifuged to remove insoluble material before adjusting the NaCl concentration to 2 M. The precipitate which formed after stirring for 24 h was recovered by centrifugation, dissolved at approximately 1 mg/ml in 0.1 M acetic acid, dialyzed against 0.1 M acetic acid, and lyophilized. This fraction, containing approximately 65% type IV, 10% AB, and 25% types I and III collagens, was redissolved at a concentration of 1 mg/ml in 0.1 M acetic acid,
and the interstitial collagens (I and III) were precipitated by adding solid NaCl to 0.7 M. Following centrifugation, the supernatant NaCl concentration was increased to 1.6 M, and the solution was stirred slowly overnight. The resulting precipitate, containing types IV and AB collagens, was recovered by centrifugation and dissolved at 1 mg/ml in 0.1 M succinic acid and dialyzed against the same buffer for 48 h. Protein at three different concentrations was read by weighing lyophilized material and by amino acid analysis.

**Treatment of Native Protein with Vertebrate and Bacterial Collagenase—**Type I collagen, isolated by standard techniques from pepsin-treated human skin, and type IV collagen were dissolved at 1 mg/ml in 0.1 M acetic acid at 4°C and dialyzed against collagenase buffer (0.15 M NaCl, 50 mM Tris-HCl, 5 mM CaCl2, 10 mM N-ethylmaleimide, 0.2 mM phenylmethylsulfonyl fluoride, pH 7.5). Insoluble protein was removed by centrifugation. Aliquots of each solution were incubated with highly purified human skin collagenase (a gift of Dr. A. Eisen, Washington University School of Medicine, St. Louis) at a collagenase/substrate concentration of 1:100 (w/w) for 24 h at 22°C.

Another aliquot of enzyme was added, producing a 1:50 ratio, and the samples were incubated for an additional 24 h at 22°C. Similarly, aliquots were also incubated with bacterial collagenase (Advanced Biofactures) at an enzyme/substrate concentration of 1:50 (w/w) for 4 h at 37°C. Each digestion was performed in parallel with control solutions containing collagenase buffer, and the reaction was terminated by addition of an equal volume of SDS-urea sample buffer containing 50 mM dithiothreitol (14) prior to analysis by SDS-electrophoresis.

**Reduction and Alkylation—**Native type IV collagen was dissolved in 8 M urea, 50 mM Tris-HCl, pH 8.0, (20 mg/3 ml) by stirring at 40°C for 10 min. Dithiothreitol (Vega-Fox) was added to a final concentration of 100 mM, and the solution was incubated at 37°C for 16 h. Iodoacetamide (Sigma) was added to produce a final concentration of 100 mM, and alklylation was performed for 1 h in the dark. The solution was then made to a concentration of 200 mM with respect to dithiothreitol and incubated for 10 min at 22°C. The reduced and alkylated type IV collagen was subsequently dialyzed against 1 M NaCl, 50 mM Tris-HCl, pH 7.5, prior to molecular sieve chromatography.

**Molecular Sieve Chromatography—**The major type IV collagen chains were resolved by chromatography on 6% agarose (Bio-Gel A-5m, 200 to 400 mesh, Bio-Rad Laboratories) as described by Cheng et al. (15). Reduced and alkylated samples which had been dialyzed against 1 M NaCl, 50 mM Tris-HCl, 100 mM NaCl, and the column (1.5 x 175 cm) and eluted at a flow rate of 10 ml/h with the same buffer. The column effluent was monitored and recorded at 206 and 280 nm by an LKB Ulvacord III. [H]Proline-labeled type I collagen, isolated from human fibroblast culture medium, was co-chromatographed with the type IV collagen sample for calibration purposes.

**CM-Cellulose Chromatography—**Lyophilized type IV chains, isolated by molecular sieve chromatography, were dissolved in and dialyzed against the column buffer (6 M urea, 40 mM sodium acetate, pH 4.8). Samples were denatured by heating at 42°C for 20 min and clarified by centrifugation before application to a column (1.0 x 11 cm) of CM-cellulose (30 column volumes of buffer/mg, maintained at 4°C). Bound protein was eluted with a linear gradient of 0 to 80 mM NaCl in 900 ml of column buffer, at a flow rate of 26 ml/h. Column effluent was monitored as described above. The column was standardized with type I collagen chains isolated from lathyritic rat skin.

**Sedimentation Equilibrium Studies—**Equilibrium sedimentation was performed with a Beckman-Spinco model E analytical ultracentrifuge operated at ultraviolet scanning absorbance, interfaced with a PDP 12 computer, as described by Teller (16). The reduced and alkylated type IV chain, which eluted from the agarose column with an apparent molecular weight of 140,000, and which was subsequently rechromatographed on CM-cellulose, was used for this study. The protein was dissolved at 40°C in 6 M guanidine-HCl (Gdish) at a concentration of 1 mg/ml and dialyzed against the same buffer for 48 h. Protein at three different concentrations was sedimented at 17,000 rpm at 20°C for 48 h. Rayleigh pleats were read by an automatic device as described by Teller (16). The apparent partial specific volume (ø) for the protein was calculated from the amino acid and carbohydrate composition to be 0.710 ml/g, according to the method of Lee and Tanasheff (17).

**Amino Acid Analysis—**Samples were dissolved in constant boiling HCl (100 to 500 µg/0.5 ml), flushed with N2, and hydrolyzed in sealed glass ampules containing 0.1 M acetic acid and 0.1 M acetic acid, and lyophilized. The protein was then redissolved at a concentration of 0.1 mg/ml in 0.1 M acetic acid, and type IV collagen was precipitated by adding solid NaCl to 0.7 M, leaving the AB collagen in solution. The latter protein can be recovered by raising the NaCl concentration to 1.2 M. The type IV collagen was redissolved in dialyzed against 0.1 M acetic acid and lyophilized. The protein isolated at this point was used for all subsequent procedures. Each precipitation step in the purification sequence was monitored by SDS-polyacrylamide gel electrophoresis, and protein recovery was quantitated by weighing lyophilized material and by amino acid analysis.

**Purification of Type IV Collagen—**Precise quantitation of type IV collagen in placenta is very difficult due to the nature of the isolation procedure, in which substantial nonsystematic losses occur at each precipitation and dialysis step. A major objective in this work was to isolate a highly purified type IV collagen, rather than to recover the pepesin-treated protein quantitatively from the tissue. We estimate that the collagen which can be recovered after 24 h of pepsin treatment comprises approximately 20% of the dry weight of the tissue. Of this collagen, 4 ± 1% is type IV. The type IV collagen isolated at the final step as outlined in Fig. 1 represents approximately half of this amount (9% total collagens; 0.1% placenta (w/w)) due to the selection of procedures which minimized co-precipitation of the interstitial collagens with type IV. For example, adding NaCl to a concentration of 1 M in the clarified pepsin digest results in precipitation of some type IV but removes larger quantities of types I and III than does precipitation of the same digest at 0.7 M NaCl, as recommended for AB collagen by Rhodes and Miller. 

**RESULTS**

**Purification of Type IV Collagen**

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Pepsin treatment of the tissue for 50 h resulted in the disappearance of all collagenous chains larger than α-chains. We have found that precipitation behavior, especially with the type IV collagens, is dependent on protein concentration and is influenced by the presence of other collagen types. In this regard, type IV collagen failed to precipitate at 0.7 M NaCl in acetic acid at concentrations of 1 mg/ml, but all the residual types I and III collagens are removed at this step. However, reprecipitation of type IV at a concentration of 0.1 mg/ml occurs at 0.7 M NaCl, resulting in complete separation from AB collagen, which remains soluble.

**Characterization of Native Type IV Collagen**

SDS-electrophoresis of the type IV collagen after purification by differential salt precipitation indicates that it consists principally of disulfide-bonded protein; after reduction, three major chains were resolved with mobilities corresponding to molecular weights, in this system, of approximately 140,000 (140K fragment), 100,000 (100K fragment), and 70,000 (70K fragment) (Fig. 2). Scanning densitometry revealed that these three fragments were recovered in the relative proportions of 1:0.05:0.72 for 140K, 100K, and 70K, respectively. Some lower

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Human Type IV Collagen

Fig. 5. CM-cellulose chromatography of type IV collagen fragments (140K, 100K, and 70K) previously isolated by molecular sieve chromatography. The column was equilibrated with 0.04 M sodium acetate, 6 M urea, pH 4.8, and maintained at 42°C. Protein was eluted with a gradient of 0 to 80 mM NaCl in 200 ml of column buffer at a flow rate of 26 ml/h. Conductivities were read at room temperature. Type I lathyritic rat skin collagen was chromatographed as a standard. Recoveries of protein were from 65 to 85%.

molecular weight material was also present. All three chains were resistant to human skin collagenase under conditions where type I collagen was cleaved by greater than 90% (Fig. 3). Fig. 3 also illustrates that the TC* peptide of the $\alpha_1(III)$ chain ($M_r = 72,000$) migrates with the 70K fragment.

Separation of the Type IV Chains

Chromatography of reduced and alkylated type IV chains on agarose A-5m resulted in separation of the three major fragments according to their apparent molecular weights, as shown in Fig. 4. The 140K fragment migrated between $\beta$ components and $\alpha$ chains of type I collagen, while the 100K fragment co-eluted with $\alpha(I)$ chains.

Ion exchange chromatography on CM-cellulose indicates that the 140K and 100K fragments elute as single peaks but at different conductivities (2.12 mmho and 2.82 mmho, respectively) (Fig. 5). The 70K component, however, eluted as two peaks, at conductivities of 2.20 (70K-I) and 2.75 (70K-II) mmho, $\alpha(I)$ chains were eluted at 2.82 mmho.

Fig. 6 shows that these four fragments are largely homogeneous by SDS-electrophoresis. There was no difference in the appearance of 140K and 100K after chromatography on CM-cellulose (data not shown). However, there appears to be a slight difference in mobility between 70K-I and 70K-II which was not apparent before CM-cellulose chromatography. At this point, we can state that we have isolated from human placenta four major collagenous components which, after pepsin treatment, contain disulfide bonds and have apparent molecular weights of 140,000, 100,000, 70,000, and slightly less than 70,000.

Sedimentation Equilibrium Studies

The molecular weight distribution of the 140K fragment in guanidine/HCl at various fringe displacements is shown in Fig. 7. The data indicate that the material was relatively homogeneous under these experimental conditions. Averages from the individual channels provided the following molecular weight and root mean square error estimates: $M_w = 143,500 \pm 2800$, $M_r = 145,000 \pm 650$, and $M_z = 146,500 \pm 450$ g/mol. The Z-average molecular weight may be somewhat high due to the presence of a small amount of aggregated material (data not shown). The average molecular weight calculated from all the data was 144,560 ± 1090.

Comparative Structural Studies on the Type IV Chains

Amino Acid Analysis—The amino acid compositions for each of the type IV fragments purified by agarose and CM-cellulose chromatography are presented in Table I. They suggest a high degree of similarity among the 140K, 100K, and 70K-I fragments, with 70K-II appearing to be substantially different. The first three fragments have characteristic type IV amino acid compositions, as compared to type I collagen: elevated levels of hydroxylysine, hydroxyproline, isoleucine, leucine, tyrosine, and phenylalanine (indicating an overall increase in hydrophobicity for these collagens), decreased levels of arginine, lysine, proline, and alanine, and the presence of 3-hydroxyproline. The overall amino acid composition of

![Fig. 6. SDS-electrophoresis of purified type IV collagen fragments.](http://www.jbc.org/)

Fig. 6. SDS-electrophoresis of purified type IV collagen fragments. Proteins were resolved on a composite 6:10% slab gel in the presence of 50 mM dithiothreitol and visualized with Coomassie blue. The 140K, 100K, and 70K fragments were analyzed after elution from agarose A-5m. The 70K-I and 70K-II fragments were analyzed after elution from CM-cellulose (CMC). The standard is human skin type I collagen. Weights are indicated as kilodaltons (Kd).
The 140K fragment is quite similar to that reported for collagen isolated from human glomerular and bovine lens capsule basement membranes (4, 25), but differences are apparent in cysteine levels (0 to 1 in lens capsule versus 3 in 140K) and in the degree of hydroxylation of proline and lysine, particularly in the 3-hydroxyproline content. The 140K amino acid composition is also similar to that of a pepsin-treated collagen isolated from a murine basement membrane tumor (10) and from amniotic fluid cells in culture (11), although differences are apparent in hydroxylysine, lysine, hydroxyproline, proline, and cysteine as well as in the levels of serine, isoleucine, and leucine.

The hydroxylysine-linked carbohydrate content of the 140K fragment reported in Table I is comparable to that determined by Kefalides (4) for the \( \alpha 1(IV) \) chain derived from glomerular basement membrane. If one calculates a carbohydrate content of 10.4% by weight for the 140K fragment and uses the molecular weight of 145,000 determined by sedimentation equilibrium, a molecular weight of approximately 130,000 can be calculated for the polypeptide chain itself. The average residue molecular weight for the 140K fragment is 99.8. The \( M_\alpha = 130,000 \) chain is therefore comprised of 1300 amino acid residues, and is approximately 30% longer than an \( \alpha \)-size chain. This figure may be an overestimate if significant amounts of nonhydroxylysine-linked carbohydrate are present. In comparison, the helical section of the \( \alpha 1(II) \) chain has a molecular weight of approximately 93,000 and contains 1014 amino acids with an average residue molecular weight of 91.

The amino acid composition of the 70K-II fragment is intermediate between those of type I collagen and the 140K fragment, as seen in the elevated arginine and alanine and decreased glutamic acid, hydroxylysine, and 3-hydroxyproline levels (compared to 140K). In addition, the increase in isoleucine, leucine, and phenylalanine suggests a unique primary structure for the 70K-II fragment. The amino acid compositions of all the type IV fragments have features in common with those reported for the collagen \( \alpha A \) and \( \alpha B \) chains (13, 26), but distinct differences between \( \alpha B \) and 140K include the degree of post-translational modification of prolyl and lysyl residues (in collagens isolated from the same tissue), elevated levels in \( \alpha B \) of arginine and alanine, and decreased levels of serine,

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the hydrophobic amino acids isoleucine, leucine, and phenylalanine.

**CNBr Peptide Analysis**—The peptide patterns produced by cleavage of the type IV fragments with CNBr, followed by SDS-electrophoresis, are shown in Fig. 8. These “maps” support the tentative conclusions drawn from the compositional data, in that the 140K, 100K, and 70K-I fragments all appear quite similar, with the 70K-II fragment displaying a markedly different pattern. All the type IV chains appear distinct from types I, III, and the A and B chains with respect to their CNBr cleavage patterns.

**Mast-cell Protease Cleavage**—Additional peptide mapping was performed using an enzyme with chymotrypsin-like activity, mast cell protease (27). This enzyme produces limited but specific and highly reproducible cleavages in denatured collagen chains. The results of a 15-min incubation with different collagen types, followed by SDS-electrophoresis, are shown in Fig. 9. Both the 140K and 100K fragments have as their major cleavage product a polypeptide chain of \( M_r = 65,000 \) on SDS-electrophoresis. This fragment is stable for over 2 h at 37°C at an enzyme/substrate ratio of 1:100 (w/w). The 70K fragments do not appear to have this cleavage product, but rather are digested to lower molecular weight fragments. Time course studies have shown that the \( M_r = 65,000 \) molecule is not an intermediate in the digestion of 70K with mast cell protease (data not shown). 70K-I (which is only partially digested in Fig. 9) has as its major cleavage product a single band which migrates considerably faster than the \( M_r = 65,000 \) cleavage product of the 140K fragment on SDS-electrophoresis. Additional lower molecular weight bands can be seen, three of which appear to co-migrate with those produced by cleavage of the 140K fragment. It is also clear that 70K-I and 70K-II are different with respect to their mast cell protease maps; 70K-II appears to be much less resistant to the enzyme since most of its cleavage products are of lower molecular weight.

Native type IV collagen was found to be resistant to chymotrypsin and trypsin at 22°C; however, digestion at 37°C for 1 h at enzyme/substrate concentrations of 1:50 and 1:100 (w/w) resulted in many cleavages and produced principally peptides of lower molecular weight (data not shown). The mast cell protease was found to digest native type IV collagen under conditions where types I and AB were uncleaved (Fig. 10). It is principally the 140K fragment which appears to be cleaved and the products are different in molecular weight from those seen with the denatured 140K fragment (see Fig. 9).

The method also demonstrates clear differences among the collagen types and suggests that, among the basement membrane collagens, type AB forms a subfamily which is structurally distinct from that to which the type IV collagens belong.

**Partial Characterization of the Major 140K Mast Cell Protease Fragment**

The \( M_r = 65,000 \) polypeptide chain, produced by cleavage of the 140K fragment with mast cell protease (140K-MCP) was purified by molecular sieve chromatography on 6% agarose (data not shown), and its amino acid composition was determined (Table I). Compared to that of the 140K fragment, the composition of the mast cell protease-produced fragment was generally quite similar, although differences can be seen in levels of serine, proline, leucine, and methionine. Further characterization of this cleavage product is in progress.

**DISCUSSION**

The data presented here are consistent with the presence of two type IV-like collagen chains in human placenta. A major difficulty associated with most studies of the type IV collagens has been the extreme insolubility of these proteins, presumably due to the presence of multimeric, disulfide-bonded aggregates composed of both collagenous and noncollagenous sequences (3). One approach has involved the use of pepsin with purified basement membranes in an attempt to isolate and characterize only triple-helical collagenous sequences. Much of the controversy about the primary structure of type IV collagen has resulted from various interpretations of data derived utilizing this methodology.

While pepsin treatment has been most useful in structural studies of types I, II, and III collagens, in which the enzyme removes only the NH₂- and COOH-terminal telopeptides of these molecules, it appears that type IV collagen contains several more centrally located pepsin-sensitive sites (10, 28). Whether these sites are comprised of nontriple helical se-

![Fig. 9. Digestion of type IV collagen fragments with mast cell protease and comparison with human types I and AB collagens. The cleavage products were analyzed by SDS-electrophoresis on a 10% slab gel in the presence of 50 mM dithiothreitol and stained with Coomassie blue. 25 µg of protein was applied to each lane. The positions of migration of the \( \alpha_1(1) \) and \( \alpha_2 \) chains and the \( M_r = 65,000 \) fragment are identified.](image)

![Fig. 10. Comparative susceptibilities of native human types I, IV, and AB collagens to mast cell protease. Collagens were digested at 22°C with mast cell protease for 48 h. The reaction mixtures were analyzed by SDS-electrophoresis on composite 7.5:12.5% slab gels in the presence of 50 mM dithiothreitol and stained with Coomassie blue. 25 µg of protein was applied to each lane. A gel of the major cleavage product of the denatured 140K fragment (140K-MCP) is shown.](image)
quences which interrupt the triple helix, or result from local instability in the helix itself, has not been demonstrated. Accordingly, the structure of type IV collagen in basement membranes has been interpreted as: 1) many different collag-

genous segments having a wide molecular weight range (after reduction and alkylation without protease treatment) (5, 22); 2) a more limited number of collagenous chains ranging in molecular weight from approximately 50,000 to 140,000 (after pepsin treatment) (6, 7, 29); and 3) a “core unit” of a chain size collagenous sequence, having a chain composition α1(IV), after pepsin treatment, with an amino acid composition distinct from those of other collagen types (4, 25).

A number of studies describing the biosynthesis of type IV-like procollagens have also been reported (8-10). Recent studies in our laboratory have indicated that amniotic fluid cells secrete a type IV-like procollagen which migrates as a closely spaced doublet on SDS-electrophoresis (11) with an apparent molecular weight for each chain of 170,000. This protein is not processed to chains of lower molecular weight and has an amino acid composition very similar to that of the mouse tumor procollagen described by Timpl and co-workers (10).

We have demonstrated that the major type IV-derived pepsin-resistant fragment isolated from human placenta has a Mₐ = 140,000, is disulfide-bonded, and contains approximately one-third glycine. Moreover, two other major fragments having a Mₐ = 100,000 and 70,000 (70K-1) appear, by the criteria of amino acid composition and CNBr peptide maps, to be derived from the 140K fragment.

We also searched for other ways to map these collagen fragments. Mast cell protease was used in this regard. A Mₐ = 65,000 polypeptide was produced from both 140K and 100K, but not from 70K-1; 70K-1, however, shared three lower molecular weight cleavage products with 140K, while 70K-11 had a different cleavage pattern altogether. From the limited and specific cleavages produced by this enzyme, we can draw three conclusions about the structural relatedness of the major type IV-derived fragments: 1) the 140K and 100K fragments arise from the same portion of a single precursor type IV molecule, suggesting that 100K is a truncated version of 140K and that this precursor has at least two pepsin-sensitive sites; 2) 70K-I does not contain, at least in toto, the Mₐ = 65,000 sequence which is common to both 140K and 100K, but it does share considerable structural homology with the two larger fragments; and 3) the 70K-II fragment appears to be derived from either a different portion of the precursor chain or, more likely, from another collagen chain as judged by the criteria of amino acid composition, CNBr peptide analysis, and mast cell protease cleavage. The two 70K chains are also readily resolved by CM-cellulose chromatography. It is, however, possible that all of these fragments are mixtures of similarly sized chains due to the existence of multiple pepsin-susceptible sites in type IV collagen.

It is of interest that Dixit (29) has isolated a 50K fragment from pepsin digests of lens capsule which is very similar in amino acid composition to 70K-II. Our data argue strongly for the presence, in placenta, of two distinct gene products belonging to the subfamily of type IV collagens. Additional support for this proposal awaits the isolation of a larger molecule from which the 70K-II fragment can be shown to be derived.

Evidence for a carbohydrate-containing triple-helical collagenous sequence in type IV collagen of Mₐ = 140,000 was provided by SDS-acrylamide gel electrophoresis and agarose chromatography using appropriate collagen standards. This determination is subject to errors resulting from the anomalous behavior of glycoproteins in these systems and from different levels of glycosylation among the collagens. Nevertheless, when provisions are made for the hydroxylsine-linked carbohydrate content of the 140K fragment and when the molecular weight of 145,000 determined by sedimentation equilibrium is used, a molecular weight of approximately 130,000, i.e. 30% longer than an α₁ chain, can be calculated for the polypeptide chain.

An interesting observation during these experiments was the apparent lack of susceptibility of native type IV collagen to human skin collagenase, under conditions where type I was specifically cleaved by greater than 90% to TC⁴ and TC⁸ peptides. We have obtained similar results with AB collagen (30). The type IV-like collagens from the mouse tumor and from amniotic fluid cells have also been found to be resistant to human skin collagenase (31, 11). These results suggest that the so-called basement membrane collagens are not degraded in vivo by the same mechanisms used for the interstitial collagens. They also suggest that the Gly-Ile/leucine site and its topography may not have been conserved or that this sequence represents a specialization confined to the interstitial collagens.

The apparent resistance to vertebrate collagenase led us to examine the action of mast cell protease toward native type IV collagen. We found that the enzyme failed to cleave pepsin-treated or lathyritic type I or AB collagen but did digest type IV, specifically the 140K fragment. The fragments produced were different from those generated from the denatured, purified type IV chains, indicating that the specificity for native molecules differs from that seen with denatured chains. The enzyme used in the present study was isolated from rat skeletal muscle and recently has been shown to be of mast cell origin (27). It is physically, chemically, structurally, and immunologically equivalent to a chymotrypsin-like serine protease isolated from rat peritoneal mast cell granules. Connective tissue mast cells are observed most frequently in the vicinity of blood vessels, and it is generally accepted that mast cell degranulation is the triggering mechanism for much of the increase in vascular permeability occurring in the early stage of inflammation. At this time, however, any proposals regarding the physiological implications of our observation that mast cell protease will degrade native type IV collagen must await further studies.

In summary, our data are consistent with the presence of two type IV-like collagen chains in placenta. Evidence has been presented for close structural homology among three fragments having unique molecular weights. The major pepsin-resistant fragment has an apparent Mₐ = 140,000, contains approximately one-third glycine residues, and is disulfide-bonded. The other two fragments, having an apparent Mₐ = 100,000 and 70,000, appear to be derivatives of the Mₐ = 140,000 chain. Evidence has also been presented for the existence of a second collagenous chain which appears to be structurally distinct.

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