The Tissue Form of Type VII Collagen Is an Antiparallel Dimer*  

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We recently reported the partial characterization of a new human collagen termed Type VII. This molecule is distinctive among the collagen family in that it contains three identical subunit α chains within a triple helical domain 424 nm in length. The molecule contains three identical α chains which are genetically distinct from other known collagens. Previous studies indicate that a portion of the limited pepsin-solubilized molecules appears to exist as antiparallel dimers associated by disulfide bonds.

In this report, we demonstrate that the major tissue form of Type VII collagen is a dimer, associated by disulfide bonds through a 60-nm overlap of the aminoterminal triple helical ends. Intermolecular disulfide bonds occur only within this overlap region. Interchain disulfide bonds exist in the carboxyl terminal 7% of the molecule and may exist within the overlap region as well. Disulfide bond-stabilized aggregates larger than dimers are not seen.

In addition to the abundant interstitial collagens, Types I, II, and III, Types IV through X are present in a variety of tissues in relatively small amounts (1). While the functions of most of these minor collagens are not known in detail, it is generally believed that each collagen has a unique function, although only Types IV, VI, and VII have been identified with specific ultrastructural entities (i.e. the basal lamina (2), beaded microfilaments (3), and the anchoring fibrils, respectively). It is unclear whether the remaining collagens form independent fiber systems, or if they are separate elements of the major collagen fiber systems. For example, Type V collagen has been implicated both as a component of the major collagen fiber system of cornea (4) and as an independent network of 12-nm fibrillar components of the pericellular interstitium of human amnion (5).

Type VII collagen is a homopolymer of three identical chains which are genetically distinct from other collagen α chains. The 424-nm triple helical domain contains a pepsin-sensitive interruption nearly midway along the triple helix.

The peptides, P1 and P2, generated by pepsin digestion of this helix are independently associated by interchain disulfide bonds (6) and are noted as (P1)α and (P2)α. Based upon ultrastructural similarities between segment-long-spacing crystallites of Type VII collagen and the banding patterns of anchoring fibrils, we recently postulated that Type VII collagen is a major structural component of these fibers found in the sub-basal laminae of certain basement membranes (7). The ultrastructural analyses suggested the Type VII collagen molecules can associate in an overlapping, antiparallel fashion to form a covalently stabilized complex 780 nm in length. In this report, we present evidence for an antiparallel dimeric aggregate of two Type VII molecules which is stabilized by intermolecular disulfide bonds at the amino terminus of the molecule.

**MATERIALS AND METHODS**

Preparation of Type VII Collagen, P1, P2, and C—Type VII collagen was isolated from human amniotic membranes as previously described (6). Fragments (P1) and (P2)n were generated from Type VII collagen by exposing partially purified molecules (1 mg/ml in 0.5 M acetic acid) to pepsin (100 µg/ml) at 4°C for 6 to 20 h. Denatured P1, P2, and α(VII) chains were separated following disulfide bond reduction with 2-mercaptoethanol by reverse-phase HPLC on C-18 (Vydac) equilibrated with 10 mM trifluoroacetic acid, 11% acetonitrile. Elution was performed using a superimposed linear gradient from 11 to 56% acetonitrile at room temperature. Alternately, denatured peptides were purified by molecular sieve chromatography by HPLC on TSK-4000 (2.15 x 120 cm) equilibrated in and eluted with 40 mM Tris/acetate, pH 6.8, 6 M urea, 60 mM Na2SO4, at 0.5 ml/min. Separation of Type VII and partial resolution of (P1)α and (P2)α without denaturation were obtained by chromatography on HPLC C-18 or C-4 (Vydac) equilibrated in 10 mM trifluoroacetic acid, 12% acetonitrile, with a superimposed linear gradient 12% to 52.5% acetonitrile. Chromatography was performed at 4°C. Type VII collagen chromatographed under those conditions can be converted to (P1)α and (P2)α, without evidence of further degradation, indicating that conditions are non-denaturing.

Peptide C-1 and clostridial collagenase were separated by velocity sedimentation as described below. Pooled fractions containing C-1 were desalted by chromatography on C-4 (Vydac) as described above.

**Disulfide Bond Reduction and Alkylation**—Samples were dissolved in 100 mM Tris-HCl, 8 M urea, 2 mM dithioerythritol, pH 8.0, and incubated for 4 h at 40°C. Iodoacetamide was then added to 5 mM and the samples were further incubated in the dark for 1 h at room temperature. Reduction without prior denaturation was performed according to the method of Risteli et al. (8) except that 90 mM iodoacetamide was used as an alkylation reagent. Prior to sedimentation, excess reagents were removed by dialysis.

*The abbreviations used are: Type VII collagen, triple helical molecule containing three identical α(VII) chains, the molecule is cleaved by pepsin at a helical disruption nearly midway along the helix producing aggregates of cleavage products, each peptide of which is designated P1 or P2 and the aggregates are indicated as (P1)α or (P2)α, the values of n are a major result of this report; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HPLC, high pressure liquid chromatography.

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Velocity Sedimentation—Sedimentation was performed in an L8-80 ultracentrifuge (Beckman) using an SW60 Ti rotor (Beckman) in polyallomer tubes (Beckman). Two hundred μl of sample were layered onto 5–20% sucrose gradients containing 2 M urea, 50 mM Tris-HCl, 0.8 M NaCl, pH 7.5. Additional runs were done without urea at 0.8 M and at 0.15 M NaCl or in 0.5 M acetic acid-buffered sucrose. Samples were centrifuged for 16 h at 54,000 rpm, 12 °C. Tubes were dripped from the bottom, 6 drops/fraction, yielding 34 to 35 fractions/tube. Fifty μl of fractions 1–20 were analyzed by SDS-PAGE after reduction as previously described (9).

Rotary Shadowing and Electron Microscopy—Rotary shadowing of molecules was accomplished by modification of standard techniques (10, 11). Samples in 0.5 M acetic acid were diluted with glycerol to a final concentration of 70%. One hundred μl of solution were sprayed through an air brush at an acute angle onto freshly cleaved 6-mm mica discs. Droplet sizes were 50 to 200 μm in diameter. Samples were dried in a Balzers MFD 010 evaporator (Torr). Platinum wire was wrapped around the carbon electrodes and the sample was placed on the stage and rotated at 100 rpm. At high voltage, the platinum was evaporated to completion at a 6° angle from the mica surface. The stage was then tilted 90° relative to a carbon source, the chamber was evacuated, and 50 Å of carbon were evaporated onto the surface of the mica. The carbon replica was immediately floated off the mica in double-distilled water and mounted onto 400-mesh grids. The samples were examined at 80 kV, with a 30-μm objective aperture.

Bacterial Collagenase Digestion—Collagenase ( Worthington CLSPA) was purified on Sephadex G-200 according to the method of Peterkofsky and Diegelmann (12). The peak was pooled, treated with 2 mM N-ethylmaleimide, and frozen at −90 °C. Samples for digestion were dissolved in 50 mM Tris-acetate, 60 mM NaSO₄, 6 M urea, pH 6.8, at ambient temperature.

Amino Acid Analyses—Amino acid analyses were carried out using the Pico Tag System (Waters) with the following modifications to achieve resolution of hydroxyproline and hydroxylysine. The reverse-phase column used was a C18 Ultrasphere, 4.6 × 150 mm (Beckman Instruments). Buffer A: 19 g/l sodium acetate trihydrate, 1 ml/l triethylamine adjusted to pH 5.7 with acetic acid. Buffer B: 60% acetonitrile in water. Elution was performed at 50 °C, 1.4 ml/min using the following linear gradient sequence: initial, 9% B; 6 min, 11% B; 11 min, 42% B; 15 min, 45% B; 18 min, 100% B; 22 min, 100% B; 22.5 min, 9% B; and 28 min, 9% B.

Amino Acid Sequences—Amino acid sequences were determined using a Gas Phase Sequencer (Applied Biosystems) Model 470A. PTH derivatives were identified following elution from a reverse-phase Ultrasphere column as described above.

Buffer A: 770 ml of 0.04 M sodium acetate trihydrate, pH 4.8, + 230 ml of acetonitrile. Buffer B: 40% isopropanol in water. Elution was performed at 1 ml/min, using a linear 0–65% B gradient, over 11 min at 50 °C.

RESULTS

Type VII collagen was previously reported to be a homopolymer of three genetically distinct collagen α(VII) chains (6). It contains a triple helical domain 424 nm in length with a pepsin-sensitive site nearly midway along this previously described domain (7). Pepsin cleavage at this site produces two fragments, (P1) and (P2), representing 55% and 45% of the helical length, respectively.

Type VII collagen was isolated from human amnion by limited proteolysis with pepsin. The native molecule precipitates from neutral solutions between 2.8 and 4 M sodium chloride, together with Type V collagen. Type VII collagen was further purified as an undenatured molecule by chromatography on CM-cellulose as shown in Fig. 1. Pooled fractions containing native Type VII collagen were finally purified by HPLC using a C18 reverse-phase column. Electrophoretic analysis of pooled HPLC fractions demonstrates a 5–10% contamination of the final product with two non-collagen proteins of Mr ≈ 100,000 and 80,000. Pure α(VII) was obtained by molecular sieve chromatography on TSK-4000 following denaturation and disulfide bond reduction.

Native (P1), and (P2), fragments were isolated by reverse-phase HPLC, and the denaturation and disulfide bond reduction products were isolated by molecular sieve chromatography on TSK-4000. Amino acid compositions of α(VII), P1, and P2 are shown in Table I. As previously indicated, the α(VII) chain is approximately 95% triple helical based on its relative content of glycine. Upon re-exposure to pepsin, the resultant fragments (P1), and (P2), are entirely triple helical. Both P1 and P2 contain cysteine, consistent with previous observations that both (P1), and (P2), are independently disulfide-bonded.

Segment-long-spacing crystallites of native Type VII collagen preparations demonstrated centrosymmetric banding patterns and lengths consistent with dimeric aggregates of

| Amino acid | Type VII | P1 | P2 |
|------------|----------|----|----|
| Aspartic acid or asparagine | 60 | 63 | 68 |
| Glutamic acid or glutamine | 94 | 94 | 102 |
| Hydroxyproline | 83 | 105 | 92 |
| Serine | 41 | 38 | 35 |
| Glycine | 303 | 327 | 338 |
| Histidine | 6.2 | Tr | Tr |
| Arginine | 65 | 67 | 59 |
| Threonine | 23 | 18 | 11 |
| Alanine | 61 | 59 | 60 |
| Proline | 82 | 91 | 81 |
| Tyrosine | 6.9 | 3.6 | 2.4 |
| Valine | 22 | 18 | 21 |
| Methionine | 8.9 | 3.9 | 6.9 |
| Cysteine | 5.0 | 6.8 | 4.7 |
| Isoleucine | 16 | 7.5 | 11 |
| Leucine | 48 | 42 | 49 |
| Hydroxylysine | 41 | 41 | 41 |
| Phenylalanine | 11 | 6.5 | 9.3 |
| Lysine | 13 | 8.7 | 8.7 |

a Tr = trace.

b Determined as carboxymethylcysteine.
Type VII collagen associated by a 60-nm overlap of the same end (7). Furthermore, our original observations indicated that the α1(VII) chains were associated by disulfide bonds into aggregates with apparent molecular weights larger than predicted for a trimer. In order to determine the size of the disulfide-bonded Type VII aggregate, a preparation of Type VII collagen which had been partially converted to (P1)₆, and (P2)₆, was analyzed by electrophoresis, velocity sedimentation, and transmission electron microscopy prior to and following disulfide bond reduction.

Type VII Collagen Is Stabilized by Interchain and Intramolecular Disulfide Bonds—Native Type VII collagen (1 mg/ml, 0.5 M acetic acid) was incubated with pepsin (100 μg/ml) for 9 h at 4 °C. These conditions of proteolysis allow a substantial conversion of Type VII collagen to the fragments (P1)₆, and (P2)₆. Undigested Type VII collagen and triple helical (P1)₆, and (P2)₆, were individually isolated from the mixture by reverse-phase chromatography as described under “Materials and Methods.” Purified Type VII collagen (Fig. 2, lanes 1 and 2), isolated (P1)₆, fragments (Fig. 2, lanes 3 and 4), and (P2)₆, peptides (Fig. 2, lanes 5 and 6) were analyzed by SDS-PAGE. Each of these samples was analyzed prior to and following disulfide bond reduction.

A comparison of lanes 1 and 2 in Fig. 2 demonstrates that, prior to reduction, the isolated Type VII exists as a heterogeneous disulfide-bonded aggregate. Little material is visualized with mobilities equivalent to the α1(VII) or α1(VII): chains. Reduction effectively dissociates the aggregate into bands with mobilities equivalent to the α1(VII).

Analysis of the (P1)₆, fragment indicates that (lane 3) the majority of the unreduced material has a mobility less than that expected for three covalently linked P1 peptides. Following reduction (lane 4), the aggregate dissociates primarily to P1 peptides. Comparison of lanes 5 and 6 indicates that before reduction (lane 5) (P2)₆, fragments migrate with mobilities expected for three covalently linked P2 peptides. Following reduction (lane 6), this aggregate dissociates entirely to P2 peptides. The data indicate that only P2 is covalently linked by disulfide bonds into the trimeric structure (P2)₆, (P1)₆, as well as whole Type VII collagen chains migrate more slowly than expected for trimers, suggesting that Type VII collagen molecules are linked by intermolecular disulfide bonds through the (P1)₆, region but not through the (P2)₆, region of the molecule.

To allow further interpretation of the size of the (P1)₆, and Type VII aggregates, nonreduced and reduced (under non-denaturing conditions) components isolated by reverse-phase HPLC were visualized by transmission electron microscopy following rotary shadowing.

In order to obtain these data, it became necessary to establish disulfide bond reduction conditions which were sufficiently rigorous to disrupt the majority of the covalent cross-links, but not disturb the triple helical conformation of the peptides. Henceforth, these materials will be referred to as “native reduced” components. As shown in Fig. 3, the nonreduced mixture of Type VII, (P1)₆, and (P2)₆, (lane 1) demonstrates heterogeneous electrophoretic mobilities slower than α1(VII), P1, or P2 peptides. Following native reduction (lane 2) or following complete reduction (lane 3), the disulfide bond-crosslinked components now migrate predominantly as α1(VII), P1, and P2 peptides.

Type VII Collagen Molecules Are Disulfide Bond-stabilized Dimers—Isolated, triple helical Type VII, (P1)₆, and (P2)₆, were visualized following rotary shadowing by transmission electron microscopy prior to and following native reduction. These results are contained in Fig. 4. As previously indicated, unreduced Type VII collagen is visualized as a dimer 785 nm in length associated by an overlap of approximately 60 nm (Fig. 4A). Its native reduction product (Fig. 4B) is seen as a 424-nm monomer indicating that disulfide bond reduction under native conditions causes separation of the dimeric structure at the overlap region. Unreduced, isolated (P1)₆, is visualized as a dimer, 405 nm in length (Fig. 4C), again associated through a 60-nm overlap. The native reduction product of this aggregate (Fig. 4D) is a monomer measuring...
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Fig. 4. Visualization of Type VII, (P1)₄, and (P2)₃ following rotary shadowing. Partially purified Type VII (A and B), (P1)₄ (C and D), and (P2)₃ (E) were visualized before (A, C, and E) and following native reduction (B and D). The images of (P2)₃ (E) were unchanged by reduction. Magnification × 59,000. Representative 231 nm. The images of (P2)₃ are indistinguishable prior to and following native reduction (Fig. 4E), supporting the hypothesis that the disulfide cross-links of the P2 peptides are only interchain and not intermolecular. However, the rotary shadowing technique allows clear visualization of only a small percentage of the total molecules in the preparation. Thus, the extent to which these dimeric structures represent the total material is undetermined. In order to evaluate the average size of the intermolecular disulfide-bonded aggregates, we have analyzed the unreduced and reduced preparations of Type VII, (P1)₄, and (P2)₃ by velocity sedimentation.

**Dimers Are the Predominant Molecular Forms of Type VII Collagen and Not Higher Order Aggregates**—Aliquots of non-reduced and native reduced Type VII collagen pepsin products were sedimented through sucrose gradients as described under “Materials and Methods.” Following sedimentation, fractions were collected dropwise from the bottom of the tube and each fraction was subjected to electrophoretic analysis following complete disulfide bond reduction. Fig. 5A represents the profile obtained from intact aggregates while Fig. 5B represents the profiles as seen following native reduction. Prior to native reduction, whole Type VII collagen sediments two to three fractions faster than its reduction product, consistent with the behavior of a dimer. Similarly, prior to reduction, (P1)₄ sediments approximately three fractions faster than its reduction product. The sedimentation pattern of only the (P2)₃ fragment is unaffected by reduction. Although the sedimentation pattern for Type VII and for (P1)₄ shows some dispersity toward faster sedimenting components, these distributions are no broader than that observed for the (P2)₃ aggregate which is entirely trimeric (see Fig. 2). The combined rotary shadowed images and velocity sedimentation behaviors of Type VII and (P1)₄ fragments are consistent with the conclusion that Type VII molecules are covalently associated by intermolecular disulfide bonds as dimers. The covalent bonds stabilizing this association are contained within (P1)₄. Likewise, (P1)₄, is a covalent dimer of (P1)₄.

The sedimentation of nonreduced and native reduced (P1)₄ dimers and Type VII dimers was repeated through sucrose gradients containing 0.15 M sodium chloride, 0.8 M sodium chloride, or 0.5 M acetic acid. Neither the Type VII dimer nor the (P1)₄ dimer remains associated under any of these salt or pH conditions, suggesting that the interaction between the molecules are compared in F1-F5. F1, Type VII dimer, length = 790 nm; F2, Type VII, 424 nm; F3, (P1)₄, dimer, 406 nm; F4, (P1)₄, 230 nm; F5, (P2)₃, 190 nm. Magnification × 112,000.
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Fig. 5. Velocity sedimentation analysis of Type VII, (P1)\textsubscript{n}, and (P2)\textsubscript{n} under nondenaturing conditions. Unreduced (A) and reduced but un-denatured (B) mixtures of Type VII, (P1)\textsubscript{n}, and (P2)\textsubscript{n} were sedimented through sucrose as described under "Materials and Methods" conditions which retain the triple helical conformation. The electrophoretic positions of α1(VII), P1, and P2 are indicated. The sucrose gradients were fractionated dropwise from the bottom of the tube and the contents of each fraction were analyzed by 5% polyacrylamide gel electrophoresis. Profiles were visualized by Coomassie Blue staining. The direction of sedimentation is from left to right as indicated (S).

Triple helices within the overlap region is not sufficient to stabilize these dimeric aggregates under the conditions of sedimentation. In contrast, the "7 S" domain of Type VII collagen sedimented under the low salt conditions has been shown to remain associated as a tetrameric structure after disulfide bond reduction (8). This aggregate dissociates during sedimentation at acid pH values or under the higher salt conditions.

Interchain and Intermolecular Disulfide Bonds Are Located at the Type VII Collagen Terminii—The above data demonstrate that intermolecular disulfide bonds occur within the 60-nm overlap region of the (P1)\textsubscript{n} fragments, and that (P2)\textsubscript{n} contains interchain disulfide bonds. The positions of these interchain covalent cross-links were then investigated, as one would predict that they occur within nonhelical regions. Cyanogen bromide peptides were prepared from whole Type VII collagen and subjected to electrophoresis before and after disulfide bond reduction. A major peptide (CB-A) has been consistently observed with an apparent Mr of approximately 82,000. This large peptide is present in a lower molar ratio than expected for a fully cleaved peptide, and it appears to contain one or more methionine residues which are relatively resistant to cleavage (data not shown). There are no differences in the patterns obtained before and after reduction for peptides with molecular weight greater than approximately 15,000 (Fig. 6). Cyanogen bromide fragments were then prepared from α1(VII), and from the P1 and P2 peptides. A comparison of the elution positions following sieve chromatography on TSK-3000 (Fig. 7) indicates that CB-A from α1(VII) is lost following pepsin-mediated conversion to P1 and P2. In its place, peptide CB-A1 segregates to peptide P2, and CB-A2 occurs in P1. The estimated Mr for CB-A1 (60,000) and CB-A2 (22,000) sum to the estimated Mr of CB-A (82,000). These data indicate that this fragment of the α1(VII) chain contains the pepsin cleavage site. CB-A is not disulfide-
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**Fig. 8.** Estimate of the relative molecular weight of the collagenase-resistant fragment (C-1) of collagen VII. Portions of unreduced and reduced but undenatured type VII were digested to completion using clostridial collagenase. Collagenase digestion of reduced collagen VII allowed recovery of only small peptides (not shown). The digestion products of unreduced collagen VII were separated from bacterial collagenase by sedimentation through sucrose gradients. The recovered peptide was desalted by reverse-phase chromatography and analyzed by 10% polyacrylamide gel electrophoresis before (lane 1) and following (lane 2) complete disulfide bond reduction using 2-mercaptoethanol (2-Me). Relative molecular weights were estimated by comparison to the electrophoretic mobilities of cyanogen bromide peptides generated from digestion of α1(II) (lane 3).

**Fig. 9.** Diagrammatic representation of the proposed structure of the triple helical domain of collagen VII. Type VII collagen can be purified from human amniotic membranes solubilized by limited digestion using pepsin. As illustrated, the vast majority of recovered molecules are dimeric, stabilized by intermolecular disulfide bonds within an overlap of 60 nm which occurs at the amino terminus. Indirect evidence suggests that nonhelical regions are likely to occur at both the amino and carboxyl termini. Complete digestion of the collagen VII dimer using pepsin produces two types of peptides: a dimer of (P1)₃ containing the overlap region and (P2)₂ peptides which consist of the carboxyl-terminal 45% of each collagen VII molecule. The conversion of collagen Type VII to [(P1)₃]₂ and (P2)₂ occurs at a site within a large peptide CB-A and cleaves this peptide into CB-A₁, representing 80% of the P2 peptide and CB-A₂. CB-A is not disulfide-bonded; therefore, the pepsin cleavage site does not contain interchain disulfide bonds. The intermolecular disulfide bonds of dimeric Type VII or of [(P1)₃]₂ are contained within fragment C-1 (Mᵣ = 18,000) which is resistant to digestion with clostridial collagenase if the disulfide bonds remain intact. Peptide C-1 accounts for approximately 90% of the amino-terminal overlap.

 bonded; therefore, there are no interchain disulfide bonds in the region of the pepsin cleavage site.

**The Overlap Region, C-1, Contains Intermolecular Disulfide Bonds**—The disulfide-bonded “7 S” region of Type IV tetramers is resistant to digestion with bacterial collagenase (8). By analogy, the isolation of the overlap region of Type VII collagen was attempted. Both whole Type VII collagen dimers and (P1)₃ dimers (not shown) were incubated with clostridial collagenase under native conditions prior to and following disulfide bond reduction. Only nonreduced Type VII dimers and (P1)₃ dimers allowed recovery of collagenase-resistant peptides (data not shown). Undigested peptides were purified from bacterial collagenase by velocity sedimentation and desalted by HPLC reverse-phase chromatography. The recovered peptide was analyzed by SDS-PAGE before and after reduction (Fig. 8). Relative molecular weights were estimated by comparison to Type I collagen CNBr peptides and to Type I collagen chains. Prior to disulfide bond reduction, the peptide migrates as a high molecular weight aggregate, Mᵣ > 100,000. Following reduction, the peptides migrate as a single major species, Mᵣ = 18,000. This collagenase-resistant peptide is termed C-1.

The overlap region containing peptide C-1 was demonstrated to be the amino terminus of Type VII collagen by sequence determinations of α1(VII), P₁, and P₂. The results (not shown) indicate that the terminal 12 residues of P₁ and α1(VII) are identical and different from those of P₂, demonstrating that the amino terminus is contained within the overlap region of the Type VII dimer.

**Discussion**

Based upon the results reported here, our current understanding of the structure of the Type VII collagen molecule is summarized in Fig. 9. As isolated by limited pepsin proteolysis, the Type VII molecule is recovered as a dimer of two extended triple helices, each 424 nm in length (2). Forty-eight percent of the helical length of each molecule is accounted for by the fragment CB-A, Mᵣ = 82,000. This peptide spans the internal pepsin cleavage site and does not contain intermolecular or interchain disulfide bonds. Therefore, this short nonhelical disruption within the major triple helix is not stabilized by covalent disulfide cross-links. The intermolecular disulfide bonds which stabilize Type VII collagen dimers are contained within a region of the amino terminus indicated as C-1. The absolute relative molecular weights of both the C-1 peptide and its disulfide-bonded analog are difficult to estimate by electrophoretic measurements since C-1 is likely to contain disruptions in the triple helix at the sites of disulfide bonding and the exact placement of disulfide bonds in the aggregate will affect its electrophoretic mobility. However, the data clearly demonstrate that the Mᵣ of the aggregate is greater than that predicted for a trimer of C-1 regions and therefore must contain intermolecular disulfide bonds. Hence, this peptide must be derived from the Type VII collagen overlap region. Assuming the Mᵣ of C-1 to be approximately 18,000, C-1 corresponds to nearly 90% of the 60-nm overlap. Intramolecular disulfide bonds occur only within this region and not at the carboxyl terminus of the molecule. Cleavage of Type VII collagen with pepsin produces two fragments, the (P1)₃ dimer and (P2)₂. This cleavage occurs within peptide CB-A. The major portion, CB-A₁, segregates with the P2 peptide where it accounts for approximately 85% of the mass of P2. The remainder of the P2 peptide is composed of at least two cyanogen bromide peptides which must contain the interchain disulfide bonding sites. In collagen sequences thus far determined, interchain disulfide bonds usually occur in nonhelical domains, or at the transition between helical and nonhelical domains (13). Therefore the terminal 28 nm of the P2 domain most likely contains a carboxyl-terminal disulfide bond or a nonhelical region. The remainder of CB-A, CB-A₂, segregates with the P1 peptide. This Mᵣ = 22,000 peptide does not contain disulfide bonds. The region of P1 between CB-
A2 and C-1 contains several additional cyanogen bromide peptides which have not yet been characterized.

Velocity sedimentation analyses indicate that the major species of Type VII and of (P1), are dimers and not higher order aggregates. It is apparent from these sedimentation profiles that the difference in sedimentation rate between (P1), dimer and its reduction product is greater than that between Type VII and its reduction product. This can best be explained by the following argument. Both the Type VII dimer and the (P1), dimer contain an overlap of 60 nm. Since the overlap region has a different rigidity and a higher mass for a given extended length, this portion of both molecules will contribute differently to the sedimentation behavior of each species than the remainder of the molecule. Because this region is more compact, one would predict that the observed sedimentation velocity would increase as the percentage of the total structure contributed by the overlap region increases. Length measurements predict that the overlap region represents 15% of the length of the (P1), dimer and 8% of the Type VII dimer. Therefore, the difference in sedimentation velocity observed between the (P1), dimer and monomer should be greater than that observed for the Type VII collagen dimer and monomer.

In addition to disulfide cross-links, Type VII collagen appears to also be stabilized by nonreducible cross-links. As shown in Fig. 2, lane 2, and Fig. 8, lane 2, both whole Type VII and C-1 contain small amounts of material which migrate more slowly than monomers. It is our impression from multiple electrophoretic analyses of similar materials that Type VI1 collagen is not extensively cross-linked by nonreducible bonds within the triple helical domain.

Indirect evidence suggests that the helical domain which we have described as Type VII collagen is a large fragment of the true matrix molecule. Based on ultrastructural studies of Type VII collagen and anchoring fibrils, it has been postulated that Type VII collagen is a major component of anchoring fibrils (2). Anchoring fibrils are accessory structures to the epithelial basement membranes of several tissues which are subjected to considerable frictional and torsional forces. These include skin, esophagus, cornea, vaginal mucosa, and the chorioamniotic membranes. These structures often appear as horseshoe-shaped fibrillar arrays which originate and insert into the lamina densa and may entrap large collagen fibers underlying the epithelial basement membrane. The (P2), terminal regions (the carboxyl termini) correspond to the sites of insertion into the lamina densa (7). Analysis of the banding patterns of Type VII collagen segment-long-spacing crystallites and anchoring fibrils indicate that the original pepsin-mediated solubilization of Type VII collagen occurs at the interface between the (P2), domain and the lamina densa (7). These observations suggest that an extension of the (P2), domain inserts directly into the lamina densa and becomes covalently cross-linked with some component thereof to provide the observed insusceptibility of this complex. In addition, we postulate that the terminal region of the (P1), domain, adjacent to the overlap region, is likely to also contain additional extensions. This conjecture is based on the observation that, following disulfide bond reduction, Type VII sediments in a nearly monodisperse fashion, as expected for monomers which show little affinity for each other even under nearly physiological conditions. Assuming no concentration effects, this observation suggests that the region of the (P1), domain involved in the overlap may not contain sufficient information to allow recognition and tight binding of these domains. This then suggests that the required aggregation and alignment of C-1 regions which presumably occurs before disulfide bond formation may be specified by a domain which has been lost either during the initial pepsin solubilization of Type VII collagen, or during post-translational proteolytic processing as occurs concurrently with fiber forming processes of other collagen types.

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