Properties of the Collagen Type XVII Ectodomain
EVIDENCE FOR N- TO C-TERMINAL TRIPLE HELIX FOLDING*

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Collagen XVII is a transmembrane component of hemidesmosomal cells with important functions in epithelial-basement membrane interactions. Here we report on properties of the extracellular ectodomain of collagen XVII, which harbors multiple collagenous stretches. We have recombinantly produced subdomains of the collagen XVII ectodomain in a mammalian expression system. rColXVII-A spans the entire ectodomain from δNC16a to NC1, rColXVII-B is similar but lacks the NC1 domain, a small N-terminal polypeptide rColXVII-C encompasses domains δNC16a to C15, and a small C-terminal polypeptide rColXVII-D comprises domains NC6 to NC1. Amino acid analysis of rColXVII-A and -C demonstrated prolyl and lysyl hydroxylation with ratios for hydroxyproline/proline of 0.4 and for hydroxylysine/lysine of 0.5. A small proportion of the hydroxylysyl residues in rColXVII-C (3.3%) was glycosylated. Limited pepsin and trypsin degradation assays and analyses of circular dichroism spectra clearly demonstrated a triple-helical conformation for rColXVII-A, -B, and -C, whereas the C-terminal rColXVII-D did not adopt a triple-helical fold. These results were further substantiated by electron microscope analyses, which revealed extended molecules for rColXVII-A and -C, whereas rColXVII-D appeared globular. Thermal denaturation experiments revealed melting temperatures of 41 °C (rColXVII-A), 39 °C (rColXVII-B), and 35 °C (rColXVII-C). In summary, our data suggest that triple helix formation in the ectodomain of ColXVII occurs with an N- to C-terminal directionality.

Collagen XVII, also known as the 180-kDa bullous pemphigoid antigen (BP180), is a transmembrane protein that is widely known as a structural component of hemidesmosomes, although structures at cell-tissue interfaces other than hemidesmosomes may also contain collagen XVII (1, 2). Mutations in the collagen XVII gene, COL17A1, lead to junctional epidermolysis bullosa, a hereditary blistering skin disease with epidermal detachment from the basement membrane (3).

The cDNA sequence of collagen XVII encodes a type II integral transmembrane protein of 1497 amino acid residues (4). It consists of an intracellular domain of 466 residues, a transmembrane domain of 23 residues, and an extracellular collagenous domain of 1008 amino acids with multiple non-collagenous interruptions. The length of the individual collagenous regions varies from 14 to 242 amino acid residues (4). Collagen XVII exists in two molecular forms, i.e. as a full-length transmembrane homotrimer of three 180-kDa α1(XVII) chains and as a 120-kDa soluble form. The latter corresponds to the extracellular domain and is presumably released from the cell surface by furin-mediated proteolytic processing (5). In some instances, an even shorter fragment with ~90–100 kDa has been observed (6).

Some information about the molecular shape of collagen XVII under physiological conditions can be deduced from rotary shadowing electron microscopy of collagen XVII from bovine cell lines or from recombinant fragments. These studies revealed asymmetric molecules with an elongated shape and a globular, ball-like structure at one end (6, 7). A 90-kDa pepsin/trypsin fragment of collagen XVII in detergent extracts of keratinocytes was resistant to further trypsin digestion at physiological temperatures, therefore suggesting that it was of triple-helical structure (5).

The entire collagen XVII as well as the C15 collagenous domain could be recombinantly produced in protease-resistant conformation, apparently not requiring propeptides (7). Triple helix formation of fiber-forming collagens was defined as a two-step process initiated by annealing and aggregation of the C-propeptides followed by C- to N-terminal folding of the three α-chains (8). A higher content of hydroxyproline residues in the C-terminal region of the amino acid sequence of fibrillar collagens has been considered as evidence that the C terminus is the locus of initiation of triple helix formation (8).

Opposite to this, there is also experimental evidence that C-propeptides can be substituted for by other peptide sequences or can be deleted without apparent harm for helix formation (9–11). In fibril-associated collagens with interrupted triple helices, C-propeptides are completely missing, and even if nonhelical ends at the C terminus are deleted in recombinantly produced collagenous molecules, as shown for collagen XII, correct triple helix formation takes place (12).

The present study was designed to address the role of subdomains of collagen XVII ectodomain in triple helix formation and stability, in particular to delineate information on the directionality of the assembly process. With a variety of recombinant polypeptides expressed in a mammalian system, we...
obtained structural information by circular dichroism studies, protease sensitivity assays, and electron microscope studies that strongly suggest triple helix formation from the N to the C terminus of collagen XVII.

**EXPERIMENTAL PROCEDURES**

*Construction of Expression Plasmids for Subdomains of the Collagen XVII Ectodomain—* Human cDNA coding for collagen XVII (4) was used as the template for amplification by the polymerase chain reaction with appropriate primers. The numbering of nucleotides in cDNAs are according to Giudice et al. (Ref. 4, GenBank™ accession number M91669), and the deduced protein sequence was counted from the methionine start codon (position 1010 in M91669). All constructs were digested by appropriate restriction enzymes and were cloned into an episomal expression vector pCEP-Pu containing the signal peptide sequence of BM-40 and a puromycin resistance gene (13). Cloning of inserts into pCEP-Pu via an NheI restriction site resulted in secreted polypeptides with four additional N-terminal amino acid residues (APla) preceding the expressed sequence (13). Sequences and correct in-frame insertions of all constructs were verified by DNA sequencing (Medigenomix).

An expression plasmid for rColXVII-A corresponding to the extracellular domain of collagen XVII (amino acid residues 527–1497) was constructed by amplification of the cDNA with the sense primer 5′-TTGCTGACTCTTTGTCTCCTTTTTCTC-3′ (nucleotides 1684 to 1707) and antisense primer 5′-ACGCGTCGACTCACGGCT-3′ (nucleotides 4574 to 4596). The NheI-Sal fragment of the product was subcloned into pCEP-Pu, resulting in plasmid pCEP-rColXVII-A. This expression plasmid generates a 971-amino acid residue protein with the N terminus within the NC16a domain and the C terminus at the cognate end of the ectodomain.

The expression plasmid for rColXVII-B (amino acid residues 527–1492) was constructed by amplification of the cDNA with the sense primer 5′-TTGCTGACTCTTTGTCTCCTTTTTCTC-3′ (nucleotides 1684 to 1707) and antisense primer 5′-ACGCGTCGACTCACGGCT-3′ (nucleotides 4574 to 4596). The NheI-Sal fragment from this product was subcloned into pCEP-Pu, resulting in plasmid pCEP-rColXVII-B. This plasmid encodes 956 amino acid residues and is identical to rColXVII-A except for a 15-amino acid deletion (NC1 domain) at the C-terminal end.

To prepare the expression plasmid for rColXVII-C (amino acid residues 527–808), the cDNA was amplified with sense primer 5′-TTGCTGACTCTTTGTCTCCTTTTTCTC-3′ (nucleotides 1684 to 1707) and antisense primer 5′-ACGCGTCGACTCACGGCT-3′ (nucleotides 4526 to 4551). The NheI-Sal fragment from this product was ligated into pCEP-Pu, resulting in plasmid pCEP-rColXVII-C. This plasmid encodes a 282-amino acid residue polypeptide corresponding to the extracellular domain of the NC16a domain and the C15 domain.

The expression plasmid for rColXVII-D (amino acid residues 1188–1497) was generated by cDNA amplification using the sense primer 5′-TTGCTGACTCTTTGTCTCCTTTTTCTC-3′ (nucleotides 1684 to 1707) and the antisense primer 5′-ACGCGTCGACTCACGGCT-3′ (nucleotides 4574 to 4596). The NheI-Sal fragment from this product was ligated into pCEP-Pu, resulting in plasmid pCEP-rColXVII-D. This plasmid encodes a 116-amino acid residue polypeptide spanning from the NC1 to the NC6 domain.

*Generation of Recombinant Cell Clones and Production of Conditioned Medium—* Human embryonic kidney 293-EBNA cells (Invitrogen) were cultivated in Dulbecco’s modified Eagle’s medium (Life Technologies) containing 10% fetal calf serum, 0.25 mg/ml G418 (Calbiochem), 0.1 mg/ml penicillin/streptomycin (Biochrom KG), and 2 ml glutamine (Biochrom KG). The pCEP-rColXVII-B, pCEP-rColXVII-C, and pCEP-rColXVII-D were separately transfected into 293-EBNA cells (one million cells/10 cm² culture dish) using a calcium phosphate precipitation method (14). After a selection with 0.5 μg/ml puromycin (Calbiochem), the transfected cells were grown to confluence, washed twice with phosphate-buffered saline, and switched to serum-free medium containing 50 μg/ml ascorbic acid (Sigma), freshly prepared. The media were collected every 24 h for 12 days (approximately 50 ml), and the cells were washed with 1 ml protease inhibitor Pefablock (Roche), and frozen at -80 °C until further use.

*Purification of Recombinant Collagen Type XVII Subdomains—* The serum-free media (2–3 liters) containing recombinant fragments of rColXVII-A and rColXVII-B were concentrated to ~80 ml by ultrafiltration and dialyzed against 20 mM Tris-HCl, pH 8.8, at 4 °C. The material was passed over an anion exchange column (HiTrapQ, 5 ml; Amersham Pharmacia Biotech) equilibrated in the same buffer and subsequently eluted with a linear 0–0.4 M NaCl gradient. Fractions containing the recombinant fragments were concentrated by ultrafiltration to ~0.5 ml and passed over a Superose 12 column (30 ml; Amersham Pharmacia Biotech) equilibrated in 50 mM sodium acetate, pH 4.8, 200 mM NaCl. Fractions were collected, and recombinant fragments were identified as described above.

*Electrophoresis and Western Blot Analysis—* SDS gel electrophoresis (Laemmli, 1970) was performed according to standard procedures. 6% polyacrylamide gels were used for rColXVII-A and -B, and 10% polyacrylamide gels were used for rColXVII-C and -D. Polyclonal rabbit antisera NC16a was used at 1:1000 diluted for rColXVII-A, -B, and -C (17), and polyclonal chicken antisera Col17ecto1–5 (15) was used at 1:20-diluted for rColXVII-D. The antisera were a generous gift of Dr. L. Bruckner-Tuderman. Goat-anti rabbit (1:1000), Dako or goat anti chicken (1:1000) linked to horseradish peroxidase was used as secondary antibodies.

*Circular Dichroism Measurements—* Purified recombinant rColXVII polypeptides were adjusted to a concentration of 20 μg/ml and dialyzed against 0.05% acetic acid. Far UV circular dichroism spectra (190–260 nm) were recorded in a 1 cm quartz cuvette on a Jasco J-715 A spectropolarimeter equipped with a temperature controller. The molar ellipticities were calculated on the basis of a mean residue molar mass of 96 g/mol. Thermal transition curves were recorded at a fixed wavelength (221 nm) by raising the temperature linearly at a rate of 30 °C/h using a Gilford temperature programmer. No deviation from the melting profiles were observed with extended temperature gradients. The degree of triple helicity at various temperatures was calculated by setting the 221-nm signal at 20 °C to 1 (maximal amount of triple helicity) and at 45 °C to zero (completely denatured collagen).

*Analysis of Hydroxylation and Glycosylation—* For amino acid analysis, purified recombinant rColXVII polypeptides were hydrolyzed with 6 N HCl for 24 h at 110 °C. Samples were analyzed on an amino acid analyzer (Biochrome 20, Amersham Pharmacia Biotech) using ninhydrin for post-column color development.

For determination of glucosylgalactosylhydroxylysine and galactosylhydroxylysine samples, up to 1 mg of purified rColXVII-C was hydrolyzed in 1 ml of 2 N KOH for 24 h at 110 °C. After hydrolysis, 100 μl of glacial acetic acid and 150 μl of 70% perchloric acid were added, mixed, and centrifuged for 10 min at 14,000 rpm. The supernatant was decanted and lyophilized. Lyophilized samples were re-dissolved in 1 ml of water and passed over a CF1 (Whatman) column to remove the majority of amino acids. Eluates were lyophilized and analyzed on an amino acid analyzer (Biochrome 20, Amersham Pharmacia Biotech) using ninhydrin for post-column color development.

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*N-terminal Sequence Analysis—* Purified native or pepsin-digested rColXVII-C were subjected to reversed-phase high-performance liquid chromatography and transferred onto a Mini Pro Blott membrane (Applied Biosystems). Protein bands were visualized by Coomassie Blue staining by comparison with immunoblotted material. Relevant protein bands were excised and loaded directly onto a Procise 494 protein sequencer (Applied Biosystems) for N-terminal sequencing.

**Enzyme Digests—** For assessment of the domain structure and stability of collagen XVII, purified recombinant rColXVII polypeptides...
were subjected to treatment by various enzymes. Collagenase treatment was used to release collagenous peptides, and pepsin treatment was used to remove protease-sensitive non-collagenous regions. Trypsin digestion was used to determine the melting temperature of the rColXVII fragments A and C.

Digestion of purified polypeptides with highly purified bacterial collagenase (Sigma) was performed with 40 units/ml enzyme in 0.2 mM NH₂HCO₃, freshly prepared, at 37 °C for 2 h (18). For pepsin digestion, 100 µl of rColXVII polypeptides (−25 µg) were acidified by dialysis against 0.05% acetic acid and incubated with 1 µg/ml pepsin (Roche Diagnostics) at 4 °C for 24 h (19). After neutralization with saturated Tris solution, samples were separated by SDS gel electrophoresis followed by Western blotting using anti-NC16a and anti-Col17ecto-1 antibodies.

For the triple-helical conformation with trypsin (20), the purified samples (−20 µg/ml) were dialyzed against 100 mM Tris-HCl, pH 7.4, 0.4 M NaCl and preheated for 5 min at each desired temperature between 20 and 46 °C (2 °C steps). An aliquot of 10 µl was removed, cooled quickly to 20 °C, and treated with 10 µl of trypsin (1 mg/ml; Sigma) for 2 min. Reactions were stopped by adding 10 µl of soybean trypsin inhibitor (5 mg/ml) to each individual sample. The incubated samples were analyzed by SDS gel electrophoresis followed by Western blotting with specific antibodies. Intensities of the immunoblotted bands were quantified with Gel-Pro-Analyzer (Media Cybernetics).

RESULTS

Production and Characterization of Recombinant Col XVII Subdomains—To investigate triple-helix formation and stability of the collagen type XVII ectodomain, we have produced several recombinant subfragments of the ectodomain in mammalian 293 cells. These subdomains included the full-length ectodomain rColXVII-A (positions 527–1497), a C-terminal truncated form thereof, rColXVII-B (positions 527–1482), the largest collagenous stretch, C15 (positions 527–808), and a short C-terminal subdomain rColXVII-D (positions 1188–1497) spanning the NC6 to the NC1 domain. These recombinant fragments are schematically summarized in Fig. 1. Each of the constructs was designed with a heterologous signal peptide to enable secretion into the cell culture medium and proper post-translational modifications. The recombinant cell clones produced each subdomain in quantities of 1–2 µg of protein/ml medium/day. Ion exchange chromatography followed by gel filtration chromatography resulted in highly purified recombinant subdomains with apparent molecular masses of 101 kDa (rColXVII-A), 96 kDa (rColXVII-B), 30 kDa (rColXVII-C), and 33 kDa (rColXVII-D), which corresponded well with the calculated values (Fig. 2). Interestingly, the collagenous domain fragment rColXVII-C migrates as a broad band, similar to what has been published recently for a corresponding construct (21). No multimers have been observed after electrophoresis by SDS gel electrophoresis (Fig. 2). N-terminal sequencing of rColXVII-C, which N-terminally starts at the same position as rColXVII-A and -B, demonstrated the expected N-terminal sequence (APLAMA) but also some minor N-terminal truncations of 2–5 amino acid residues (Table I). Sequencing of the C-terminal construct rColXVII-D resulted in a sequence (APGNSWSSIS) that was three amino acid residues shorter than expected (Table I). These results indicate that the sequence APLA, which represents an artificial sequence due to the expression strategy (see “Experimental Procedures”), is not stable in the recombinant constructs. The authentic collagen type XVII sequences, however, were stable.

The rColXVII-A and rColXVII-D polypeptide were further analyzed by amino acid analysis. Within the limits of error, the amino acid compositions determined correlated well with the expected compositions calculated from the cDNA (Table II). For rColXVII-A, about 30% of the total prolyl and 34% of the total lysyl residues were hydroxylated. For rColXVII-C, about 29% of the total prolyl and 36% of the total lysyl residues were hydroxylated (Table II). Some of these residues in rColXVII-C were also further modified by the attachment of a small but significant number of mono- and disaccharides (0.01 monosaccharide and 0.17 disaccharide per molecule; Table II).

Pepsin and Collagenase Degradation of Recombinant Col XVII Subdomains—Limited enzymatic digestion with pepsin converted rColXVII-A into a ~30-kDa fragment via an intermediate protein of the apparent molecular mass of 58 kDa (Fig. 3, panel A, lane 2), whereas digestion with bacterial collagenase completely degraded this recombinant ectodomain under the experimental conditions. Similar data were obtained for the C-terminally truncated rColXVII-B (Fig. 3, panel B). The 30-kDa pepsin fragments corresponded to the size and the immunoreactivity of rColXVII-C (collagen XVII domain C15), indicating that this domain adopts a stable triple-helical conformation in rColXVII-A and -B and, thus, resists degradation by pepsin. Recombinant rColXVII-C itself was also resistant to limited degradation with pepsin, whereby a slightly (~3 kDa) faster electrophoretic mobility of the pepsin-treated material was observed (Fig. 3, panel C). N-terminal sequencing of the pepsin-treated rColXVII-C revealed a N-terminally trun-
tion of 32 amino acid residues, corresponding to parts of the non-collagenous NC16a domain (Table I). Accordingly, the Gly-X-Y repeats of rColXVII-C were not degraded by pepsin. These results suggest a triple-helical conformation for rColXVII-C and indicate that more C-terminally located domains are not required for triple helix formation of domain C15. Treatment of the C-terminal rColXVII-D with pepsin resulted in a complete degradation, indicating the absence of a stabilizing triple-helical conformation (Fig. 3, panel D). Controls with bacterial collagenase demonstrated complete degradation of all recombinant polypeptides (Fig. 3, lanes 3).

Ultrastructural Analysis of Recombinant rColXVII Subdomains—To obtain information of the molecular shape, the recombinant rColXVII subdomains were analyzed by electron microscopy after rotary shadowing (Fig. 4). The full-length ectodomain rColXVII-A displayed a long extended shape with a globular portion at one end (Fig. 4A). Measurement of the lengths of individual particles revealed 3 groups of about 60–70 nm, 130–140 nm, and 240–250 nm. A length of 130–140 nm corresponds well with expected values for the ectodomain (22). A length of 240–250 nm may represent dimers that occur through lateral alignment, whereas shorter fragments may derive from proteolytic cleavage of the polypeptide. The globular domain at one end is about 11–12 nm in diameter. The widths of the rotary-shadowed molecules (~ 4 nm) are consistent with the width of triple-helical collagen molecules, accounting for about 2 nm for the platinum coat. The molecules often adopt relatively rigid conformations including some wave-like regions but without the presence of sharp kinks or bends.

Rotary-shadowed molecules of rColXVII-C display an extended thread-like shape with a uniform length distribution of 63 ± 5 nm (Fig. 4B). The extended shape and the width of the molecules clearly indicate a triple-helical conformation of rColXVII-C. No globular regions nor obvious kinks were observed. Since this construct starts at the same amino position as rColXVII-A, we conclude that the globular domain in rColXVII-A represents the C-terminal end of the molecule.

The C-terminal rColXVII-D always resulted in globular particles of about 10–12 nm in diameter, with some resemblance to the globular domain at the C-terminal end of rColXVII-A (Fig. 4C). No extended regions, suggestive for triple-helical collagenous domains, were observed. Since rColXVII-D contains five collagenous regions (C1–C5), we concluded that this recombinant construct was not able to adopt triple-helical conformations and instead assembled into a globular pepsin-sensitive aggregate (see also Fig. 3D, lane 2).

Conformation and Stability of the Recombinant Collagen XVII Fragments—To further investigate structural aspects, the recombinant subdomains rColXII-A, -B, and -C were analyzed by spectral and thermal circular dichroism analyses. For rColXVII-C, a spectrum typical for a collagen triple helix with a maximum at 221 nm was observed (Fig. 5A). Purified molecules that include non-collagenous domains (rColXII-A and -B) demonstrated shoulders at 221 nm (Fig. 5A). These shoulders did not reach values of positive ellipticities typical for triple helices. However, when rColXVII-A (Fig. 5A) or -B (not shown) were treated with pepsin to remove the non-collagenous regions before analysis, typical maxima at 221 nm were observed. These data demonstrate that pepsin-resistant portions of rColXVII-A or -B adopt a triple-helical conformation. We concluded that in intact rColXVII-A or -B the ellipticities originating from non-collagenous regions interfered with those originating from collagenous triple-helical regions to produce shoulders instead of maxima at 221 nm.

Thermal denaturation profiles of the recombinant fragments were recorded as a decrease of ellipticity at 221 nm (Fig. 5B). Polypeptide rColXVII-C demonstrated a melting temperature of about 35 °C and a melting range from 30 to 40 °C. Full-length ectodomain rColXVII-A melted at about 41 °C, with a melting range of 35 °C to 46 °C. After treatment of rColXVII-A with pepsin, a biphasic melting curve was observed. The first phase of this profile resembles the denaturation profile of rColXVII-C and represents likely the intact C15 triple-helical domain in the proteolytic degradation mixture. The second phase demonstrated a somewhat higher melting temperature of about 38 °C and probably represents domain C15 plus additional C-terminal regions corresponding to the 58-kDa fragment obtained after limited pepsin degradation of rColXVII-A (Fig. 3A, lane 2). The denaturation profile of rColXVII-B was, as expected, very similar to the results obtained with rColXVII-A, with a range of melting temperature between 34 and 46 °C. After pepsin degradation of rColXVII-B, a biphasic melting curve was observed, and the melting temperature decreased from 39 to 34 °C (data not shown).

The results obtained by circular dichroism analysis were further confirmed by using a temperature-dependent trypsin digestion assay (20). In this test, denaturation of the triple-helical regions of rColXVII-A and -C was measured at various temperatures by monitoring the degradation of the protein by trypsin. The results of this assay are shown in Fig. 6. Melting temperatures of about 43 °C for rColXVII-A (Fig. 6A) and of about 33 °C for rColXVII-C (Fig. 6B) are in good agreement with data obtained by circular dichroism (Fig. 5B). The rColXVII-A is not degraded by trypsin at temperatures below ~35 °C, although many non-collagenous domains containing arginine and lysine residues are present in this polypeptide. This result indicates that the non-collagenous domains are protected against proteolytic attack by trypsin under the conditions applied.

**DISCUSSION**

Collagen XVII is a transmembrane component of hemidesmosomes and other cell-matrix interfaces. Although the function of the collagen XVII intracellular region has been the focus of intensive analyses (23–25), little is known about structure and function of the ectodomain. Here we report on the struc-

![Fig. 2. Purification of recombinant extracellular subdomains of human collagen type XVII.](http://www.jbc.org/content/105/5/1597)
We have recombinantly expressed four constructs composing various regions of the collagen XVII ectodomain: the full-length ectodomain (rColXVII-A), the ectodomain lacking the short C-terminal NC1 domain (rColXVII-B), the longest collagenous region C15 plus 40 N-terminal amino acid residues from the NC16a domain (rColXVII-C), and a C-terminal fragment spanning NC1 to NC6 (rColXVII-D). These recombinant polypeptides were expressed in mammalian 293 cells, a system that previously has been reported to produce correctly folded recombinant collagen XVII constructs (21). All recombinant constructs were designed with a heterologous sequence for a signal peptide to ensure that the recombinant polypeptides pass through the endoplasmic reticulum for post-translational modification. The secreted polypeptides were purified to homogeneity from the cell culture medium by conventional chromatography procedures.

The structure of the recombinant collagen XVII fragments were extensively studied. Analysis of circular dichroism spectra and limited enzymatic digestion with pepsin and trypsin clearly demonstrate that the large fragments rColXVII-A and -B and the much shorter fragment rColXVII-C adopt triple-helical conformations. Based on hydrodynamic properties of the ectodomain, recombinantly expressed in a transient system with COS cells, the ectodomain of collagen XVII has been described as elongated, and a trimeric assembly of \( \alpha \)-chains was expected from sedimentation analysis (7). Furthermore, limited trypsin digestion of authentic collagen XVII suggested a triple-helical conformation of the ectodomain (5). In our studies, we analyzed for the first time the recombinant ectodomain of collagen XVII, represented by rColXVII-A and -B, by circular dichroism. The typical maxima for triple helices at 221 nm were less prominent as compared with “pure” collagenous proteins. However, when rColXVII-A and -B were digested with pepsin, the typical maxima at 221 nm could be observed. These results indicate that the signals from triple-helical regions and non-triple-helical regions interfere, providing an explanation for the reduced maxima at 221 nm. Thermal transition curves recorded at 221 nm showed a melting temperature of 41 °C for rColXVII-A, demonstrating a stable triple-helical conformation at physiological temperatures. These data are in good agree-
ment with data obtained by a trypsin protection assay of the ex vivo preparations (5). Since the C-terminally truncated version rColXVII-B of the recombinant ectodomain displayed very similar circular dichroism spectra and transition profiles, we conclude that the NC1 domain (position 1483–1497) at the C-terminal end does not have a major impact on the stability of the ectodomain. Furthermore, these results indicate that the NC1 domain is apparently not necessary for the formation of the triple-helical conformation. Comparison of thermal denaturation profiles of rColXVII polypeptides determined at 221 nm as a function of temperature. The signal at 20 °C was set to 1 and represents the maximal amount of triple helicity in the polypeptides, and the signal at 45 °C was set to 0 and represents completely denatured triple-helical regions. rColXVII-A (dotted line), rColXVII-A treated with pepsin (dashed line), rColXVII-B (dash-dot-dash line), rColXVII-C (solid line), rColXVII-C treated with pepsin (dash-dot-dot-dash line).

Rotary-shadowed images of rColXVII-A demonstrated extended molecules with a globular domain at one end. The molecules demonstrated several regions of flexibility but no kinks. These results are in agreement with data from sedimentation studies suggesting more flexibility than a rigid rod (7). Electron micrographs from rColXVII-C, the long collagenous stretch, also revealed a long extended shape of 63 nm. Although the molecules appeared relatively rigid, some curved molecules were observed. Based on the absence of a globular domain in rColXVII-C, we can now unambiguously assign the globular domain in rColXVII-A to the C-terminal end, since both fragments start at the same N-terminal position. This C-terminal globular domain was either not present or only occasionally present in other studies (6). In this regard it is interesting that rColXVII-D, corresponding to the C-terminal end, ultrastruc-
We suggest that the three domains contribute somehow to the stability of the C15 domain, additional residues (positions 527–566) N-terminal to the C15 temperature (26.5 °C; Ref. 21). This comparison suggests that the transition curve revealed a significant lower melting temperature than that of rColXVII-A (53% in rColXVII-C, 74% of the eligible prolyl residues versus 53% in rColXVII-C), the transition curve revealed a significant lower melting temperature (26.5 °C; Ref. 21). This comparison suggests that the 40 residues originating from the NC16a domain but is otherwise identical to rColXVII-D is able to adopt its native conformation and rather aggregates to a globular structure.

For rColXVII-C, comprising 81 Gly-X-Y (including one Gly-Ser-Gly) repeats plus 40 additional N-terminal amino acid residues from the NC16a domain, clear evidence for triple-helical conformation was obtained by circular dichroism analysis or by enzyme resistance experiments. These data may indicate that fragment rColXVII-D is not able to adopt its native conformation and rather aggregates to a globular structure.

![Figure 6: Temperature-dependent trypsin digestion assay of rColXVII-A (A) and rColXVII-C (B). Samples were pre-heated to discrete temperatures in 2 °C steps from 20 to 46 °C and then incubated with trypsin. Degradation was monitored by Western blotting after gel electrophoresis (upper panels). Note that rColXVII-A and -C were only degraded at elevated temperatures. The intensity of the bands was quantified by densitometry. The protein amounts in arbitrary units are plotted as a function of temperature (lower panels).](http://www.jbc.org/)

FIG. 6. Temperature-dependent trypsin digestion assay of rColXVII-A (A) and rColXVII-C (B). Samples were pre-heated to discrete temperatures in 2 °C steps from 20 to 46 °C and then incubated with trypsin. Degradation was monitored by Western blotting after gel electrophoresis (upper panels). Note that rColXII-A and -C were only degraded at elevated temperatures. The intensity of the bands was quantified by densitometry. The protein amounts in arbitrary units are plotted as a function of temperature (lower panels).

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For rColXVII-C, comprising 81 Gly-X-Y (including one Gly-Ser-Gly) repeats plus 40 additional N-terminal amino acid residues from the NC16a domain, clear evidence for triple-helical conformation was obtained by circular dichroism analysis or by enzyme resistance experiments. These data may indicate that fragment rColXVII-D is not able to adopt its native conformation and rather aggregates to a globular structure.

The triple-helix formation of fibril-forming collagens starts at the C-terminal end of the molecule (8, 27). Collagen XVII is a type II transmembrane protein with an intracellular N and an extracellular C terminus. It has been suggested that unlike other collagens, the membrane-bound collagens fold from the N- to the C-terminal end (28). The data presented here support this hypothesis for several reasons as follows. (i) The deletion of the C-terminal end (28). The data presented here support this hypothesis for several reasons as follows. (i) The deletion of the C-terminal NC1 domain does not abolish triple-helix formation of rColXVII-B. (ii) The small C-terminal fragment rColXVII-D, without the context of more N-terminal sequences, does not adopt a triple-helical structure despite the presence of several collagenous stretches. (iii) The N-terminal C15 collagenous domain can be recombinantly expressed as a triple-helical polypeptide, although it lacks the entire C-terminal portion of the ectodomain. (iv) The stability of this polypeptide increases when N-terminal amino acid residues from the NC16a domain are present.

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Properties of the Collagen Type XVII Ectodomain: EVIDENCE FOR N- TO C-TERMINAL TRIPLE HELIX FOLDING
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