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The NC16A domain of collagen XVII plays a role in triple helix assembly and stability ☆,☆☆

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Abstract

Collagen XVII/BP180 is a transmembrane constituent of the epidermal anchoring complex. To study the role of its non-collagenous linker domain, NC16A, in protein assembly and stability, we analyzed the following recombinant proteins: the collagen XVII extracellular domain with or without NC16A, and a pair of truncated proteins comprising the COL15-NC15 stretch expressed with or without NC16A. All four proteins were found to exist as stable collagen triple helices; however, the two missing NC16A exhibited melting temperatures significantly lower than their NC16A-containing counterparts. Protein refolding experiments revealed that the rate of triple helix assembly of the collagen model peptide GPP_{10} is greatly increased by the addition of an upstream NC16A domain. In summary, the NC16A linker domain of collagen XVII exhibits a positive effect on both the rate of assembly and the stability of the adjoining collagen structure.

Keywords: BP180; Transmembrane collagen; Protein stability; Protein folding; Nucleation site

Collagen XVII (also known as BP180 or BPAG2) is a major transmembrane component of the epithelial anchoring complex [1,2], a structure that functions in maintaining the adhesion of cells in the basal layer of stratified epithelia to the underlying basement membrane. The importance of collagen XVII in maintaining tissue architecture is underscored by genetic and acquired skin diseases in which its loss or lack of function leads to blister formation at the level of the dermal–epidermal junction (reviewed in [3]).

Biochemical studies from several groups have established that the collagen XVII protein exists as a transmembrane homo-trimer, with an N-terminal globular head region comprising the intracellular domain, and a long, highly extended, semi-rigid extracellular domain corresponding to a series of collagen triple helices (Gly–Xaa–Yaa tripeptide repeats) separated by non-collagenous interruptions (see Fig. 1) [4].

It has been hypothesized that collagen XVII and other members of the membrane-associated collagen sub-family undergo triple helix assembly in the N-terminal (membrane-proximal) to C-terminal direction [5], rather than in the C-terminal to N-terminal direction as is typical for the "classical" collagens. Therefore, the membrane-proximal non-collagenous linker region of collagen XVII (designated NC16A; see Fig. 1) has been identified as a likely candidate for the site of association and initiation of triple helix assembly.

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☆☆ *Abbreviations*: AA, amino acid; CD, circular dichroism; Col, collagen; NC, non-collagen; DSS, disuccinimidyl suberate; T_m, melting temperature.
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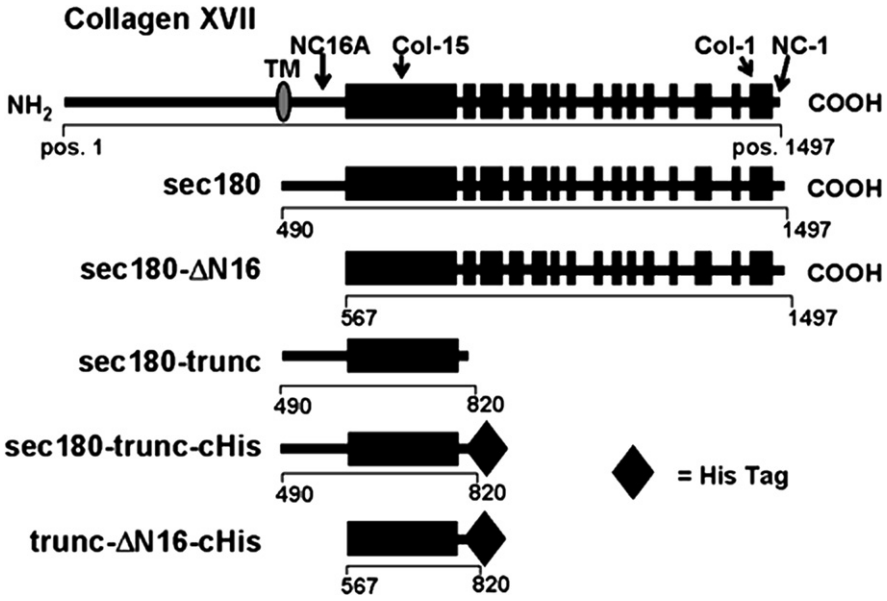

Fig. 1. Schematic representation of recombinant proteins of collagen XVII. The extracellular domain of type XVII collagen (top diagram) consists of 15 collagenous domains (solid boxes) and 16 non-collagenous domains (horizontal lines). Also shown are schematic representations of the recombinant proteins used in this study. The corresponding AA positions are indicated for the termini of each protein (from GenBank Accession NM_130778). The lengths (in AA) of sec180, sec180-ΔN16, sec180-trunc, and trunc-ΔN16 are 1008, 931, 331, and 254, respectively. The His-tag, when present, adds 13 AA to the length of the protein. TM, transmembrane domain; pos, position.

Supporting this idea is the fact that NC16A contains a predicted α-helical coiled-coil structure [4], as do the linker regions of the other members of the membrane-associated collagen sub-family [6]. Also it has been demonstrated that the carboxy-terminal region of collagen XVII is not required for triple helix formation [7]. More recently, Franzke et al. [8] reported that deletion of NC16A from full-length collagen XVII decreases its thermal stability. A disadvantage of using a transmembrane form of the protein for such an analysis is that a detergent is required for solubilization. In the present study we further probed the effects of NC16A on the stability and refolding of collagen XVII using soluble recombinant forms of the ectodomain of this protein.

Materials and methods

Generation of recombinant forms of the collagen XVII ectodomain. The DNA vector pCEP4-sec180, which is based on the pCEP4 episomal expression vector (Invitrogen, Carlsbad, CA) and which encodes a secreted form of the collagen XVII ectodomain, was described previously [4,9]. pCEP4-sec180 was used as the template for PCR amplification of the segments of collagen XVII represented in Fig. 1.

For use in the refolding experiments, a collagen model protein, consisting of 10 GPP triplets (GPP_{10}), with an N-terminal NC16A moiety, was produced as a GST fusion protein using the pGEX-2T bacterial expression system (Pharmacia Biotech, Piscataway, NJ).

Peptide synthesis. A GPP_{10} synthetic peptide ($sGPP_{10}$) was synthesized using Fmoc chemistry on an Applied Biosystems 432A Synergy peptide synthesizer (Applied Biosystems, Foster City, CA). Peptide purity was confirmed by MALDI-TOF (matrix-assisted laser desorption time-of-flight) mass spectrometry.

Immunodetection. Three rabbit antisera reacting with distinct epitopes on the extracellular region of collagen XVII were used in this study. Rabbit sera R594 and R136 react with sites in NC16A and near the C-terminus of collagen XVII, as previously described [9]. Rabbit antiserum, R5151, a generous gift from Dr. Kim Yancey, recognizes sites within the C-terminal two-thirds of the collagen XVII ectodomain.

Immunoblotting was carried out as previously described [9]. When the His-tag was needed as a means of detection, a mouse anti-penta-His antibody (Qiagen, Valencia, CA) was used.

Concentration by immunoprecipitation or by Nickel–NTA affinity-purification. Immunoprecipitation was performed as described previously [9] to detect small amounts of the metabolically radiolabeled recombinant proteins in column fractions. Alternatively, when the His-tag was needed as a means of concentration, the samples were incubated for 2 h with Ni–NTA agarose beads (Qiagen, Valencia, CA).

Gel filtration chromatography. The recombinant forms of collagen XVII were analyzed by gel filtration chromatography using a Superose-6 column (Amersham, Piscataway, NJ), as previously reported [9].

Calculations. The frictional ratio (f/f_0) was obtained using the following equations [4]:

$$M_r = 6\pi N\eta r_s s_{20,\omega}/(1-\ddot{v}\rho) \quad (1)$$

$$f/f_0 = r_s(4\pi N/3M_r\ddot{v})^{1/3} \quad (2)$$

where N is Avogadro's number (6.022×10^{23} mol^{-1}), η is the viscosity of water at 20 °C (1×10^{-3} kg m^{-1} s^{-1}), $\ddot{v}$ is the partial specific volume, ρ is the density of water at 20 °C (0.998 g/ml), r_s is the Stoke's radius, and $s_{20,\omega}$ is the sedimentation coefficient. The axial ratio, P, was calculated from the frictional ratio by fitting the protein as a cylinder using the equation:

$$f/f_o = (2/3)^{1/3}[P^{2/3}/(-0.3+\ln(2P))] \quad (3)$$

Glycerol gradient sedimentation. The sedimentation properties of the collagen XVII recombinant proteins were analyzed by glycerol gradient sedimentation (linear gradient, 10–30% v/v), as previously described [4].

Chemical cross-linking. The various truncated forms of collagen XVII were subjected to chemical cross-link analysis using DSS (disuccinimidyl suberate; Pierce, Rockford, IL), as previously described [9].

Sensitivity to proteolysis. The thermal stability of the recombinant proteins was determined as previously described [9]. When the affinity purification was done by Ni–NTA purification, the reaction was