Crystal structure of the noncollagenous (NC1) domain of human placenta collagen IV

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The heterotrimeric protomers of collagen IV form a network in all basement membranes and provide an anchoring scaffold and mechanical strength to it. Each protomer consists of a long triple-helical collagenous domain and a globule non-collagenous (NC1) domain at the C-terminus. Via this NC1 domain two protomers dimerize and build a hexameric complex consisting of four α1 and two α2 chains.

Collagen IV protomers were isolated from human placenta and the triple helix was digested by collagenase. Subsequently the NC1 hexamers were purified by anion exchange chromatography and were crystallized. The grown crystals had an irregular shape and belonged to the space group R3 with the cell constants a = b = 234.59 Å, c = 99.49 Å, α = β = 90°, γ = 120°. The crystals were mounted in cryo-loops directly from the mother liquor and were shock frozen in liquid nitrogen. X-ray data were recorded at 100 K. In cause of strong pseudo R32 symmetry as well as non-isomorphy problems between native and heavy atom soaked crystals it was not possible to solve the structure with data sets, measured with the in-house rotating anode generator. The structure was solved by MAD experiments at the wigglar beamline BW6 using the 165 mm MarCCD detector. Initial and final experimental phases were obtained by three-wavelength MAD experiments using crystals soaked in 8 mM K₂[OsCl₆] and 1 M NaBr, respectively. The phases from the K₂[OsCl₆] soaked crystal were indispensable for the determination of the large number of Br⁻ sites found within the NaBr soaked crystals. Subsequently, solvent flattening, 2-fold non-crystallographic symmetry averaging, automatic model building and phase combination resulted in an excellent electron density map at 1.9 Å resolution. The model was completed by several cycles of manual inspection, model building and refinement.

Figure 1: Ribbon plot of the NC1 hexamer. The intrachain disulfide bridges are indicated by two yellow balls and the covalent cross-links between Met93 and Lys211 by ball-and-stick representations.
The asymmetric unit of the trigonal crystals contains one NC1 hexamer with four \( \alpha_1(IV) \) and two \( \alpha_2(IV) \) NC1 chains. This hexamer has an ellipsoidal shape and exhibits a virtually exact 2-fold rotation axis (Fig. 1). Two caps, made up of the trimeric NC1 domains of two opposing collagen IV protomers, juxtapose each other via a central planar interface. In each cap, two \( \alpha_1 \) chains (A/B\( \alpha_1 \)-1 and A/B\( \alpha_1 \)-2) and one \( \alpha_2 \) chain (A/B\( \alpha_2 \)) are segmentally arranged, displaced by 120° around a central tunnel, which resulted in a pseudo 3-fold rotation symmetry transforming (on each trimer side) the \( \alpha_1-1 \) chain, the (chemically identical, but environmentally only similar) \( \alpha_1-2 \) chain and the (chemically different) \( \alpha_2 \) chain upon each other.

All six NC1 chains start and end at the pole of the trimeric caps. Due to similarity in sequence and topology, each chain can be divided into the N-terminal subdomain I and the C-terminal subdomain II (Fig. 2). Each subdomain is organized into nine \( \beta \)-strands (I\( \beta_1 \) to I\( \beta_9 \) and II\( \beta_1 \) to II\( \beta_9 \)), one helical turn (I\( \alpha_1 \) and II\( \alpha_1 \)) and connecting segments. In each subdomain, four strands of subdomain I (I\( \beta_4 \), I\( \beta_3 \), I\( \beta_7 \) and I\( \beta_8 \)) form an incomplete \( \beta \)-sheet, which is complemented to a 6-stranded antiparallel sheet by the II\( \beta_5 \)−II\( \beta_6 \) finger-like hairpin loop from subdomain II. Equivalently, strands of subdomain II form also a fragmentary \( \beta \)-sheet, which is completed by insertion of a hairpin loop from the adjacent chain monomer. Within each subdomain three disulfide bridges exist, but no disulfide bridges could be found between two subdomains or between two chains. A quite planar large trimer-trimer interface is responsible for the dimeric interaction between adjacent protomers. Across this interface, the two \( \alpha_2 \) chains mainly juxtapose each other, while the \( \alpha_1-1 \) chains mainly oppose the \( \alpha_1-2 \) chain and vice versa.

![Figure 2: Topological diagram of the \( \alpha_1-1 \) chain showing the subdomains I (N-terminus) and II (C-terminus) in black and grey, respectively. The residues Met\(^{93} \) and Lys\(^{211} \), responsible for the covalent cross-link and the intrachain disulfide bridges (double circles) are explicitly marked. The \( \beta \)-strands provided from adjacent chain monomers to complete the 6-stranded \( \beta \)-sheets are drawn in white.](image-url)

Interestingly, each \( \alpha_1-1 \), \( \alpha_1-2 \) and \( \alpha_2 \) chain makes very close contacts to the \( \alpha_1-2 \), \( \alpha_1-1 \) and \( \alpha_2 \) chain of the opposite trimer, respectively, via their residues Met\(^{93} \) and Lys\(^{211} \) of the II\( \beta_8 \)/II\( \alpha_1 \) and the I\( \beta_7 \)/I\( \beta_8 \)-loops. Crystallographic and biochemical analyses provides strong evidence for a novel covalent side-chain cross-link between methionine and lysine, but the exact chemical nature of the cross-link could not be identified yet. The existence of such a covalent cross-link would also explain the former observation, that purified and dissociated NC1 hexamers can be separated into both, monomers and stable, non-reducible dimers of alpha chains.

**References**
