COMMENTARY

Collagen pretzels revealed by electron microscopy

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Collagen XV is a million-dalton protein with a structural role in skeletal muscle and capillaries. As with all collagens, studies of its function are hindered by the absence of good structural data: collagens are triple-helical, non-crystallizable, multidomain proteins with extensive post-translational modification that are refractory to analysis by high-resolution structural techniques. For collagen XV, this situation is compounded by the fact that it is also a proteoglycan. In this issue of the *Biochemical Journal*, Myers and her colleagues use rotary shadowing electron microscopy to obtain images of purified collagen XV molecules that are sufficiently detailed to show the three-lobed structure of the N-terminus and individual glycosaminoglycan side chains. Individual molecules appear as knotted strands resembling a

pretzel (a pastry snack folded in a unique figure-of-eight), which contrasts with our conventional image of collagen molecules as semi-rigid rods. Importantly, collagen XV multimerizes into cruciform structures in which simpler forms have two to four molecules per complex. Immunoelectron microscopy revealed knotted collagen XV complexes bridging collagen fibrils adjacent to basement membrane. These accomplishments are made all the more impressive by the fact that collagen XV was purified from human umbilical cord, in which the protein is represented at only $(1-2) \times 10^{-4}$ % of dry weight!

Key words: basement membrane, collagen, electron tomography, endostatin, extracellular matrix, fibril, rotary shadowing.

Understanding the structure and assembly of collagens has occupied the attention of matrix biologists and structural biologists for many years. X-ray fibre diffraction studies in the 1950s and recent X-ray crystallography of collagen-like peptides have provided information about the collagen triple helix at atomic resolution (for reviews, see [1,2]). However, most collagens function as part of supramolecular assemblies, which are refractory to study by conventional high-resolution techniques. Consequently, important questions about the structure of native collagens and how they assemble *in vivo* remain unanswered.

Studies in the 1960s–1980s used electron microscopy to record detailed images of type I collagen (the major collagen, found in skin and tendon) and the 67 nm D-periodic fibrils that are formed by self-assembly of type I collagen molecules (for a review, see [3]). In subsequent years other collagens were identified that assemble into extended networks (e.g. collagens IV, VIII and X), anchoring filaments (collagen VII), beaded narrow-diameter microfibrils (e.g. collagen VI), and FACIT (fibril-associated collagens with interrupted triple helices) collagens that bind to the surfaces of collagen fibrils and provide binding sites for ECM (extracellular matrix) macromolecules (for a review, see [4]). We could be forgiven for thinking that we had seen the complete array of supramolecular assemblies that collagen could form. Then along came the paper by Myers and colleagues in this issue of the *Biochemical Journal* [5], which shows that collagen XV assembles into novel and varied conformations including pretzel-shaped monomers and cruciform-shaped complexes. We are given a new insight into the remarkable versatility of the triple helix to generate a plethora of protein conformations that are adapted to the mechanical requirements of the tissue in which collagens occur. We are left asking the question: “How are we every going to discover the structural rules that determine collagen assembly?” But we will come back to this question later.

Collagens are a large family of triple-helical proteins that are crucial for tissue scaffolding, cell adhesion, cell migration, mol-

ecular filtration, tissue morphogenesis and repair [4]. Key components of the ECM, at least 28 different collagens occur in vertebrates (numbered I–XXVIII). The collagens are classified according to their function and domain homology. However, collagen XV and its structural homologue collagen XVIII stand out because they appear to have dual structural and paracrine functions. A major hurdle to understanding the function of these collagens has been to understand their structure and assembly *in vivo*.

Collagen XV was first identified by Myers et al. [6] when a novel collagen cDNA clone was isolated from a human placenta library. It was identified on the basis of a characteristic signature of repeating Gly-X-Y triplets in the primary structure of collagen, in which X and Y can be any amino acid but are often proline and hydroxyproline respectively. The predicted collagenous domain had numerous interruptions, some of which had consensus sequences for attachment of serine-linked GAGs (glycosaminoglycans) and asparagine-linked oligosaccharides, implying that this collagen is extensively post-translationally modified. We now know that collagen XV is a ‘card-carrying’ proteoglycan, as well as being a ‘fully paid-up member’ of the ‘collagen club’. The collagenous domain is flanked by an N-terminal domain that shares sequence homology with thrombospondin-1 repeats found in other proteins and a C-terminal domain that is highly homologous with the endostatin domain of collagen XVIII.

Collagen XV is closely homologous with collagen XVIII, which is important for retinal vascular development and is indispensable for angiogenesis in the eye [7]. The importance of collagen XVIII in the eye has been exemplified by the identification of inactivating mutations in the human COL18A1 gene in people with Knobloch syndrome, which is characterized by age-dependent vitreoretinal degeneration and occipital encephalocele [8]. Collagen XVIII is restricted to BMs (basement membranes) and only sometimes carries a GAG chain. Proteolytic cleavage within the C-terminal domain releases endostatin, which has been reported to have anti-angiogenic properties.

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Gene disruption studies in mice show that collagen XV functions as a structural protein that is needed to stabilize skeletal muscle cells and microvessels. COL15A1-deficient mice show progressive muscular disease and are more vulnerable to exercise-induced muscle injury than wild-type mice. Despite the anti-angiogenic role of collagen XV-derived endostatin, the development of the vasculature is normal in the null mice. Nevertheless, collagen XV-null mice exhibit collapsed capillaries and endothelial cell degeneration in the heart and skeletal muscle [9].

These exciting discoveries about the function of collagen XV and XVIII collagens cry out for detailed structural data on the assembly and organization of these molecules *in vivo*. Myers et al. [5] take the first and important step towards achieving this goal. They show that monomers (i.e. triple-helical homotrimeric molecules) of collagen XV are ~200 nm in length and have a knotted, pretzel-shaped conformation. The N-terminal end has three 7.7 nm-diameter spheres corresponding to the thrombospondin-like domains. Interestingly, collagen XV self-assembles into high-order structures, which can adopt a cruciform pattern with intermolecular binding sites. Myers et al. [5] propose that collagen XV might function as a biological spring to stabilize and enhance resistance to compression or expansion forces. This agrees with their identification of collagen XV-bridging neighbouring collagen fibrils in the vicinity of BMs. It has to be said that their observations were only possible after tenacious efforts to purify the collagen from human umbilical cord, in which it is represented at only a minuscule fraction of 1 % dry weight.

It clearly would have been easier for Myers and co-workers to generate a recombinant form of collagen XV carrying an affinity purification tag. But to do this, they would have had to use a cell that expresses prolyl 4-hydroxylase, was capable of adding GAG chains of the correct size and composition, and secretes the collagen without proteolytic degradation. In addition, the presence of a purification tag might well have altered the ability of collagen XV to assemble into multimers, such as, for example, the pretzel- and cruciform-like assemblies.

And so, returning to my question of how we are going to understand how collagen assembles into supramolecular structures that interact with other macromolecules *in vivo*. The study by Myers et al. [5] demonstrates the power of studying individual molecules that have been isolated from tissue. The availability of purified collagen XV molecules that self-assemble into larger structures

opens up new opportunities for high-resolution imaging, perhaps using atomic force microscopy, mass mapping scanning transmission electron microscopy, deep-freeze etch, and particle averaging for cryoelectron microscopy. The structure of the endostatin domain of collagen XVIII is known. So too are the structures of thrombospondin-1 domains. Therefore it should be possible to combine information from X-ray structures of individual domains with data from atomic force microscopy and electron microscopy to predict (to a close approximation) the structure of the pretzel-like collagen XV molecules and cruciform shapes. Ultimately, we will want to know the precise location of collagen XV in the three-dimensional space between basement membranes and collagen fibrils. One approach that holds enormous promise is cryoelectron tomography of unstained, frozen-hydrated tissue or cells in culture (for a review, see [10]).

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