X-ray diffraction analysis of the 3D organization of collagen fibrils in the wall of the dogfish egg case

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The wall of the egg case of the dogfish, *Scyliorhinus canicula*, contains a network-forming collagen assembled into a regular three-dimensional (3D) structure. It accomplishes supportive, protective and filtering functions for the embryo contained within it. The collagen molecules in the egg case are organized into a body-centred unit cell of dimensions (mean $\pm$ s.d.) $(11.6 \pm 1.0) \text{ nm} \times (11.6 \pm 1.0) \text{ nm} \times (81.6 \pm 3.2) \text{ nm}$, which belongs to the I422 space group. At a higher hierarchical level, the collagen molecules assemble into parallel arrays of fibrils, *ca.* 100 nm in diameter, which aggregate to form laminae *ca.* 0.5 μm thick. These laminae are organized into a plywood-like structure and account for 98% of the thickness of the wall of the egg case. X-ray diffraction patterns of the wall of the egg case were taken along mutually perpendicular directions, one being perpendicular to the surface of the egg case. Three different kinds of diffraction pattern were observed. One of the patterns was characteristic of an X-ray direction perpendicular to the laminae in the egg case (along the *x*-direction). The two other patterns were obtained with the X-rays directed parallel to the plane of the laminae, either along the capsule long axis (*z*) or perpendicular to this (*y*). These two patterns were observed interchangeably in either of the *x*- or *y*-directions depending on the specimen. The diffraction patterns were analysed and interpreted taking into consideration the 3D electron microscope data of the egg case. The results confirm and extend previous findings from transmission electron microscopy and low-angle X-ray diffraction and they suggest that there is only one major type of ordered collagen arrangement in the wall of the egg case.

Keywords: dogfish; egg-case wall; X-ray diffraction; network-forming collagens; type IV collagen; basal membrane

1. INTRODUCTION

The egg-case wall of the dogfish, *Scyliorhinus canicula*, is mainly composed of collagen (Knight & Hunt 1974; Rusaouen *et al.* 1976). It plays a supportive, filtering and protective role for the embryo contained within it. With the naked eye the dogfish egg case appears as an oblong capsule (figure 1*a,b*), *ca.* 50 mm by 20 mm, whose four corners are elongated into tendrils that are fastened to seaweed when the eggs are laid. The egg-case wall is *ca.* 2 mm thick when wet and for approximately 98% of its thickness comprises laminae that are each *ca.* 0.5 μm thick (figure 1*c*; Knight & Hunt 1976). The laminae are formed by parallel arrays of fibrils, each *ca.* 100 nm in diameter (Knight & Feng 1994*b*; Knupp *et al.* 1996). The orientation of the fibrils is reported to change from lamina to lamina with a successive rotation of 45° between the long axes of the fibrils (figure 1*d*; Knight & Hunt 1976). The outer surface of the egg case, accounting for *ca.* 2% of the thickness of the wall, is made of a more darkly coloured and refringent layer that grades into a layer containing many protein granules (Knight & Feng 1994*a,b*; Knight *et al.* 1996). In the bulk of the wall, each collagen fibril contains a quasi-crystalline molecular organization. The

unit cell of this structure was studied using transmission electron microscopy (TEM) and was found to belong to the I422 space group with dimensions of *ca.* 11 nm $\times$ 11 nm $\times$ 74 nm (Knupp *et al.* 1996). A three-dimensional (3D) reconstruction was obtained by combining electron microscope data from different views through the unit cell (figure 1*e*; Knupp *et al.* 1996), and a model for the organization of the molecules within the unit cell was suggested (Knupp *et al.* 1998).

Several X-ray diffraction studies have been done on the dogfish egg case. High-angle experiments, carried out by Knight & Hunt (1974) and Rusaouen *et al.* (1976), reported a 2.9-Å meridional arc typical of a collagen triple helix. Low-angle X-ray diffraction studies were reported by Gathercole *et al.* (1993), who proposed a unit cell with $a=b=22.6 \text{ nm}$, $c=39.3 \text{ nm}$, and also by Hepworth *et al.* (1994) in which the tensile properties of the egg wall were related to the capsule's multilaminate construction.

Here we have used synchrotron radiation to study in detail the low-angle X-ray diffraction patterns from laminae of fresh egg-case wall along mutually perpendicular directions, and we have interpreted these patterns in the light of the calculated Fourier transforms obtained from electron micrographs of different views through the unit cell (Knupp *et al.* 1996). We also recorded

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Figure 1. (a) Dogfish *Scyliorhinus canicula* egg case. The capsule is *ca.* 50 mm $\times$ 20 mm and its corners are elongated into four tendrils that attach to seaweeds when the eggs are laid. (b) Portion of the capsule. The part of the wall highlighted is enlarged in (c). (c) Diagram of a section of the dogfish egg wall. The wall consists of a series of laminae in which the collagen fibrils assume different orientations. (d) Electron micrograph of a zone showing two abutting laminae with different fibril orientations (bar, 50 nm). (e) Three-dimensional reconstruction of the collagen arrangement in the fibrils that form the laminae (bar, 10 nm) (Knupp *et al.* 1996).

comparable data from egg-case wall previously fixed in 2.5% glutaraldehyde for several months. This served as a control for our published 3D reconstruction using electron micrographs from fixed tissue in that it monitored any possible structural changes that might have arisen from the fixation process.

2. MATERIAL AND METHODS

Fresh *Scyliorhinus canicula* dogfish eggs were obtained from the University Marine Biology Station in Glasgow and from the London Aquarium (London, UK). Pieces of egg-case wall were removed and washed in distilled water. Successive laminae were stripped off these and cut into small strips (*ca.* 0.5 mm wide in the plane of the egg-case wall and *ca.* 300–400 μ m thick in the direction perpendicular to the wall) to fit glass capillaries that were 0.5 mm in diameter. The capillaries were then mounted on beamline 2.1 at the CCLRC Daresbury synchrotron radiation source (X-ray wavelength = 1.54 Å; see Harford & Squire (1997) for details of the beam line). The camera length was 2.5 m and patterns were collected on a multiwire area detector. The acquisition time varied from a few seconds to 5 min. During this time the specimens were kept moist by a water reservoir in the capillaries. The orientation of the specimens was chosen so that the laminae building the egg-case wall were either perpendicular to or parallel to the X-ray beam direction. Occasionally, diffraction patterns from different views of the same specimen were taken after the specimen capillary had been rotated by 90° around its long axis. A similar set of observations was made on pieces of egg-case wall that had been fixed and stored in 2.5% glutaraldehyde for several months.

The X-ray patterns were processed and analysed using the BSL data manipulation package (see <http://wserv1.dl.ac.uk/SRS/NCD/manual.bsl.html>). The Fourier transform model was built using Design CAD for Windows. The computed diffraction patterns in figure 4 were obtained by calculating the fast Fourier transforms of different views of dogfish egg-case TEM micrographs of plastic sections taken with a JEOL 1200EX TEM. Specimen preparation procedures and data analysis have been described in detail in Knupp *et al.* (1996).

3. RESULTS AND DISCUSSION

(a) Description of the diffraction patterns

Figure 2 shows representative low-angle X-ray diffraction patterns obtained with the X-ray beam aligned along different directions through the egg-case wall. Figure 2a shows a diffraction pattern typical of one of those patterns obtained with the X-ray beam direction parallel to the laminae in the wall (*y*- or *z*-direction in figure 1c). The meridian of this pattern shows even orders of an 81.6 ± 3.2 -nm repeat (arrowheads). On the equator there is a strong reflection corresponding to an 8.0 ± 1.0 -nm repeat (chevron), and on the first layer-line there are two reflections corresponding to an 11.6 ± 1.0 -nm lateral spacing (arrow). The 11.6-nm reflections appear connected by almost complete circular arcs, the only region missing being close to the equator. The 8.0-nm reflection also shows arcing, but not a gap.

If the specimen is rotated by 90° and X-rays are directed perpendicular to the laminae (*x*-direction in figure 1c), a diffraction pattern of the kind shown in

Figure 2. (a) Low-angle X-ray diffraction pattern from the dogfish egg-case wall obtained with the X-ray beam along a direction perpendicular to the laminae (y - or z -direction in figure 1c). On the meridian are even orders of an 81.6-nm repeat (arrowheads). On the equator there is a strong reflection corresponding to an 8-nm spacing (chevron). The first layer-line has two reflections corresponding to an 11.6-nm spacing (arrows). The 11.6-nm reflections are connected by an almost complete arc, the only gap being across the equator. The 8.0-nm equatorial reflections also smear into arcs. (b) X-ray diffraction pattern obtained with the X-ray beam perpendicular to the laminae in the egg-case wall (x -direction in figure 1c). Rings of intensity corresponding to an 81.6-nm repeat are seen (arrowheads). Inset: the same diffraction pattern plotted using different grey intensities. The curved arrow highlights an 11.6-nm spacing ring. (c) Alternative X-ray diffraction pattern to (a), obtained with the X-ray beam along a direction almost parallel to the

figure 2b is obtained. Here rings of intensity corresponding to an 81.6-nm repeat are observed (arrowheads). It is also possible to recognize an 11.6-nm ring whose intensity is weaker with respect to the other features in the pattern (inset; curved arrow).

Figure 2c shows the second type of X-ray diffraction pattern obtained with the X-ray beam along a direction parallel to the laminae (y - or z -direction). This kind of pattern occasionally arises in place of the one shown in figure 2a. It is believed to originate when the X-ray direction is not exactly parallel to the laminae. In this third kind of pattern, it is still possible to detect reflections corresponding to an 81.6-nm repeat on the meridian (arrowheads). On the equator and above and below this position there are three reflections (arrows) whose equatorial spacing corresponds to 11.6 nm and which lie on a complete circle of intensity. In the different patterns recorded in this study, the upper and lower reflections were observed to vary in their distance from the equator in the range $1/55$ to $1/32 \text{ nm}^{-1}$. In addition, the 11.6-nm equatorial reflection changes its relative intensity from pattern to pattern. Further out, there are two off-equatorial reflections corresponding to an 8-nm spacing (chevrons).

(b) Comparison with the results of Gathercole *et al.* (1993)

The diffraction patterns described above are, in essence, of the same kind as those described by Gathercole *et al.* (1993). We do not have any evidence, though, that supports a dimension for the unit cell of $22.6 \text{ nm} \times 22.6 \text{ nm} \times 39.3 \text{ nm}$. We believe that the doubled spacing reported by Gathercole for the a - and b -dimensions of the unit cell may arise from an extension into arcs of the fourth order of the 81.6 (ca. 20.4)-nm meridional reflections caused by the multiplicity of orientations of the collagen fibrils within the laminae. Inaccuracy in the measurement of the spacing of such arcs owing to their broadening and high intensity might account for the discrepancy. The halved spacing reported for the c -dimension is easily accounted for by the body-centred nature of the unit cell; in fact, all the meridional reflections are even orders of the 81.6-nm fundamental repeat. After these corrections, the Gathercole *et al.* unit cell becomes $11.3 \text{ nm} \times 11.3 \text{ nm} \times 78.6 \text{ nm}$ in dimensions that are consistent, within experimental error, with the dimensions of the unit cell found in this work.

(c) X-ray patterns from specimens stored in a 2.5% glutaraldehyde solution

X-ray diffraction patterns were also taken from specimens that had been stored in a 2.5% glutaraldehyde solution for several months to check the state of preservation and any structural alteration of the collagen arrangement within the egg-case wall. Two representative

laminae in the egg-case wall (y - or z -directions in figure 1c). Reflections corresponding to even orders of an 81.6-nm repeat are observed on the meridian (arrowheads). On the equator and on a layer-line, it is possible to observe three reflections corresponding to 11.6 nm, which lie on a complete circle of intensity. The chevrons highlight two weak reflections corresponding to 8.0 nm.

Figure 3. (a, b) Two representative diffraction patterns (similar to figure 2a, b, respectively) obtained from specimens fixed in 2.5% glutaraldehyde for several months. Diffraction patterns from such specimens do not differ significantly from those obtained using fresh specimens.

patterns are shown in figure 3. Qualitatively, these patterns show no significant differences from equivalent patterns using fresh specimens. Within experimental error the dimensions of the unit cell are unchanged.

(d) *Explanation of the X-ray diffraction observations*

Calculated Fourier transforms of the TEM data of the I422 unit cell have been used for the interpretation of the X-ray diffraction patterns described above. By taking the Fourier transform data from electron microscopy and allowing for different orientations of the collagen fibrils in the egg case, it is possible to create a model of the Fourier transform of the whole piece of egg-case wall exposed to the X-ray beam. The intersection of the model with the Ewald sphere orientated along the x -, y - or z -axes generates different diffraction patterns. Only fibril orientations needed to reproduce all of the observed diffraction patterns recorded are included in the construction of the final model. This line of thought presupposes that the collagen arrangement seen with the electron microscope is the only ordered collagen arrangement present in significant quantity in the egg-case wall. It therefore tests this assumption: if the model thus created is consistent with all of the X-ray diffraction data, we can conclude that the collagen arrangement seen with the electron

Figure 4. Diffraction patterns obtained by Fourier transform computations from areas of electron micrographs showing different views through the dogfish egg-case wall (Knupp *et al.* 1996). (a) Computed Fourier transform of a (1,0,0)-type projection through the unit cell. The meridian shows even orders of the 81.6-nm axial repeat. The row-lines correspond to the 11.6-nm spacing. (b) Computed Fourier transform of a (1,1,0)-type projection. The row-lines correspond to an 8.0-nm spacing. (c) Fourier transform of a (0,0,1)-type (transverse section) projection.

microscope is representative of the major population of collagen throughout the egg-case wall.

Figure 4a shows the calculated Fourier transform of a (1,0,0)-type longitudinal projection through the unit cell

Figure 5. (a) Model of the Fourier transform of the dogfish egg-case fibrils. On the fibril axis (parallel to the vertical arrow) are orders of the 81.6-nm repeat (see figure 4*a,b*). These reflections are arced across the meridian owing to fibril disorientation. The chevrons indicate rings arising from the (1,0,1), (1,0,-1), (1,1,0) and their symmetry-related reflections (see figure 4*a-c*). These rings arise from the random orientation of fibrils around their axes. Other reflections seen in figure 4 are not considered in this model as there is no evidence of them in the X-ray diffraction patterns. (b-e) Models obtained by allowing two main fibril orientations *ca.* $\pm 15^\circ$ apart along four main directions which are themselves 45° apart. The final Fourier model of the fibrils in the dogfish egg-case wall is given by the sum of the models represented in (b-e), when sampled in different ways by the Ewald sphere. Although this model assigns the same weight to all the fibril directions, this is not always the case, and different population densities are encountered in different specimens. The (1,1,0) rings are omitted for clarity.

obtained from plastic sections (Knupp *et al.* 1996). On the meridian we can observe the even orders of the 81.6-nm repeat. The row-lines correspond to the 11.6-nm lateral spacing. Figure 4*b* is the computed Fourier transform of a (1,1,0)-type longitudinal view. Once again the reflections on the meridian are the even orders of the 81.6-nm reflections and the first row-line spacing corresponds to 8.0 nm. Figure 4*c* is obtained after computing the Fourier transform of a (0,0,1)-type transverse view. The unit cell spacing of this view corresponds to a square lattice spacing of 11.6 nm $\times$ 11.6 nm. The three Fourier transforms can be combined together in 3D Fourier space and back-Fourier transformed to obtain the reconstruction of the molecular arrangement in the fibrils (figure 1*e*; Knupp *et al.* 1996).

Figure 5*a* illustrates part of the Fourier transform model used to explain the observed X-ray diffraction data. The model is obtained by combining the calculated diffraction patterns of figure 4. Its rotational symmetry is a product of the assumption that the fibrils have random orientations around their long axes. This implies that the I422 unit cells also have random orientations around the *c*-axis. Accordingly, the Fourier transforms of the unit cells assume random positions around the *c*^{*}-axis in reciprocal space. If many unit cells are sampled by the X-ray beam, the equivalent reflections from different transforms become distributed on rings around the meridian. On the *c*^{*}-axis of the Fourier model (parallel to the vertical arrow on the top left side of figure 5) are orders of the 81.6-nm repeat. These reflections are shown slightly broadened as a result of disorder in the directions of the fibrils. The chevrons indicate rings arising from the (1,0,1), (1,0,-1) and (1,1,0) rings and their symmetrically related reflections (see also figure 4*a,b*). Some of the weaker, outer reflections seen in figure 4*a-c* are not included in this model since there is no evidence for them in the X-ray diffraction patterns. As discussed later, this is probably due to limited packing order within the fibrils in the egg capsule.

The final Fourier transform model can now be generated if different orientations of the fibrils within the laminae of the egg case are taken into account. Figure 5*b-e* shows the different sets of Fourier transform (hence fibril) orientations postulated to explain the diffraction patterns obtained experimentally. The basic repeating object is an assembly of fibrils with a small range of long-axis orientations up to *ca.* $\pm 15^\circ$ apart. The $\pm 15^\circ$ values, measured directly from the X-ray diffraction patterns, should be regarded as the upper and lower limits for an almost continuous series of fibril orientations between the two $\pm 15^\circ$ extremes, all fibrils still remaining approximately parallel to the plane of the laminae. The continuous set of orientations is not reproduced in the drawing for sake of simplicity. This repeating object, with a small range of fibril tilts, is then transformed by 45° rotations between successive laminae (figure 5*b-e*). The contribution of each fibril direction to the final patterns is proportional to the number of laminae with fibrils along that particular direction. For simplicity, the model described above assigns the same weight to all the fibril directions. However, in reality this is not always the case and different population densities are encountered in different specimens. This leads to a different contribution to the

Figure 6. Suggested origin of the diffraction pattern in figure 2a. (a) When the X-ray beam is along z (arrow), fibrils orientated parallel to y generate the pattern in (b) when it intersects the Ewald sphere (a portion of which is drawn in light tones in (a)). (c) A fibril with preferred orientation along y generates the pattern in (d). If both fibril directions are present in the specimen, the sum of the two diffraction patterns will be recorded, as in (e). The 11.6-nm arcs do not continue to the equator as they are generated partly by the $(1,0,1)$ reflection ring of the Fourier transform and partly by the $(1,0,-1)$ reflection ring (see figure 5). The gap between the two rings results in the gap between the two 11.6-nm arcs.

Figure 7. Suggested origin of the diffraction pattern of figure 2*b*. When the X-ray beam is perpendicular to the laminae (i.e. it is along *x*, arrow), the Ewald sphere has a position that is perpendicular to the one in figure 6. The Fourier transform described in figure 6*a* now intersects the Ewald sphere as in (a) giving rise to the pattern in (b). (c) Fourier transform generated by fibrils whose directions are at 90° to those in (a) around the *x*-axis. (d) Pattern generated by the intersection of (c) with the Ewald sphere. (e) Sum of a few possible patterns at 0°, 45°, 90° and 135°. The 81.6-nm rings begin to appear. The 11.6-nm reflections increase their population density and begin to look like a ring (see inset to figure 2*b*).

final recorded pattern intensities from fibrils along different directions and easily accounts for the variability seen in the patterns recorded experimentally.

Figure 6 illustrates the origin of the X-ray diffraction pattern in figure 2*a*. A pair of fibrils orientated horizontally (figure 6*a*) generates the pattern in figure 6*b* when it intersects the Ewald sphere, a portion of which is drawn in lighter tones in figure 6*a*. Here figure 6*a,c* assumes that the X-ray beam direction is nearly vertical. The reflections on the meridian are generated by the fibrils whose directions lie between $\pm 15^\circ$ to the horizontal. Similarly, a set of fibrils orientated at 90° to the first set, this time being almost parallel ($\pm 15^\circ$) to the X-ray beam direction (figure 6*c*), produces the pattern in figure 6*d*. If both sets of fibril directions are present in the specimen, the Fourier transform model is given by the sum of the models of figure 6*a,c* and the diffraction pattern recorded is similar to that in figure 6*e*. The 11.6-nm arcs do not extend to the equator as they are generated partly by the (1,0,1) reflection rings and partly by the (1,-1,0) reflection rings (see figure 5). The gap between the two sets of rings results in the gap between the two 11.6-nm arcs. The sets of orientation at $\pm 45^\circ$ to the vertical position (figure 5*c,d*) intersect the Ewald sphere only at the positions of the 11.6-nm and 8.2-nm spots, thus reinforcing those particular features. This model (figure 5*b-e*) possesses fourfold rotational symmetry around the *x*-axis. This means that the model generates the same kind of diffraction pattern when the Ewald sphere corresponds to X-rays along the *y*-axis or along the *z*-axis.

Figure 7 illustrates the origin of the diffraction pattern of figure 2*b*. When the X-ray beam is rotated by 90° relative to the specimen position in figure 6, the Ewald sphere, now in the plane of the page, intersects the Fourier transforms as in figure 7, where the X-ray beam direction is perpendicular to the page. The Fourier transform models described in figure 6*a,c* now intersect the Ewald sphere in a manner shown in figure 7*a,c*, giving diffraction patterns as in figure 7*b,d*. Figure 7*e* is the sum of the diffraction patterns generated by laminae containing fibrils as in figure 7*a,c*, together with another set of laminae at 45° to the first set around the X-ray beam direction (figure 5*c,d*). In this simulated diffraction pattern the 81.6-nm rings begin to appear and the 11.6-nm reflections increase their population density and begin to look like a ring (see figure 2*b* inset, curved arrow).

Figure 8 illustrates the origin of the diffraction pattern in figure 2*c*. This kind of pattern arises if the X-ray beam direction is nearly but not exactly parallel to the *y*- or *z*-axes. In figure 8*a,c* the corresponding orientations of the Ewald sphere are represented. Figure 8*b* shows the simulated diffraction pattern obtained from the intersection of the Ewald sphere with the portion of the model shown in figure 8*a*. Depending on the exact angle of the X-ray beam with respect to the *y*- (or *z*-) direction, the Ewald sphere will change its inclination and the position of its intersection with the model. This gives rise to a different spacing for the off-equatorial 11.6-nm reflections and a different relative intensity for the equatorial reflection, as observed. Figure 8*d* is the simulated pattern obtained from the intersection of the (1,0,1) and (1,1,0) rings (and related ones) of the model of figure 8*c*. Figure 8*e* is the

sum of the patterns in figure 8*b,d*. The fibril models of figure 5*c,d* intersect the Ewald sphere only at the 11.6-nm ring positions, thus helping to reinforce this feature.

4. CONCLUSIONS

In summary, the observed X-ray diffraction patterns in figure 2 can be explained in a simple way taking into account the structure seen with the electron microscope and a few preferential directions for the collagen fibrils within the laminae. There are no observed X-ray diffraction features that cannot be accounted for by various orientations and sampling of the Fourier transform of the I422 unit cell. In addition, figure 3 shows that the processing of the dogfish egg case in a 2.5% glutaraldehyde solution for electron microscopy does not significantly alter the collagen arrangement in the egg-case wall. Therefore the observed I422 structure seen using the electron microscope is representative in structure, symmetry and spacing of the native collagen unit cell.

Figure 9 combines all of the X-ray diffraction and electron micrograph information discussed in this paper into a model for the anatomy and ultrastructure of the dogfish egg-case wall. The X-ray diffraction observations show that fibrils containing the I422 substructure are all orientated roughly along the same preferred direction within a single lamina. However, in views down the *x*-direction, each lamina contains fibrils grouped with the observed $\pm 15^\circ$ spread of angles around this preferred direction (figure 9*b*). In views along the *y*- and *z*-directions the fibrils must be more nearly parallel (figure 9*c*). Successive laminae then have their preferred directions separated by multiples of *ca.* 45° . Many adjacent layers appear at 45° (135°) separations (figure 9*a*). Others show a 90° separation between adjacent laminae, with good longitudinal and transverse views in the same section.

Apart from the $\pm 15^\circ$ spread, these results confirm the microscopy studies carried out by Knight & Hunt (1976), which describe the fibrils as grouping side-by-side along a common direction and forming uniaxial laminae. The fibril orientations, in turn, are described as changing their direction by 45° from lamina to lamina.

Unfortunately, this new X-ray diffraction study has not provided higher-resolution data on the 3D collagen structure within the I422 unit cell in the dogfish egg-case wall. However, it has shown that only a single molecular arrangement is needed to explain all of the X-ray diffraction data. We ascribe the lack of high-resolution information in the X-ray diffraction pattern to variable degrees of molecular order within different fibrils. Image processing of our electron micrographs (Knupp *et al.* 1996) was obviously carried out on the most highly ordered regions that we could find. The X-ray study, on the other hand, samples everything in the beam. Intrinsic variability in the degree of local order within different fibrils would then wipe out weaker, high-resolution X-ray reflections, as observed.

It is apparent from this and previous studies that the open molecular arrangement within the I422 unit cell of collagen molecules provides a structure that is both strong and at the same time permeable to water, solutes and small proteins (Knight *et al.* 1996). As in the case of plywood, the assembly of such fibrils into laminae that

Figure 8. Origin of the diffraction pattern in figure 2c. This pattern can be explained in a way that is analogous to figure 6, but this time the X-ray beam is not exactly along the y - (or z -) directions but is close to them (arrow). (a, c) Examples of how the slightly tilted Ewald sphere intersects the Fourier transform model. (b, d) X-ray patterns generated by the model in (a, c). (e) The final, fully reconstructed diffraction pattern.

Figure 9. Fibril arrangement in the egg-case wall. (a) Portion of the wall showing the changing preferred orientations of fibrils in successive laminae. Such laminae are *ca.* 0.5 μm thick and the orientation change is usually an odd multiple of 45° . (b) Fibril arrangement in a portion of a lamina viewed in the *x*-direction. The fibrils, each *ca.* 100 nm in diameter, show preferred orientation with a large spread around this direction of up to *ca.* $\pm 15^\circ$. Along each fibril one occasionally sees (1,0,0) or (1,1,0) views through the I422 unit cell, but there is only short-range order along the fibrils and they show a variable amount of local twisting. Successive pairs of stripes are *ca.* 40 nm apart. (c) Side view of the arrangement in (b) (as in particular views along *y* or *z*), in which the fibril orientation is almost parallel to the plane of the laminae with only a small degree of disorientation.

pack together at different angles will confer further rigidity to the egg-case wall so that the embryo inside is well protected. Such protection, both from predators and fouling, may be needed for up to a year. There is evidence that in addition to this structural role, the egg case, built mainly from oxidatively cross-linked collagen molecules, provides antibacterial and general antifouling activity (Thomason *et al.* 1996). This may be partly due to heavy-metal scavenging and partly due to the action of tanning agents, such as dihydroxyquinones, which react with Fe^{2+} to produce hydroxy radicals that act as deterrents.

Although the collagen molecule of the dogfish egg case is very short (*ca.* 45 nm; Knight *et al.* 1996) and therefore substantially different from any other kind of collagen, it shares some basic characteristics with type IV collagen and with other network-forming collagens. In particular, like collagen IV in the basal lamina, it creates an open network with supportive, filtering and particular chemical properties (Timpl *et al.* 1981; Yurchenco & Furthmayr 1984; Yurchenco & Ruben 1987). However, unlike type IV collagen, the dogfish collagen is arranged

at least locally in a rather regular way, thus permitting effective analysis by electron microscopy and X-ray diffraction. It is possible that insights gained about the dogfish collagen may help in the understanding of the analogous function of the much larger and less well-ordered type IV collagen in the basal lamina.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.