MOLECULAR TILTING IN DRIED ELASTOIDIN AND ITS IMPLICATIONS FOR THE STRUCTURES OF OTHER COLLAGEN FIBRILS

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Summary Wet elastoidin spicules (fish fin rays) yield low-angle meridional X-ray diffraction patterns which resemble those from tendons. However, when the spicule dries the meridian splits into the arms of a diagonal cross (sometimes only one arm appears). Of the possible explanations we reject shearing of the axial arrangement of molecules but confirm tilting. We suggest that, in three dimensions, the molecules are tilted at angles which vary from 0° at the centre to some maximum value at the surface of the spicule, resembling torsion of the array of molecules. Molecular tilting probably occurs in other collagen fibrils.

Introduction We have used elastoidin spicules, which form fish fin rays as a model system for understanding the structure of collagen fibrils (1). The meridional low-angle X-ray diffraction pattern of native elastoidin consists of orders of a 67 nm periodicity and closely resembles that of rat tail tendon (1-5). There can be little doubt (5) that this observation is explained by an axially projected arrangement of molecules in the spicule closely resembling that proposed by Hodge and Petruska (6) for other collagens. The equatorial diffraction patterns of both native and dried elastoidin show that there is no long-range lateral order in the arrangement of molecules (1). (Absence of long-range lateral order may be a feature of the arrangement of molecules in other collagen fibrils (7)).

When the spicule dries the meridian splits into a diagonal cross, whose internal angle is about 68° (2-5) and the layer line spacing increases from 1/67 to 1/59 nm^{-1} (5). It has also been reported that, instead of splitting in two directions to form a cross, the meridian occasionally tilts in one direction only i.e. one arm of the cross is missing (3). We have confirmed these observations with dried elastoidin. More importantly we provide an

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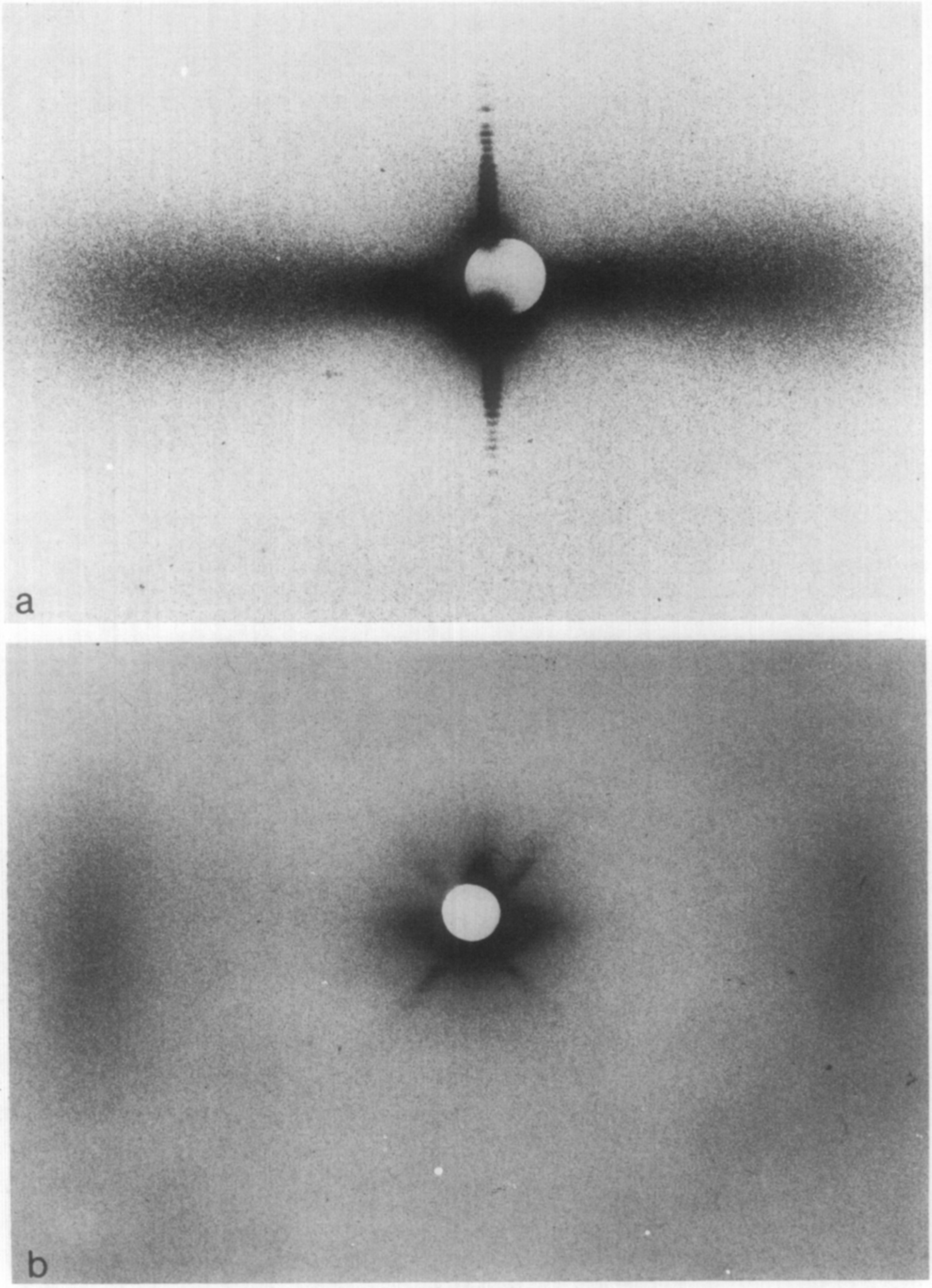
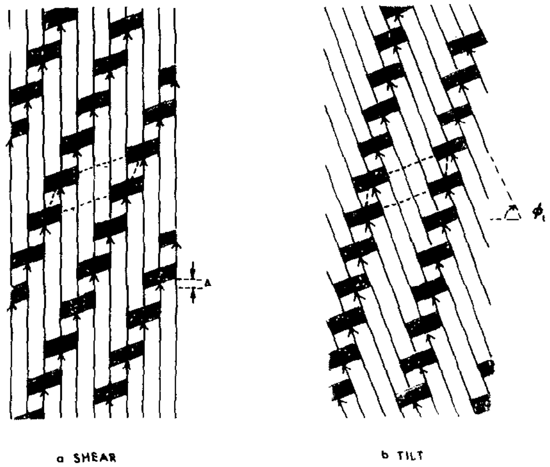


Figure 1. Low-angle X-ray diffraction patterns of (a) native and (b) dried elastoidin spicules from Squalus acanthias. The incident beam was normal to the central region of the spicule. In (a) the meridian (vertical) consists of orders of a 67 nm periodicity and in (b) it is seen to have split into a diagonal cross (layer line spacing 59.8 ± 0.6 nm). Details of materials and experimental methods will be published (1).



a SHEAR b TILT

Figure 2. Schematic representation of models based on (a) shear and (b) tilt. In both models molecules are 4.4 D long and are packed a distance *a* apart. Parallelograms marked on (a) and (b) represent the unit cell of each net; the dimensions of these cells (obtained from the figures by simple geometry) give rise to the parameters of the diffraction patterns shown in Figure 3. In (a) each molecule is staggered a distance D + Δ with respect to its predecessor; in (b) it is simply staggered by D = 67 nm.

explanation for the splitting and then suggest its significance for understanding the structure of other collagen fibrils.

Tilting or shearing? The tilting of the meridian could be caused by either an axial shearing, the molecules remaining parallel to the spicule axis, or by tilting of the molecules relative to the axis.

We can rule out all models based on shear; these are represented schematically by Figure 2(a). In the representation of the Hodge-Petruska model given here (Figure 2), each molecule of length 4.4 D (D = 67 nm) is translated with respect to its preceeding neighbour by D. However, in Figure 2(a) this translation is increased to D + Δ to propagate a shear. The effect on the diffraction pattern would be to tilt the meridian through an angle of $\phi_s = \tan^{-1}(\Delta/a)$ where a is the lateral intermolecular spacing. According to this model the spacing between layer lines would still be 1/D (i.e. $1/67\,\mathrm{nm}^{-1}$) whereas experiment shows that it actually changes to $\sim 1/60\,\mathrm{nm}^{-1}$ (see Figure 1 and 3(a)). This disparity between theory and experiment allows us to eliminate models based on shear.

Models based on tilt, which are represented by Figure 2(b), are acceptable. The meridian tilts by the same angle ϕ_t as the molecules and now the layer line spacing alters to $1/D \cos \phi_t$ (Figure 3(b)). We favoured this model initially by a process of elimination. Further support comes from the

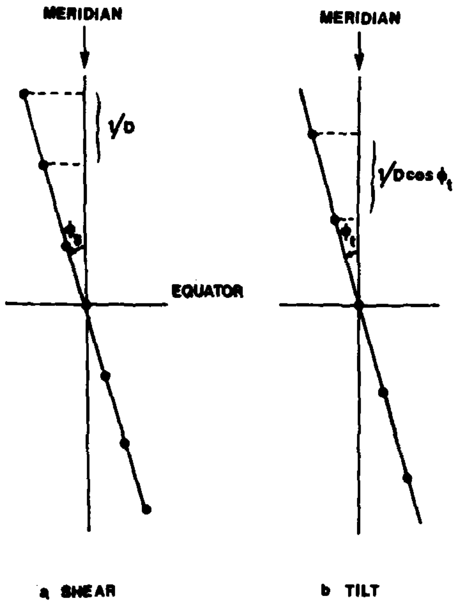


Figure 3. Layer line spacings and meridional tilts predicted by (a) shear and (b) tilt models for the structure of dried elastoidin. In (a) $\phi_s = \tan^{-1}(\Delta/a)$ and in (b) ϕ_t is the molecular tilt; in both D = 67 nm is the periodicity of the native spicule. These spacings and angles are derived from Figure 2 which, although based on a special case of the Hodge-Petruska model, provides a general result. In essence Figure 2 represents a projection of the structure on a plane containing the spicule axis and therefore the validity of this approach depends on point (c) of the section on three-dimensional models in the text.

meridional tilt and the change in layer line spacing providing independent measures of ϕ_t. Values are, from meridional tilt, $\phi_t = 68/2 = 34^\circ$ and, from layer line spacing, $\phi_t = \cos^{-1}(60/67) = 27^\circ$; the close similarity between these results is consistent with a tilt model. (We note that the value of 27° is likely to be a much less accurate estimate since it involves finding the cosine of the quotient of two similar numbers).

Three-dimensional models To understand how tilting is incorporated into the three-dimensional arrangement of molecules we note the following:-

(a) Elastoidin appears to contain no intermediate level of organisation from molecule to spicule (1,5).

(b) If the spicule is rotated about its axis the arrangement of molecules must always appear the same. We have two reasons for making this statement. One, orientation about this axis is not taken into account in X-ray diffraction experiments. Two, the absence of lateral long-range order means that the lateral distribution of molecules must be isotropic.

(c) Our data yield information about the structure projected on to a plane containing the spicule axis. At low angles (corresponding to at least the first 20 orders of the meridional diffraction pattern) the Ewald sphere is approximately a plane so that we are examining the section of reciprocal space which corresponds to this projection.

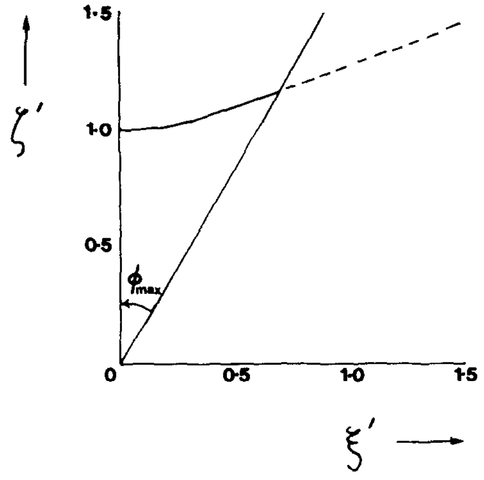


Figure 4. Shape of the ℓ th layer line in the diffraction pattern expected from a three-dimensional model of the kind derived in the text. If ξ and ζ are the equatorial and meridional spacings (in nm^{-1}), then the equation of the layer line is

$$\zeta' = \left\{1 + \sqrt{1 + 4\xi'^2}\right\}^{\frac{1}{2}}$$

where $\zeta' = D\zeta/\ell$ and $\xi' = D\xi/\ell$. The derivation of this equation follows from Figure 3(b) by Pythagoras' theorem. Since the spicule is roughly cylindrical there are far more molecules near the surface than near the axis; therefore the majority of molecules have a tilt approaching ϕ_{max}. Thus the intensity along a layer line reaches a maximum as ϕ_t approaches ϕ_{max}. In all four quadrants of the X-ray diffraction pattern these maxima give rise to a cross. Exact calculation of intensity along the layer line would require a detailed model for the dependence of ϕ_t on the distance of the molecules from the spicule axis.

Had lateral long-range order been present the most obvious model satisfying these criteria would have been an arrangement of molecules resembling a screw dislocation which is a cylindrical shear. Whether one can consider a screw dislocation to exist in an arrangement without long-range order is a very interesting but difficult question which need not concern us unduly here since we have already eliminated this kind of model in the previous section.

The simplest three-dimensional model based on molecular tilt is one in which molecules are tilted from the spicule axis at an angle ϕ_t increasing from 0^o at the centre to a value of ϕ_{max} at the surface. This model implies that far more molecules in the spicule will have high tilt angle values, approaching ϕ_{max}, than lower ones. If all molecules had the same tilt of ϕ_t the inside of the spicule would have to be highly strained to specify the Hodge-Petruska axially projected structure. The nearest analogue is provided by torsion of a cylinder although we do not suggest that the outside of the cylinder is more stretched than the inside but merely that its molecules are more tilted. Figure 4 shows the effect of this range of tilt values on the layer lines of the X-ray diffraction pattern.

The torsion model accounts for the observation that when a spicule dries, its tip rotates in a counter-clockwise direction. We do not as yet have a convincing explanation for the occasional disappearance of one arm of the diagonal cross in Figure 1(b).

Implications for other collagens In X-ray diffraction patterns of wet rat tail tendon, under tension, the meridian and a series of well developed row lines are split into a cross with an internal angle of about 6° (8). This effect was attributed to a shear in the arrangement of molecules (8) but we suggest that molecular tilting ought also to be considered as a possible explanation (see also references 9 and 10).

Electron micrographs have been published showing what appears to be tilting of the molecules on the surface of the collagen fibril (11-14). These are interesting for two reasons. First, the degree of tilt is large (up to ~40°) but no evidence for such a large effect is found in X-ray diffraction patterns of tendon. Second, a heavy metal stain is required to reveal this tilting which is curiously inconsistent with the observation (8) that stain removed the only feature of the X-ray diffraction pattern of rat tail tendon which could be ascribed to tilting. Finally we note that tilting has been suggested as a mechanism for limiting fibril size (15,16).

In conclusion it appears that in at least some collagen fibrils the molecules may not be strictly parallel to the axis but are slightly tilted. Elastoidin provides an extreme example in which, when it is dried, the molecules tilt by ~30°.

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