X-RAY DIFFRACTION BY COLLAGEN TAPE SHOWS THAT TYPE I COLLAGEN FIBRILS NEED NOT HAVE A THREE-DIMENSIONAL LATTICE

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Summary X-Ray diffraction patterns show that collagen tape, manufactured from beef extensor tendon, consists of highly oriented collagen fibrils. Meridional low-angle patterns show that the fibrils have one-dimensional order in the axial direction with a periodicity of 64 nm. Absence of Bragg reflections in the equatorial direction shows that the arrangement of molecules in a fibril has no long-range three-dimensional order. Similar results have been obtained from other oriented collagen fibrils although there is some three-dimensional order in the arrangement of molecules in moist rat-tail tendon under tension. Models for the collagen fibril in which all the constituent molecules are arranged in an extended three-dimensional lattice cannot provide a complete description of its structure under all conditions.

Introduction I have obtained X-ray diffraction patterns from highly oriented collagen tape, made of beef extensor tendon, which provide further information on the arrangement of molecules in type I collagen fibrils. Grynpas (1) has recently published medium-angle X-ray diffraction patterns of bone collagen which closely resemble those from other oriented collagen fibrils in that the meridian (parallel to the fibril axis) consists of discrete Bragg reflections. This result demonstrates that the arrangement of molecules in a fibril has one-dimensional order in the axial direction, presumably because of the axially projected structure described by Hodge and Petruska (2). However his patterns did not have any Bragg reflections in the equatorial direction. He attributed this absence of reflections either (a) to their being obscured by lack of orientation or (b) to lack of long-range three-dimensional order in the arrangement of molecules.

The X-ray diffraction patterns from collagen tape resemble those from bone, and most other oriented collagen fibrils, in showing only diffuse scatter on the equator. Both tendon and bone contain type I collagen in which the triple-helical molecules consist of two identical $\alpha 1$(I) chains and a somewhat different $\alpha 2$ chain (3). Because the molecules in the tape are so well oriented, the ambiguity which hindered Grynpas in his interpretation of diffraction patterns from bone collagen does not arise. Therefore the tape provides an example of a system in which molecules of type I collagen are arranged in fibrils without any sign of long-range three-dimensional order.

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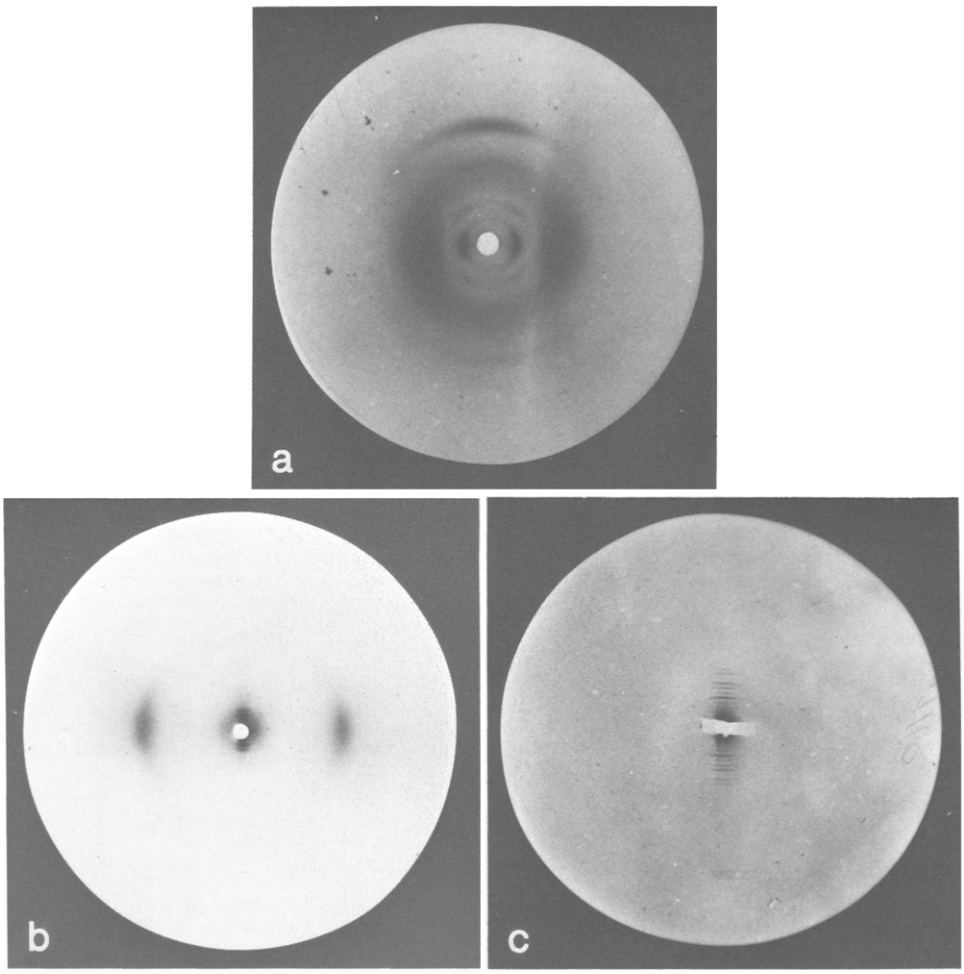

Figure 1 X-Ray diffraction patterns of collagen tape : (a) high angle, (b) medium angle and (c) low angle. The meridian is vertical in all patterns. In (a) the sample is tilted 15° from the vertical to the X-ray beam to show the 0.29 nm meridional reflection. In (c) the backstop obscures the first diffraction order.

Previously the significance of the diffuse equatorial scatter and its implications for the structure of collagen fibrils has been largely ignored. Collagen tape consists of highly oriented fibrils and therefore provides high quality X-ray diffraction patterns which emphasise the importance of the diffuse scatter.

Methods Collagen tape is manufactured by Ethicon Inc. (Somerville, NJ, U.S.A.). The tape is prepared by dispersing beef extensor tendon in acid solution to produce a gel of small fibrils; the fibrils are oriented to form a tape which is "tanned" (4), maintained under tension and dried (D. S. Jackson, personal

communication). Fibrils are not disrupted but remain intact during the preparation of the tape.

X-Ray diffraction experiments are classified according to the approximate specimen-to-film distance as high-angle (3 cm), medium-angle (10 cm) or low-angle (30 cm). In all experiments the X-ray source was an Elliot GX6 rotating anode generator (with a nominal 100 μm point focus) used with a Searle X-ray diffraction camera (both from Marconi-Elliot Avionics Systems, Borehamwood, Herts.). Different X-ray focussing systems were used for the three kinds of experiments : a toroidal mirror for high angles (5); glass mirrors for medium angles (6) and a glass mirror with a quartz crystal monochromator for low angles (7). Measurements on X-ray diffraction patterns were made using a Mitutoyo PJ-250C optical comparator (Mitutoyo Manufacturing Co. Ltd., Tokyo, Japan). Low-angle patterns were calibrated by accurate measurement of the specimen-to-film distance. The calculated meridional periodicity could then be used to calibrate medium-angle patterns. High-angle patterns were calibrated by dusting the specimen with calcite (which yields an intense ring of spacing 0.3035 nm).

The collagen in the tape was confirmed to be type I, i.e. to consist of two different kinds of chains, by gel electrophoresis (8). For this technique tape (1 mg) was digested by pepsin (0.1 mg) in acetic acid (2 ml; 0.4 M) for 3 days at room temperature.

Results High-, medium- and low-angle X-ray diffraction patterns from collagen tape are shown in Figure 1. The high-angle pattern is typical of what one would expect from highly oriented collagen molecules, particularly in having an intense meridional reflection corresponding to a spacing of 0.29 nm (9).

The meridional direction in both the medium- and low-angle patterns consists of discrete Bragg reflections which correspond to a periodicity of 64.0 ± 0.4 nm. This result demonstrates that the molecules are organised in fibrils. Similar results have been obtained for tendons from a variety of sources since the original work of Bear (10) and can be explained by the axially projected arrangement of parallel molecules in the collagen fibril (2). Intensities of meridional reflections in Figure 1(c) are comparable with those obtained from dry tendons (11).

The equatorial direction in the medium-angle diffraction pattern shows no signs of any Bragg reflections; therefore, there is no evidence for any long-range three-dimensional order in the organisation of molecules in a fibril. The diffuse peak, corresponding to a spacing around 1.1 nm, closely resembles that observed in the diffraction pattern of dried elastoidin (12) and is in roughly the same position as the maximum in the diffuse equatorial scatter from tendon (13). A detailed analysis of the elastoidin shows that such peaks can be explained by a parallel array of single collagen molecules with no long-range three-dimensional order (14).

Discussion Collagen tape consists of highly oriented fibrils in which the parallel molecules are not arranged on an extended three-dimensional lattice. Evidence for three-dimensional long-range order in collagen fibrils has only been obtained from moist rat-tail tendon when under tension (15, 16). Even in moist rat-tail tendon under tension many of the molecules are not ordered in three dimensions (13). Calculations show that the diffuse equatorial peaks

in the diffraction patterns from these disordered phases can be explained by an array of single collagen molecules which are ordered in one but not in three dimensions; there is no evidence for any form of microfibril (13). When rat tail tendons are dried all evidence for a three-dimensional lattice disappears (15) although this effect appears to be reversible (17). The 64 nm periodicity and the relative intensities of the meridional reflections suggest that the structure of the fibrils in the tape is the same as those in dried tendon. If drying causes loss of Bragg reflections it would appear that electron microscopy is unlikely to yield information on the ordered phase since drying is a prerequisite of this technique.

These results show that long-range three-dimensional order is not an essential feature of the arrangement of molecules in fibrils of type I collagen. Whether or not the molecules are capable of forming more ordered fibrils than those observed does not affect the validity of this conclusion. Therefore models which resemble crystals cannot provide a complete description of the structure of the collagen fibril under all conditions. No long-range three-dimensional order was observed in the structure of either native or dried elastoidin spicules although elastoidin differs from the other collagens considered here in that it is not type I (14). This conclusion is not inconsistent with the proposal that, although type I collagen molecules are capable of being ordered in a three-dimensional lattice, other collagen molecules with three identical chains are not (18). However it does show that observation of lack of long-range order in a few examples of collagens with three identical chains does not prove the proposal.

The occurrence of collagen fibrils in which the arrangement of molecules is highly ordered in one dimension, but lacks long-range three-dimensional order, leads to the idea that collagen fibrils are type A smectic liquid crystals (19). In the past undue emphasis has been given to the ordered phase which appears in moist rat-tail tendon under tension. However we have seen that, even under these conditions, only some of the molecules in tendon collagen fibrils are ordered in three dimensions. The appearance of the ordered phase when the fibrils are subjected to tensile stress is consistent with a liquid crystal model (19, 20). It would appear that in most collagen fibrils under most conditions there is a regular arrangement of molecules in one but not in three dimensions.

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