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ENERGY DISPERSIVE X-RAY DIFFRACTION BY COLLAGEN FIBRILS IN COSTAL CARTILAGE USING SYNCHROTRON RADIATION

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Summary Low-angle X-ray diffraction patterns from the isotropic distribution of collagen fibrils, which occur in low concentrations in costal cartilage, were recorded using synchrotron radiation. An energy dispersive technique was used to exploit the properties of synchrotron radiation to the full. The third, fourth, fifth and sixth diffraction orders from the axial periodicity of the fibrils were recorded and used to calculate a value for this periodicity of 67 ± 1 nm. This result is in good agreement with measurements made on amianthoid areas as well as from fibrils in tendon, which consist of a chemically distinct form of collagen.

Introduction We have used an energy dispersive technique with synchrotron radiation to obtain low-angle X-ray diffraction data from the low concentration of disoriented collagen fibrils in normal costal (rib) cartilage. Meridional X-ray diffraction patterns from tendons which contain a high concentration of oriented collagen fibrils consist of discrete reflections because of the 67 nm axial periodicity of the fibrils (1). The concentration of collagen in costal cartilage is much lower (less than 10%; refs. 2, 3 and 4) than in adult tendon (30%; 70% when dried; ref. 5) and the fibrils in cartilage have random orientations. Our attempts to measure the fibril periodicity by conventional X-ray diffraction techniques were unsuccessful.

We wished to compare the periodicity of collagen fibrils in normal costal cartilage with those in amianthoid areas. High-angle X-ray diffraction patterns have shown that during ageing amianthoid areas appear in costal cartilage in which the normal isotropic distribution of collagen fibrils becomes markedly anisotropic (6). The amianthoid areas also contain a much higher concentration of collagen than the rest of the cartilage (7). Because of the high concentration of oriented collagen fibrils it was possible to obtain low-angle X-ray diffraction patterns which showed that their axial periodicity in the amianthoid areas was 67 ± 1 nm (6). This result was in good agreement with the periodicity of the fibrils in tendon; we are now able to compare it with the value for normal costal cartilage.

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Methods Bone was trimmed from transverse sections (1 mm thick) of amianthoid-free human costal cartilage and the specimens were mounted in air-tight cells, with Melinex (12 μm thick) windows, in contact with physiological saline. X-Ray diffraction patterns were obtained by an energy dispersive technique, which exploits to the full the properties of the intense synchrotron radiation (8,9,10), in a total time of 20 minutes. (Actual exposure to X-rays was for 20/9 minutes because of the periodic nature of the synchrotron beam.) The specimen was moved every 2 minutes to prevent radiation damage; no colouration of the specimen, which would have signified damage, was observed. After the diffraction pattern had been recorded a selected area of the specimen was exposed to the beam for 4 minutes and some signs of slight colouration were observed then. Integrated intensities were obtained by the methods of references 8 and 9; no disorientation correction is required in the energy dispersive technique.

Results Diffraction patterns of two of the three specimens used showed peaks which could be attributed to orders of the axial periodicities of the collagen fibrils. Only the third, fourth, fifth and sixth order peaks were observed. A typical result is shown in fig. 1(a). For the purpose of comparison fig. 1(b) shows a diffraction pattern obtained from a sample of stretched wet rat tail collagen (4 min exposure). Both patterns are raw data (unnormalised) (8,9). The poor quality of the patterns can be explained by the very high diffuse background contributed, presumably, by other extracellular protein, polysaccharides and the cellular components of the cartilage. Thus the poor quality of the pattern arises from the nature of the material and is not a consequence of the technique. Energy dispersive techniques yield the intensity of photons scattered at a fixed angle as a function of their energy whereas conventional techniques measure the photon intensity as a function of angle and all photons used have the same energy, i.e. the radiation is monochromatic. The plots of fig. 1 are qualitatively very similar to conventional plots against angle (8); this allows us to represent them as a function of reciprocal spacing.

Peak positions in the diffraction patterns yielded a value of 67 ± 1 nm for the periodicity of collagen fibrils in normal costal cartilage. This result agrees with the value obtained for amianthoid regions and supports the conclusion that the fibrils in this region have the usual periodicity (6). It is also in good agreement with the values of around 67 nm obtained from a variety of tendons (1). Good agreement with the tendon result was not a foregone conclusion. Most of the fibrils in costal cartilage contain type II collagen which is chemically distinct from the type I collagen of tendon (4,11).

Fig. 1(a) shows that the signal-to-noise ratio was too poor for accurate intensity measurements and, in any case, we were unable to record sufficient orders for a proper analysis. We note that the relative intensities, on an arbitrary scale, of the third, fourth, fifth and sixth order peaks were of the order of 30, 8, 40 and 9. These results do not differ

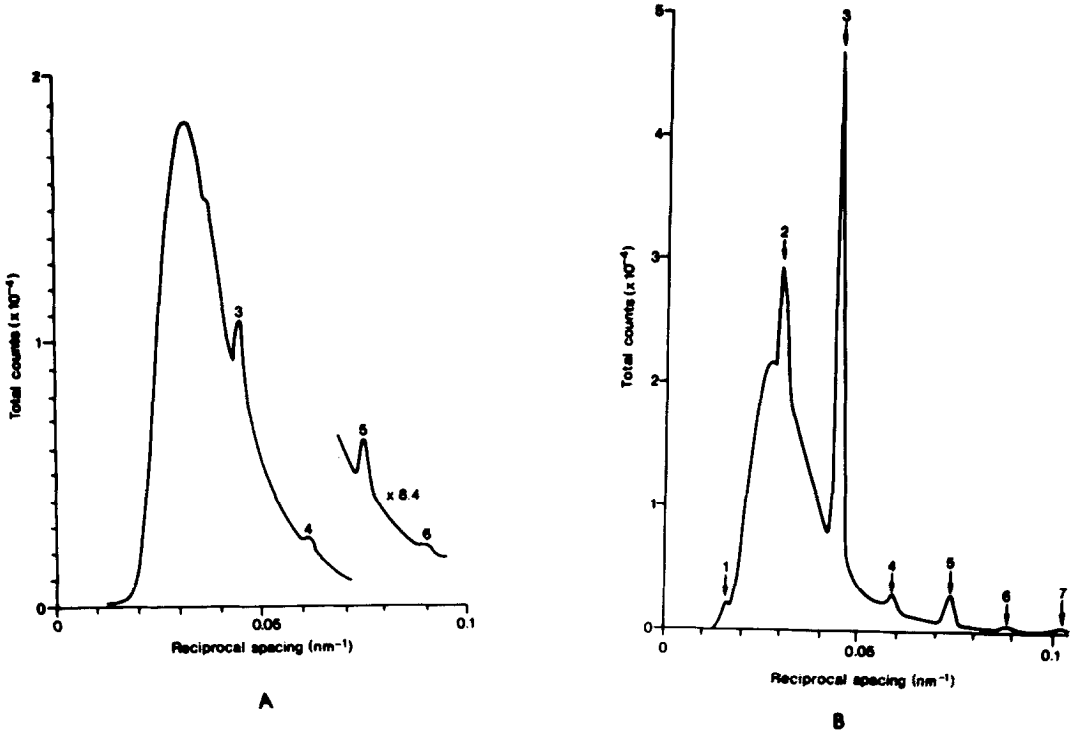

Fig. 1 Intensity of photons scattered from (A) costal cartilage and (B) rat tail tendon as a function of calculated reciprocal spacing (in reciprocal nm). Detection channel numbers were converted to reciprocal spacings using (B) in which the peaks have a periodicity of 67.1 nm (1).

greatly from those obtained from tendon collagen (1), although there is no reason to suppose that different collagen types will yield peaks with the same intensities. One sample yielded a fifth diffraction order which was surprisingly intense (180 on our arbitrary scale). We are unable to explain this result satisfactorily although it might arise from a contribution to the peak by fluorescence from tin or indium seals in the X-ray beam line which was not eliminated properly by our apparatus; this peak was omitted in our calculations of the fibril periodicity.

Discussion Our results demonstrate the usefulness of synchrotron radiation in diffraction studies of disordered biological material. It would not have been possible to measure the native fibril periodicity by electron microscopy because the necessary chemical treatment and subsequent dehydration inevitably shrinks the fibrils. Shrinkage usually reduces the fibril periodicity to around 64 nm (1). We were unable to make the measurements using conventional X-ray sources; this is not surprising when we note the low concentration and lack of order of the collagen fibrils in the cartilage or when we note the poor

definition of peaks in fig. 1(a). X-Ray diffraction patterns recorded from collagen fibrils using conventional techniques have been confined to tissues like tendon where there is a high concentration of oriented fibrils. Because of its very high intensity, synchrotron radiation allows us to obtain patterns from tissues with a low concentration of disordered fibrils in a short time.

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