Twisted Fibrils are a Structural Principle in the Assembly of Interstitial Collagens, Chordae Tendineae Included

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X-ray diffraction analysis of connective tissue samples, which contain type I and type III collagen shows that twisted collagen fibrils are a general principle of assembly. The occurrence of twisted fibrils in native wet Chordae tendineae, skin and Aorta is combined with a shorter axial periodicity of about 65 nm. This shorter D period is shown to be directly related to the tilt of the molecules, which have to be curved to build-up twisted fibrils.

Introduction

Twisted collagen fibrils have been observed earlier in the electron microscope. The first observations were done on connective tissue samples, which had been altered by swelling agents [1, 2]. After first doubts [3], the observation of twisted structures in replicas of freeze-fractured collagen fibrils [4, 5] gained therefore in significance. Nevertheless, the final piece of evidence had not been found, which proved that these findings were relevant for the native structure of collagen fibrils.

Recently we reported about a structural evidence, which showed that twisted fibrils are an integral part of the structure of native interstitial collagens [6].

Materials and Methods

The samples used in this study were from victims of accidents aged between 22 and 62 years. All samples were stored in Ringer at 4 °C and exposed to X-rays in native wet state.

Chordae tendineae were cut-off from the mitral valve. *Finger flexor tendons* were dissected into smaller fibres. Strips of *skin* were cut from the subcutis. Samples of blood vessels were longitudinal strips cut from the *Adventitia of the Aorta*.

The samples of skin and Adventitia were delipidated with chloroform/methanol 2:1, washed with Ringer and stored in Ringer again. The orientation of collagen fibrils was considerably improved in these samples by stretching.

Wide angle X-ray diffraction patterns were recorded on OSRAY M3-DW Film with Cu K$_\alpha$ radiation using a Kiessig camera with pin hole collimation. The specimen to film distance was 0.2 m.

Small angle meridional diffraction spectra were recorded on the double focusing camera X33 of the EMBL using synchrotron radiation provided by the storage ring DORIS of the DESY/Hamburg [7]. The specimen to detector distance was 4.45 m. The intensities were registered with a linear detector with parallel readout [8]. The samples were immersed in Ringer in a closed cuvette with Hostaphan R6 foils as windows for the X-ray beam.

The analysis of the small angle meridional diffraction spectra was done by a non-linear regression analysis which includes a fitting of the peaks by Gaussian functions, the subtraction of the background [9] and a correction for the detector width.

Model building calculations were done according to the method of Hulmes [10]. The collagen type I amino acid sequence was partly from calf [11] and where it is available from human cDNA sequencing [12, 13], whereby posttranslational modifications were assumed to be the same as in calf collagen.

Calculated intensities were corrected by a temperature factor B, according to $\exp(-Bh^2)$, whereby h is the order of the meridional reflection. The R factors were calculated from the observed amplitudes (F_o, square root of the intensities) and the calculated amplitudes (F_c) by $R = \Sigma \left| F_o - F_c \right| / \Sigma F_o$.

Results and Discussion

X-ray evidences

Fig. 1a shows the X-ray diffraction pattern of a stretched Chorda tendinea of a human mitral valve. The 1.5 nm reflection is not centred on the equator, but is split into two reflections above and below it. This splitting is not observed in diffraction patterns of human or animal flexor tendons. The splitting angle is approximately 9°. The same splitting is also observed in diffraction patterns of human Adventitia of the Aorta [6] and in human, calf and lamb skin. This indicates that the molecules of these interstitial collagens are tilted, or coiled around the fibril axis, respectively (Fig. 1b). The small angle meridional intensities, which reflect the axial periodicity of the fibrils, on the other hand, are centred on the meridian, indicating that the fibrils are not tilted. This is to be distinguished from cases where an equatorial splitting is due to tilted fibrils [14].

The diameters of the fibrils of the interstitial collagens described here are too small to accommodate molecules with a linear tilt. The molecules therefore have to be curved to build-up twisted fibrils.

The Chorda whose diffraction pattern is shown in Fig. 1a also gives rise to a crystalline reflection at 3.7 nm, which is centred on the equator. This reflection changes into a Debye-Scherrer ring when the fibre is relaxed, while the splitting of the 1.5 nm reflection is still visible, but smeared. The 3.7 nm reflection disappeared after the sample was treated with organic solvents. It indicates therefore epitactic lipid deposition between fibrils in this Chorda tendinea of a man aged 62 years. These oriented lipid depositions, which give rise to a reflection centred on the equator are a further, although indirect indication for the axial orientation of the collagen fibrils. The diffraction pattern in Fig. 1a therefore shows on the equator the different orientation of the molecules relative to the fibrils.

Oriented lipid deposition in the Achilles tendon of a man aged 107 years also led to crystalline equatorial reflections [15]. In the diffraction pattern of this sample all reflections were centred on the equator, because the molecules in Achilles tendons are oriented nearly parallel to the fibrils.

The collagen fibrils of skin [16, 17], Adventitia and Chordae tendineae also differ from those of tendons in their axial periodicity of about 65 nm. The corresponding meridional small angle X-ray diffraction spectra (Fig. 2) also show differences in the intensities. The very low first order reflection in spectra of Chordae tendineae (Fig. 2d) indicates a decreased step function between the overlap region and the gap region in the axial electron density distribution of these collagen fibrils. A low first order reflection was attributed to a partially filled gap region in tendons of dermatosparactic animals [18].

Model calculations

We have shown with the help of model calculations that the shorter D period in skin collagen can be attributed to a uniform shortening of the axial structure due to a twisting of the fibrils [6]. We have extended these calculations for Chordae tendineae and include now the human typ I amino acid sequence [12, 13].

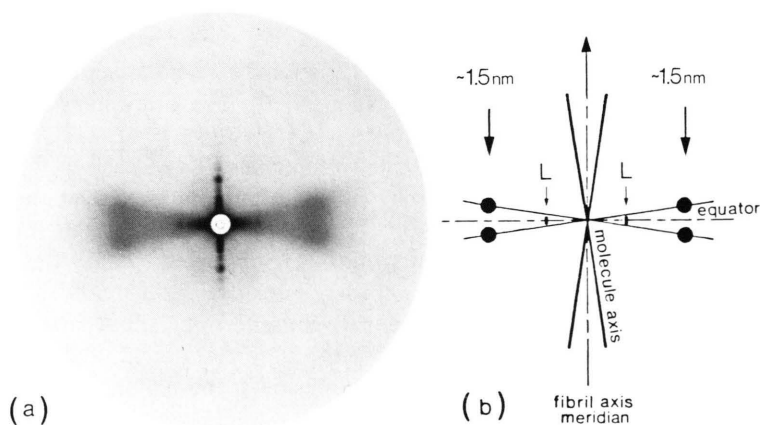(a)   (b)

Fig. 1. (a) Wide angle X-ray diffraction pattern recorded from a native Chorda tendinea of the mitral valve of a man aged 62 years. The fibre is oriented vertically, stretched and immersed in Ringer. (b) Diagram of the splitting of the 1.5 nm reflection and of the orientation of the collagen molecules with respect to the fibril axis. The D periodic fibrils are not tilted, as shown by the unsplit meridional reflections in (a). L: crystalline reflection at 37 nm, which originates from lipids.
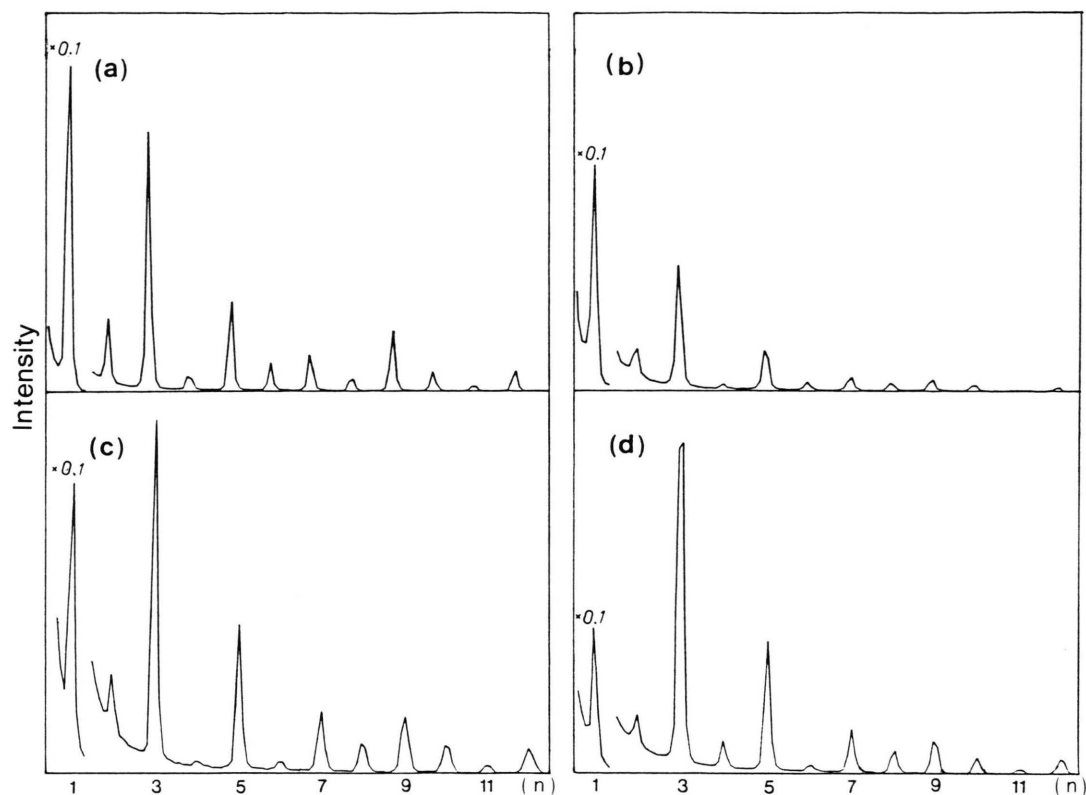
Fig. 2. Meridional small angle X-ray diffraction spectra of native wet human connective tissue samples. (a) Finger flexor tendon D period = 67.0 nm; (b) skin D = 65.2 nm; (c) Adventitia of the Aorta D = 65.8 nm; (d) Chorda tendinea D = 65.3 nm. The samples in (b) and (c) were slightly stretched to improve fibril orientation, the D period in (c) is therefore larger than in the unstretched samples (D = 65.5 nm). The first order reflection is plotted reduced by a factor of 10. n is the order of the meridional reflection.

Fig. 3 shows the corrected meridional small angle spectrum of a Chorda tendinea compared to the model calculations. The structural parameters of the model are very similar to those of our models of tendons [19] and skin [6] collagen. An R factor of 0.11 was calculated from the 2nd to the 12th order,
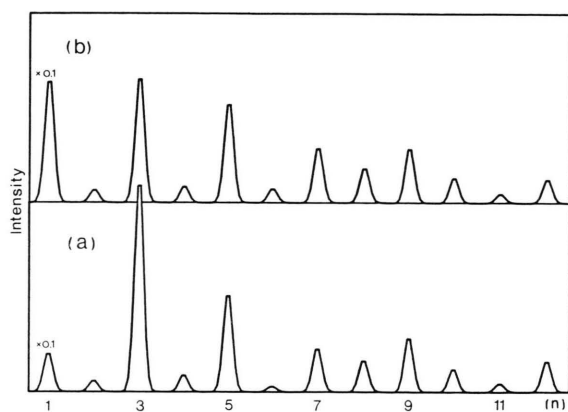

Fig. 3. (a) Corrected meridional small angle intensities of a native wet Chorda tendinea with a D period of 65.3 nm. (b) Meridional intensities calculated by Fourier analysis from a molecular model based on the amino acid sequence of type I collagen. The intensities were corrected by a temperature factor of 0.008. The structural parameters of the model are the D stagger of 235 amino acid residues and the axial extension of both telopeptides of 0.80, expressed as a fraction of the triplehelical extension. The first order reflection is plotted reduced by a factor of 10. n is the order of the reflection.

because the model does not take into account the low first order reflection, which is not dependent on the type I collagen contribution to X-ray diffraction.

The content of type III collagen in Chordae tendineae amounts to 20% of total collagen. We intend to investigate the influence of type III collagen on the meridional intensities when we have more data of Aorta, where type III collagen amounts to about 30%.

We conclude from our model building calculations that the shorter D period in Chordae tendineae is due to the tilt of the molecules, which are curved to build-up twisted fibrils.

Conclusions

Our X-ray investigations show that twisted collagen fibrils are a general structural principle in connective tissues which contain type I and type III collagen. Type III collagen nevertheless is not the cause for the coiling. Samples of the Aorta of a patient with type IV Ehlers-Danlos-Syndrome, lacking type III collagen, also show split 1.5 nm equatorial reflections in the diffraction patterns (Nemetschek, unpublished results).

The measured tilt angle of 9° is a mean value, because molecules at the periphery of the fibrils have a greater tilt than molecules inside fibrils*. The peripheral molecules, which are therefore under stress most likely exert force on the inner molecules and cause an additional axial contraction of the fibrils. This additional contraction is suggested because a tilt of 9° would result in a D period of only 66 nm, whereas the measured values are smaller.

Twisted fibrils exhibit a high degree of axial order. This indicates that the lateral interactions between neighbouring molecules are strong enough to hold a fibril together, although the individual molecules are differently tilted and stressed, depending on their radial position in the fibril.

The stress of the peripheral molecules most likely is the decisive factor for the self-regulation of the fibril diameter of the interstitial collagens described in this study. The diameter of twisted fibrils is limited by the maximum possible stress of the molecules, which the lateral intermolecular interactions can withstand, as suggested by Chapman in a model [20].

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* *Note added in proof:* A recent report provide a plausible explanation for the cause of a molecular tilt [21].

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