The Macromolecular Structure of Collagen in Tendon Fibres of Dermatosparactic Animals

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Small-angle x-ray diffraction spectra of dermatosparactic tendon collagen show a decreased intensity of the first order reflection. We interprete this finding to be due to the N-terminal propeptide which fills the intermolecular gap region partially.

Introduction

Dermatosparaxis is observed in calves and lambs [1, 2]. It is due to a deficiency of a N-procollagen-peptidase and a therewith correlated disturbance during the cleaving of N-terminal propeptides (pN) from collagen [3]. X-ray diagrams [4, 5] of these functionally disturbed fibres [4] show the typical medium and wide angle pattern of collagen and in the small-angle diagram the 67 nm-reflection with its intense odd orders of reflection which indicate an unchanged ratio of the intermolecular overlap to gap region.

Material and Methods

Tail tendons of normal and dermatosparactic calves (Liège) and lambs (Oslo) were examined. We had tail tendons of two 1-day old dermatosparactic calves and controls from a normal 1-day old and a normal 3-months old calf. The dermatosparactic lambs were 1 and 3 days old and we had controls from normal lambs which were 1 day, 3 days and 3 months old.

For the x-ray measurements the fibres were held in a closed cuvette between double hooks by knots tied at both ends [6]. The fibres were stored in Ringer's solution where a few drops of toluene had been added.

The spectra were recorded on the double-focusing camera X33 of the EMBL in the HASYLAB using synchrotron light provided by the storage ring of DORIS of the Deutsches Elektronen-Synchrotron (DESY) Hamburg [6]. The advantage of the camera X33 is the high resolution due to the camera length of 3.8 m and the good focusing.

The evaluation of the recorded spectra is described elsewhere [6].

Electron densities were calculated on the basis of the amino acid sequences of calf collagen α1(I), α2(I) and the pN-peptide. Calculated spectra were obtained by the Fourier transform of the electron density.

Results and Discussion

In our combined mechanical and x-ray diffraction measurements [6] we recorded small-angle spectra from native tail tendons of dermatosparactic lambs (D-LTT) and calves (D-CTT) using synchrotron light. The intensity of the first order reflection of the dermatosparactic lamb (Fig. 1b) has decreased by a factor of 3 compared to the normal lamb (Fig. 1a) (this corresponds to 33% of the control value). In both spectra the intensities of the odd orders are similarly intense. The intensity of the first order reflection of the dermatosparactic calf (Fig. 1d) has decreased by a factor of 1.4 compared to normal calf (Fig. 1c) (this corresponds to 70% of the control value). Spectra of 13 tendon fibres of two different dermatosparactic lamb tails and 17 fibres of two different calf tails were recorded and confirm this observation. N-LTT and N-CTT fibres show a reduced D-period of 66.5 ± 0.2 nm compared to RTT 67.0 ± 0.2 nm and slightly changed intensities [7]. The D-periods of dermatosparactic fibres also show shortened values of 66.5 ± 0.2 nm. In stretching experiments the tension-induced increase of the D-period up to 1% is not correlated with changes in the intensities in the diffraction spectra from tendon fibres of

Abbreviations: RTT, rat-tail tendon; N-CTT and D-CTT, normal and dermatosparactic calf-tail tendon; N-LTT and D-LTT, normal and dermatosparactic lamb tail tendon; pN-peptide, N-terminal propeptide.

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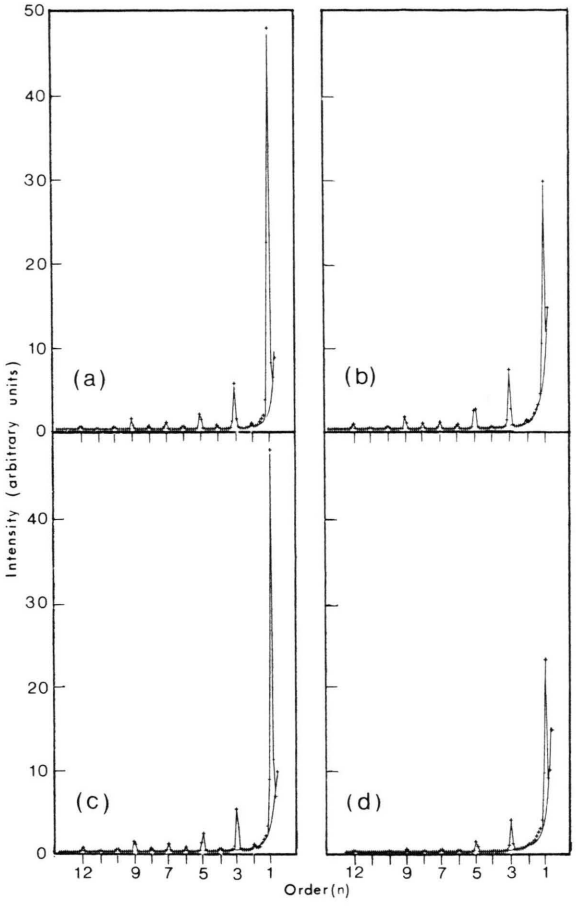

Fig. 1. Meridional small-angle spectra of native tail-tendon recorded from: a) normal lamb; b) dermatosparactic lamb; c) normal calf; d) dermatosparactic calf. The original spectra are shown with a fit of the background and the peak positions and intensities. Note the decrease of the first order reflection in Fig. 1b and 1d. Measuring time: 5 sec per spectrum.

normal and dermatosparactic lamb and calf, similar to native RTT [8].

An uniform filling of the intermolecular gap region of the parallel associated triple helices with a substance more electron dense than water, like sugar or protein, lowers the intensity of the first order reflection but also changes the ratio of the intensities of the other odd orders.

Therefore we interprete the lowering of the intensity of the first order and the nearly unchanged other intensities by a partially filled gap, what means that the pN-peptide does not extend over the whole length of the gap. The pN-peptide most likely adapts a conformation so that the hydrophobic and electrostatic interactions to the neighbouring chains are optimized. Modelbuilding calculations were done according to the method described by Hulmes *et al.* [9] on the basis of the amino acid sequence of N-CTT [10, 11]. The parameters of the model for the normal collagen are the D-stagger D and the axial residue translation of the telopeptides r_N and r_c (N and C terminal) as a fraction of the triple helical axial residue translation (0.286 nm). The optimized D-stagger of the N-CTT spectrum is with D = 233 lower than that of RTT, what agrees with the measured shortened D-period and the changed intensities of reflection. The intensities calculated from the electron density distribution of the native collagen model of normal CTT are shown in Fig. 2a (bottom). The R-value is 0.10 for 12 orders of reflection. The variable parameters of the complete pN-peptide (Fig. 2b) are

1) the conformation of the non-triple-helical region A, which comprises the back-folded chain,
2) the length of the triple helical region B of the pN-peptide and
3) the non-triple helical linkage between the triple helix of the pN-peptide and of the collagen molecule including the N-terminal telopeptides and
4) the content of pN-peptide itself.

The best agreement between measured and calculated intensities of dermatosparactic tendon fibres (*R*-value 0.15 for 12 orders of reflection) was achieved for the collagen molecule plus the pN-peptide with the conformation plotted in Fig. 2b. This provides a decreased intensity for the first order reflection (Fig. 2a top), without altering the strong intensities of the odd orders, corresponding to our measurements. Fig. 2c shows the electron density distribution derived from the calculated intensities and phases with and without the pN-peptide. The axial residue translation in the "globular" domain A is 0.9 compared to the residue translation in the triple helix, and 0.5 in domain C. The triple helical part B is 45 amino acid residues long. The conformation of the N-terminal telopeptides is unchanged. The spectra of D-LTT can be fitted with a content of (30 ± 5) % pN-peptide and those of D-CTT with (15 ± 5) % pN-peptide. This means that every third or seventh collagen molecule respectively contains a pN-peptide, which disturbs the fibrillar assembly.

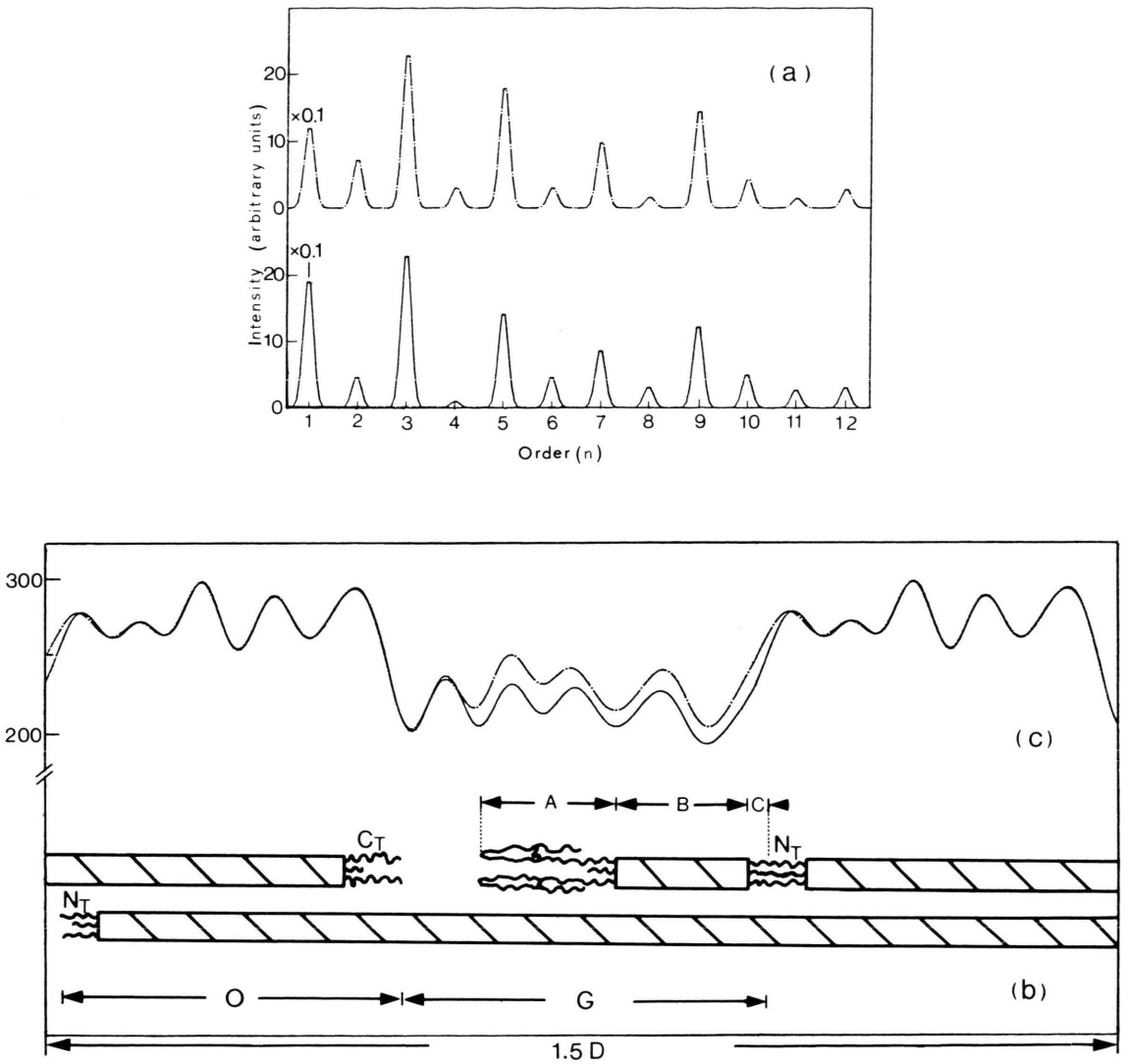

Fig. 2. a. Calculated spectra of the native collagen model without (———) and with 25% pN-peptide (−·−·). The spectra are normalized to the intensity of the third order reflection.
b. Parallel association of collagen molecules with the incorporation of a pN-peptide under consideration of the conformation proposed by Kühn *et al.* [12]. The 139 amino acid residue long pN-peptide of α 1(I)-chain [13] consists of a N-terminal "globular" domain A, a triple helical part B and the short non-triple helical region C which is the connection to the telopeptide of the α1(I)-chain. The pN-peptide of the α2(I)-chain consists of the domains B and C but of only a few amino acids of domain A. D, D-period; G, gap region; O, overlap region; C_T, C terminal telopeptide; N_T, N terminal telopeptide.
c. Electron density distribution (above the water background) derived from the amino acid sequence of collagen (CTT) without (———) and with one complete pN-peptide (−·−·) on every fourth collagen molecule. The ordinate is the net number of electrons of the five segments, that form the D-period, per amino acid residue translation. The D-stagger is here 233 residues.

The results of our measurements from the tendon fibres of dermatosparactic animals and the preliminary interpretation on the basis of the amino acid sequence show, that the remaining pN-peptide can be detected by x-ray diffraction in native tendon fibres. We are therefore in the position to get informations about the native structure of the N-terminal procollagen-peptide. This opens new perspectives

for the investigation of other pathological connective tissues and beyond that also for the function of the intermolecular gap region as a host for guest molecules.

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