The Tubular Structure of Collagen Fibril

By Masao HAISA

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According to Arndt and Riley[1], X-ray powder patterns obtained from all sorts of proteins have two characteristic scattering maxima, the principal and the packing peaks. However, the patterns obtained from collagen have, in addition, another peculiar meridional peak which has the 2.86 Å spacing and which seems to be constant whatever the origin of the collagen fibrils. In this case, the equatorial packing peak varies its spacing from 11 to 17 Å with an increase in the water content of the material and it is accompanied with the gradual appearance of streaking[2]. Although it is generally agreed that the collagen molecule consists of a three-stranded helix, it is not always certain whether the fibril is made up of "Collagen II" or of the "Collagen I" proposed by Crick and Rich[3].

On the other hand, small-angle diffraction studies of dry collagen[4,5] have shown that the meridional spacing is about 640 Å and that the layer lines on the meridian exhibit fanning. It was postulated that a particular kind of disordering of the structure produced such a fanning effect. Tomlin and Ericson[6] have shown that the fanning is due to the regular shearing of the fibrils. On inspection of electron micrographs, Schmitt and Gross[7] have found that the axial repeat of 640 Å contains five primary staining levels.

Although it is now permissible to define collagen as any fibrous protein of the collagen group which gives the above features of X-ray wide-angle as well as small-angle patterns[8], there has yet been reached no general agreement about the interpretation of these patterns. An attempt is made in the present paper to elucidate the general features of the X-ray patterns of collagen-class proteins in terms of the helically grooved tubular structure. Some electron-microscopic findings are also given which support this structure.

1) U. W. Arndt and D. P. Riley, *Phil. Trans. Roy. Soc.*, **A247**, 409 (1955).
2) W. T. Astbury and R. Lomax, *J. Chem. Soc.*, 1935, 846.
3) F. H. C. Crick and A. Rich, "Recent Advances in Gelatin and Glue Research", Ed. by G. Stainsby, Pergamon Press, London (1958), p. 20.
4) O. E. A. Bolduan and R. S. Bear, *J. Polymer Sci.*, **5**, 159 (1950).
5) R. S. Bear, *Advances in Protein Chem.*, **7**, 69 (1952).
6) S. G. Tomlin and L. G. Ericson, *Acta Cryst.*, **13**, 359 (1960).
7) F. O. Schmitt and J. Gross, *J. Am. Leather Chemists Assoc.*, **43**, 658 (1948).
8) W. T. Astbury, *Trans. Faraday Soc.*, **34**, 378 (1938).

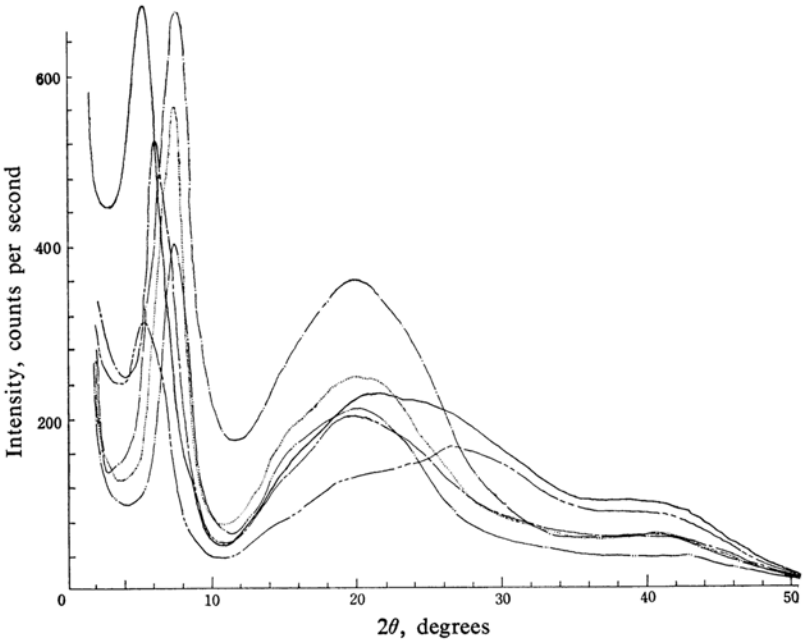

Fig. 1. Wide-angle equatorial spectra of fowl-neck tendon collagen.

- Native, moist collagen
- Native, dried collagen
- Moist collagen treated with formaldehyde
- Dried collagen treated with formaldehyde
- Moist collagen treated with acetic acid
- Dried collagen treated with acetic acid

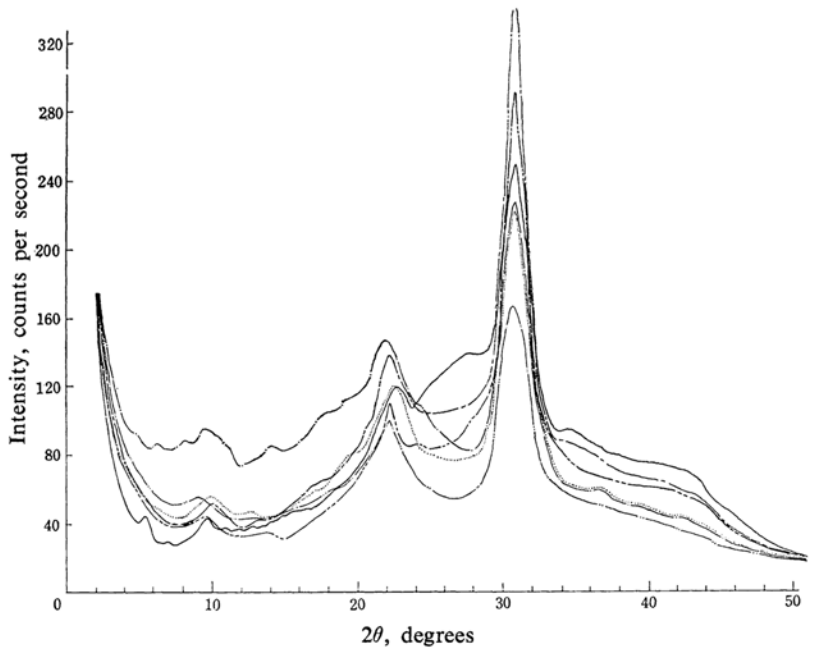

Fig. 2. Wide-angle meridional spectra of fowl-neck tendon collagen. Remarks are shown in Fig. 1.

Wide-angle X-Ray Diffraction

All experiments described in this paper were made on moist and dried fowl-neck tendon collagen, untreated or treated with formaldehyde or acetic acid. Figures 1 and 2 show the equatorial and meridional spectra respectively, obtained by means of a Geiger-counter X-ray diffractometer, "Geigerflex", using Cu radiation filtered by nickel. It will be seen that the meridional peaks are unaffected in either the intensity or the position of the maxima by treatment with formaldehyde or acetic acid, whereas the equatorial peaks are remarkably affected. It should be noted that the principal peak at 4.4 Å spacing on the equator is stronger and broader than that at 4.0 Å spacing on the meridian and that the water halo is remarkable for moist materials. The streak for moist collagen treated with formaldehyde or acetic acid is weaker than that for untreated collagen. On the assumption of the validity of von Laue's method[9], the linear dimension of the crystalline region along the fiber axis is estimated to be 76 Å for the peak at $2\theta=31.3°$.

A closer examination of Figs. 1 and 2 would lead us to suppose that the tanning with formaldehyde provides interchain cross-linking and therefore prevents the intrusion of water molecules and that the swelling by means of acetic acid is caused by breaking the lateral aggregation of polypeptide chains.

Some Fourier Transforms Related to the Small-angle Pattern of Collagen Fibril

The Fourier transform of a uniform helix of infinite length, radius r and pitch P, takes the form

$$J_n(2\pi Rr)\exp[in(\Psi+\pi/2)] \tag{1}$$

where r, φ and z are the cylindrical coordinates in real space, R, Ψ and Z, those in reciprocal space, and J_n denotes the nth order Bessel function.

In the case of a discontinuous helix with u units per t turns, the orders of the Bessel functions occurring in the lth layer line are given by those values of n satisfying the relation

$$l=tn+um \tag{2}$$

where the m's are integers[10]. In a fiber diagram, only the cylindrically averaged intensity is relevant; hence, the cross-terms arising from (1) disappear upon this operation. With $t=1$ and $u=5$, for example, we have

$$\left.\begin{array}{lll} \text{when} & l=0,5,\ldots, & J_0^2+2J_5^2+2J_{10}^2+\ldots \\ \text{when} & l=1,4,\ldots, & J_1^2+J_4^2+J_6^2+J_9^2+\ldots \\ \text{when} & l=2,3,\ldots, & J_2^2+J_3^2+J_7^2+J_8^2+\ldots \end{array}\right\} \tag{3}$$

The transform of a thin disc of radius a is given by[11]

$$\pi a^2\frac{2J_1(2\pi Ra)}{2\pi Ra} \tag{4}$$

The Fourier transform of a helically grooved rod can be obtained by the convolution of those of disc and helix, and it is integrated by the separation of the coordinates. The integration along the fiber axis z is obtained by considering the disc of thickness fP spaced uniformly in a period of P, f thus being a fraction. This is given by, on the lth layer line,

$$2\pi f\frac{\sin lf\pi}{lf\pi} \tag{5}$$

The transform of the central core of the rod takes the form 4. For the part of the residual helical ridge with an inner radius a and an outer one $a+b$, b thus being the depth of ridge, the integration with respect to r is given by the form:

$$\int_a^{a+b}J_n(2\pi Rr)r\mathrm{d}r \tag{6}$$

This can be calculated by using the recurrence formula:

$$\int xJ_n(x)\mathrm{d}x=xJ_{n+1}(x)+n\int J_{n+1}(x)\mathrm{d}x$$

$$xJ_{n+1}(x)+xJ_{n-1}(x)=2nJ_n(x)$$

and the identity:

$$\int J_n(x)\mathrm{d}x=2\sum_{j=0}^{\infty}J_{n+2j+1}(x)$$

where x is the argument of the Bessel function. That is,

$$\int xJ_n(x)\mathrm{d}x=4(n+1)J_{n+2}(x)$$

$$-4\sum_{j=1}^{\infty}[2jJ_{n+4j}(x)-(n+2j+1)J_{n+4j+2}(x)] \tag{7}$$

The first maxima of the integral 7 lies at (see Appendix)

$$x=n+2+0.81\times\sqrt[3]{n+2}$$

Small-angle X-Ray Diffraction

One of the most striking differences between the small-angle X-ray patterns of moist and dry collagens lies in the fanning effect of the layer lines. Two distinct types of reflection confirmed by the author are depicted schematically in Fig. 3. The first type, which appears

9) M. von Laue, *Z. Krist.*, **64**, 115 (1926).
10) W. Cochran, F. H. C. Crick and V. Vand, *Acta Cryst.*, 5, 581 (1952).
11) R. S. Bear and O. E. A. Bolduan, ibid., 3, 236 (1950).

Fig. 3. Schematic illustration of small-angle diffraction patterns from collagen-class proteins. (a) Moist tendon collagen. (b) Dried tendon collagen. (c) Moist elastoidin. (d) Dried elastoidin.

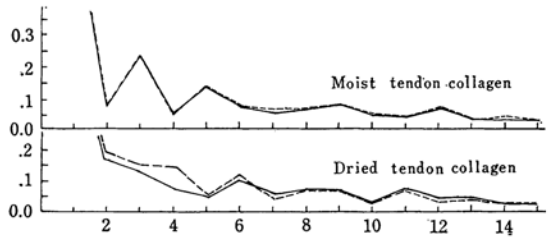

Fig. 4. Agreement of the calculated transform (broken line) and the square root of the observed intensity (full line) for moist and dried fowl-neck tendon collagens.

only on the meridian and is characteristic of moist materials, corresponds to a structure of smooth cylinder scatterers, while the second one, the fanning effect obtained from dry materials, might be attributed to a structure with a helical ridge. As may be readily seen from the theory developed in the foregoing section, the Fourier transform of the former is obtained by the product of (4) times (5). The alteration of intensity with the order of the layer lines may be expressed in excellent agreement with the observation by (5), in which $f=0.47$ (or 0.53) for moist collagen and $f=0.41$ (or 0.59) for dry collagen, as is shown in Fig. 4, (a) and (b) respectively. From a consideration of the transform 6, it is clear that the helical ridge structure is entirely adequate to account for the appearance of fanning and for the phenomenon of complex intensity distribution along the respective layer lines. The point of particular interest is that the intensity distribution of the layer lines is in accord with the transform 3, in which their first maxima occur at the arguments $x=0$, 1.8 and 3.4 respectively.

Electron Micrographs

The possibility of collagen fibrils being of a tubular[12] or a helical[13] structure has been suggested, but there is little conclusive evidence to support such structures. In the present investigation, collagen fibrils obtained from fowl-neck tendon were examined thoroughly. Specimens were picked up on an electron-microscopic grid without use of any supporting film and were shadowed with chromium. Typical electron-micrographs showing the membraneous structure of collagen fibril are reproduced in Figs. 5 to 8. They are cross-striated,

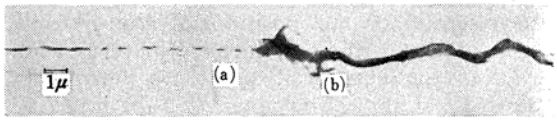

Fig. 5

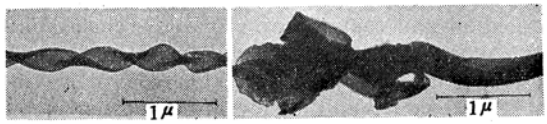

Fig. 5 (a) Fig. 5 (b)

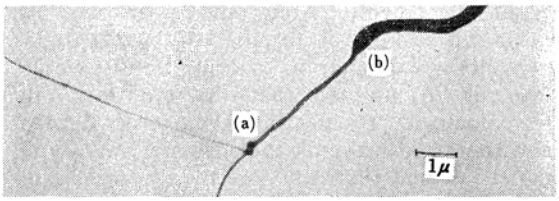

Fig. 6

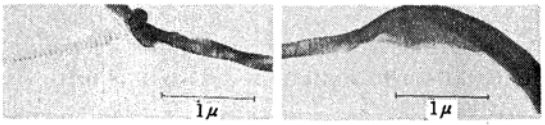

Fig. 6 (a) Fig. 6 (b)

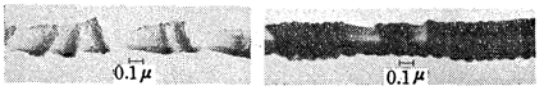

Fig. 7 Fig. 8

the period of the striations being of the order of 650Å, i.e., typical of collagen. A feature of interest in relation to this structure is the frequent occurrence of a stepwise transition from an assembly of filaments to a single one. Figs. 5 and 6 show such a transition and higher magnifications of some parts of the specimens. Further evidence for this view is provided by their pronounced tendency to aggregate side to side, with a slight displacement relative to one another, as is shown in Figs. 7 and 8. On the basis of the morphological evidence presented above, the electron-microscopic structure of collagen fibril is considered to be a kind of tubular structure with a circular or flat and coiled cross section.

12) J. J. Kennedy, *Science*, **121**, 673 (1955).
13) R. Reed, M. J. Wood and M. K. Keech, *Nature*, **177**, 697 (1956).

Structure of Collagen

In view of the experimental and theoretical results obtained above, it may be concluded that the collagen fibril consists of two phases alike in quantity, the crystalline phase characteristic of collagen-class and the amorphous phase, which has more hydration sites and, upon drying, loses water molecules to leave the helical groove. These considerations lead us to suppose that the collagen filament may be composed of coiled coils of a large number of polypeptide chains as follows:

$$([-P-P'-M-A-A'-]_4)_n$$

where P and P′ stand for the crystalline part and are rich in sequences of gly-pro-hypro, gly-pro-ala and other similar ones respectively. A and A′ form the amorphous part and are rich in sequences including gly and two other amino-acid residues. M is the transition part, about which the hydration sites are considered to be crowded. The striations are supposed to arise from the periodical occurrence of the P, P′, M, A and A′ parts, of which the distance between successive repeats causes the meridional identity period of 640Å. The fact that the tanning and the swelling do not alter the intrinsic spacing of the wide-angle reflections suggests that they occur outside the P and P′ parts.

Further evidence adduced to support this structure is as follows:

(a) The analytical data[14] provide some evidence that the sequence gly-pro-hypro may be of frequent occurrence in gelatin and collagen. According to Huggins[15], glutamic and aspartic acids can be in the form of condensed rings similar to the pyrrole ring; hence, they can take the place of pro or hypro in the P part. Thus, half of the total amino-acid residues in collagen can form a sequence of the gly-pro-hypro type.

(b) There are some evidence showing that, in collagen fibril, the amino-acid residues occur in groups. For example, Kuhn et al.[16] have stated from electron-microscopic studies that most of the basic residues are crowded in the α- and β-striations. Very likely they correspond to the A and A′ parts.

(c) The effective length of the side chain of pro and hypro is that of the pyrrole rings; hence, they can constitute the crystalline phase. On the other hand, the fully stretched side-chains of the basic residues are fairly long (ca. 8.8Å), whereas that of non-polar residues found in collagen is very short (ca. 3.4Å).

(d) The results obtained by Boedtker and Doty[17] and by Schmitt et al.[18] are in agreement with the view that the collagen molecule can be considered to have a length of the order of 2800Å.

(e) It is likely that the collagen filaments aggregate to each other by means of electrostatic forces, including hydrogen bonding, laterally. The lateral aggregation of the smooth cylinders having like diameters effects the streaking in the X-ray pattern as shown in Fig. 1. This view is supported by the findings that the crystal energy of the collagen fibrils does not always depend on the hydroxyproline content[19] and that the sorbed water is linked by hydrogen bonds to the CO group projecting outwards from and perpendicular to the fiber axis[20].

(f) A plaid pattern observed by Gross[21] perhaps results from a sheet with a helical structure.

Structure of Elastoidin

Elastoidin, a member of the collagen-class proteins obtained from shark fins, gives a small-angle diffraction pattern in which the expansion of the layer lines with an increase in l is outstandingly prevalent, whereas the central region fades out, as shown in Fig. 3(d). This fact may well be interpreted by assuming that a in transform 6 is very small compared with b. This supports the view that the collagen-class proteins have the tubular structure with a helical groove. It has been pointed out that the hydroxyproline content of elastoidin is but two-thirds that of collagen. The X-ray pattern from collagen has the packing peak, whereas elastoidin exhibits no additional bulbous terminal spot of the streak. This is in agreement with the view that elastoidin fibril is short of the regular lateral arrangement of chains because of a relative lack of the crystalline P and P′ parts and that it hence forms a deep groove on drying.

Summary

1. The X-ray scattering has been measured for moist and dry collagens of fowl-neck tendon, untreated or treated with formaldehyde

14) R. E. Schrohenloher, J. D. Ogle and M. A. Logan, *J. Biol. Chem.*, **234**, 58 (1959).
15) M. L. Huggins, *Proc. Natl. Acad. Sci., U. S.*, **43**, 209 (1957).
16) K. Kuhn, U. Hofmann and W. Grassmann, *Naturwissenschaften*, **46**, 512 (1959).
17) H. Boedtker and P. Doty, *J. Am. Chem. Soc.*, **78**, 4267 (1956).
18) F. O. Schmitt, J. Gross and J. H. Highberger, *Symp. Soc. Exptl. Biol.*, **9**, 148 (1955).
19) P. Doty and T. Nishihara, "Recent Advances in Gelatin and Glue Research", Ed. by G. Stainsby, Pergamon, London (1956), p. 92.
20) R. D. B. Fraser and T. P. MacRae, *Nature*, **183**, 179 (1959).
21) J. Gross, *J. Biophys. Biochem. Cytol.*, **2**, Suppl. 261 (1956).

or acetic acid. Some modifications of the collagen fibril were also examined by electron microscope.

2. The wide-angle scattering data are compatible with a structure consisting of crystalline and amorphous parts. The intensity distribution of the small-angle diffraction is interpreted by a helical groove structure. The Fourier transform calculated for this model is shown.

3. The electron micrographs show that collagen fibril has a kind of tubular structure with a circular or flat and coiled cross section.

4. The data are discussed with respect to the assumption that collagen fibril is composed of a woven sheet of polypeptide chains in which the crystalline and amorphous parts occur alternatively.

5. A reasonable assignment of hydration to the helical groove is possible; this gives the fibril an approximately uniform electron density in the moist state.

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Department of Chemistry
Faculty of Science
Kwansei Gakuin University
Nishinomiya, Hyogo-ken

Note added in proof

Reference 3 appeared in complete form:

A. Rich and F. H. C. Crick, *J. Mol. Biol.*, **3**, 483 (1961).

Appendix

$$x^{-2}\int_0^x J_n(x)x\mathrm{d}x$$