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THE STRUCTURE OF FEATHER RACHIS KERATIN

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The rachis of feathers gives rise to x-ray diffraction patterns of great complexity—they have been described as the most complex known for the naturally occurring fibrous substances. For their interpretation there is required a unit of structure with dimensions at least 9.5 A × 34 A × 94.6 A. In the following paragraphs we propose a structure for this protein that accounts for the principal features of the x-ray pattern and for some physical properties of the substance.

Astbury and other workers in the field have mentioned that the pattern somewhat resembles that of stretched hair, stretched muscle, and other proteins with the β-keratin structure, but that the x-ray diagram indicates that the length per residue is only 3.07 A, somewhat shorter than expected for an extended polypeptide chain, about 3.6 A, and than observed for silk fibroin, about 3.5 A, and for the β-keratin proteins, about 3.3 A. Astbury suggested that the chains might be in a somewhat collapsed β-keratin configuration, and pointed out that the reversible extensibility of feather keratin through about 7 per cent supported this assumption.[1] We were struck by the identity of the indicated fiber-axis residue length, 3.07 A, and the corresponding length predicted for the undistorted pleated-sheet configuration of hydrogen-bonded polypeptide chains, described in the preceding paper, and we investigated the possibility that feather keratin is composed of these pleated sheets in parallel orientation. This can be ruled out as unsatisfactory, however, in that, although the predicted distance between chains in the direction of the hydrogen bonds, 4.75 A, agrees closely with that indicated by the x-ray diagram, about 4.68 A, the other equatorial

reflections on the diagram cannot be accounted for by such a structure. The diagram[3] shows four equatorial reflections, with spacings 34 A, 17 A, 11 A, and 8.56 A, that seem to be the first four orders from a unit with edge about 34 A.   The magnitude of this dimension suggests three layers of protein, each about 11 A thick, and these layers could not be identical, inasmuch as all four orders of reflections are observed, rather than only the third order.   Consideration of alternative possibilities led us to the conclusion that the layers consist of a pleated sheet and two layers of α helixes, these helixes having the configuration described in recent papers.[4]

A plan of the proposed structure is shown as figure 1, and a schematic drawing as figure 2.   The chains of the pleated sheet are at the positions $x = 0, y = 0$, and $x = \frac{1}{2}, y = 0$, the base of the unit of structure having the dimensions $a_0 = 9.5$ A and $b_0 = 34.2$ A.   The centers of the α helixes
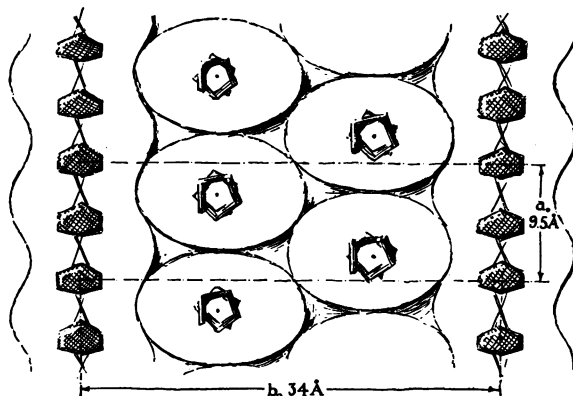

FIGURE 1

Plan of the proposed structure for feather rachis keratin.
The structure consists of pleated-sheet layers, between
which there are double layers of 3.7-residue helixes.

are at $\frac{1}{4}, y$ and $\frac{3}{4}, \bar{y}$, with y approximately $\frac{1}{3}$.   The α helixes are indicated to be in the close-packed arrangement given by these parameters by the absence of an equatorial reflection at 9.5 A.   A diffuse reflection corresponding to the second order is observed at about 4.68 A.

We have found that the intensities of the equatorial reflections can be rather well explained by this structure, with consideration only of the atoms of known fixed position in the pleated sheet and in the α helixes. The atomic parameters and also the form factors for the pleated sheet have been reported in a preceding paper,[2] and those for the α helix are given in the following paper.   The value of the parameter y indicated by the data is 0.275.   The structure factors calculated with use only of the atoms of known position, including the β carbon atom for each residue in both the

pleated sheet and the α helixes, and with the approximation of cylindrical symmetry for the α helixes, are given in table 1 under the heading F_1.

Although these structure factors are in rough agreement with the observed intensities, somewhat improved agreement is obtained by making a correction for the remaining atoms of the side chains of the pleated-sheet residues. We have carried out this calculation by assuming two carbon atoms, or atoms of equivalent scattering power, per residue, arranged about the positions $y = +\frac{1}{12}$ and $-\frac{1}{12}$. These positions, at 2.85 A from the center of the pleated sheet, are those expected for the pleated-sheet side chains. The distribution of the atoms about these positions has been approximated by using an F-factor for these atoms proportional to that calculated for the pleated sheet.[2] The values F_2 in table 1 were calculated with inclusion of these side-chain atoms, and the quantity $I_{calc.}$ given in the following column was obtained by multiplying the square of F_2 by the Lor-
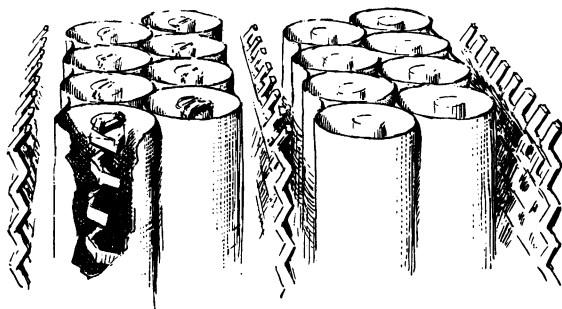

FIGURE 2

Drawing representing the proposed structure for feather rachis keratin.

entz, polarization, and frequency factors. The calculated intensity for (040) seems to be small; however, this calculated intensity is increased to the value 330 by inclusion of the intensity, 175, calculated for the form $\{110\}$, which is the only important diagonal reflection in this region of the equatorial plane. The structure factor for $\{120\}$ is small. The strong, diffuse reflection at 4.68 A is due to $\{200\}$, $\{210\}$, and $\{220\}$, with calculated spacings 4.75 A, 4.71 A, and 4.58 A. The structure factor for the α helix is very nearly 0 for this interplanar distance, and the form factor for these reflections is that of the chain in the β sheet, with contributions from the side-chain atoms. The value of F_2 has been obtained by assuming that the main-chain and side-chain atoms have the same effective distribution about their central axis as has the pleated sheet in the y direction. The general agreement of observed and calculated intensities is seen to be satisfactory.

In addition to these equatorial reflections, Corey and Wyckoff[3] reported three others, at 51.0 A (faint), 81.8 A (medium), and 115 A (medium). Bear[5] has pointed out that the central regions of the x-ray pattern given by feather rachis are confused by radiation artifacts related to the strong reflections, and that this is probably the source of these three large equatorial spacings. If we accept this interpretation, the equatorial reflections of feather keratin are all accounted for by our unit.

The meridional reflections observed by Corey and Wyckoff[3] and by Bear[5] are given in table 2. They can nearly all be accounted for as orders of a large identity distance, 94.6 A. In addition to the meridional reflections given in table 2, Bear reported a number of small-angle near-meridional reflections, corresponding to other orders of the identity distance 94.6 A.

TABLE 1

COMPARISON OF CALCULATED AND OBSERVED INTENSITIES OF EQUATORIAL X-RAY REFLECTIONS FOR FEATHER RACHIS KERATIN

PSEUDO-ORTHORHOMBIC, TRICLINIC UNIT WITH $a_0 = 9.50$ A, $b_0 = 34.2$ A, $c_0 = 94.6$ A, $\alpha \cong 90°$, $\beta \cong 90°$, $\gamma \cong 90°$

hkl	dcalc.	F_1	F_2	Icalc.	Iobs.[a]	dobs.[a]
010	(34.2 A)	22.8	42.6	730	Strong	33.3 A
020	17.1	−20.8	−10.4	24	Faint	17.1
030	11.4	45.5	45.5	295	Medium	11.0
110	9.14	−30.0	−30.0	210⎫	Medium	8.56
040	8.56	46.1	37.6	155⎬		
050	6.84	9.4	−3.2	1	...	...
060	5.70	12.3	0.4	0	...	...
070	4.89	11.4	3.1	1	...	,..
200	4.75	...	25	38⎫		
210	4.71	...	25	75⎬	Strong	4.68
220	4.58	.·.	25	75⎭	(diffuse)	
080	4.28	10.6	6.6	2		
090	3.80	16.4	16.4	12		
0·10·0	3.42	9.0	12.2	6		

[a] Corey and Wyckoff.[3]

For the ideal pleated sheet we have calculated a fiber-axis distance of about 3.07 A per residue, which agrees in numerical value with the spacing of the outermost meridional reflection. We think it likely, however, that the fiber-axis length per pleat in the pleated sheet in this substance is in fact not 6.14 A, but 6.30 A, and that there are fifteen of these units (thirty amino-acid residues) in the unit with $c_0 = 94.6$ A. The corresponding fiber-axis distance per residue, 3.15 A, can be achieved by a small deformation of the ideal pleated sheet, amounting to rotation by 7° around the C—C* axes of the amide groups, as described in the preceding paper.[2] A possibility that seems to us less likely is that there are $15^1/_2$ units of the pleated sheet in the distance 94.6 A, in which case the length per residue would be 3.05 A, and the value of c_0 would be twice as great, 189.2 A.

The fiber-axis length per residue for the α helix in substances so far investigated ranges from 1.53 to 1.56 A. These values correspond to the integers 62 and 61, respectively, as the number of residues in unit length 94.6 A along the c axis, the value 62 giving 1.525 A and 61 giving 1.551 A as the length per residue. The number of residues per unit turn is predicted to be close to 3.69, and values between 3.6 and 3.67 have so far been reported. With 17 turns in c_0, 61 residues would give 3.59 residues per turn, and 62 residues would give 3.65 residues per turn; no other value than 17 turns seems likely, inasmuch as it is improbable that the α-carbon bond angle would be strained enough to give 3.81 residues per turn (61 residues in 16 turns) or 3.45 residues per turn (62 residues in 18 turns). The most likely possibility is thus the 62-residue 17-turn helix, with 3.65 residues per turn. The distribution of side chains along the polypeptide chain presum-

TABLE 2

MERIDONAL REFLECTIONS FROM FEATHER RACHIS KERATIN

ORDER OF REFLECTION	BEAR		COREY AND WYCKOFF	
	d	INTENSITY	d	INTENSITY
4	23.6 A	10	23.1 A	Strong
			17.2	?
8	11.90	3	...	...
9	10.46	3	...	...
?	...	..	9.08	Faint
15	6.30	6	6.20	Strong
17	5.53	3	...	...
19	4.98	6	4.90	Strong
21	4.45	4	4.37	Medium
24	...	..	3.95	Faint
27	...	..	3.52	Faint
?	...	..	3.22	Faint
31	...	..	3.07	Medium

ably is such as to stabilize this helix, and the scattering of x-rays by the different side chains, in positions that remain to be determined, gives rise to the true meridional reflections, with contributions from the atoms of the main chains in the helix and the pleated sheet for some reflections.

It is interesting to note that the reflection $(0 \cdot 0 \cdot 17)$ is reported by Bear, at 5.35 A; this would correspond to one turn of the α helix. A strong reflection at 6.20 A (Corey and Wyckoff) or 6.30 A (Bear) is interpreted by Bear as the fifteenth basal plane reflection, $(0 \cdot 0 \cdot 15)$. We interpret this as involving collaboration of the 15 units of the pleated sheet.

The identity distance 94.6 A receives a rational explanation as resulting from the presence of two structures, the slightly distorted pleated sheet with identity distance along the a axis of 6.30 A, and the α helix with 5.57 A per turn. The simplest ratio of integers approximating the ratio of these numbers is 17:15, corresponding to the mutual identity distance

94.6 A. These two structures accordingly might well be expected to form a protein such as feather keratin, with a triclinic unit with $a_0 = 9.50$ A, $b_0 = 34.2$ A, $c_0 = 94.6$ A, $\alpha \cong 90°$, $\beta \cong 90°$, $\gamma \cong 90°$.

It seems likely that the pleated sheets are all oriented similarly in the structure—there is no significant indication of a unit with $b_0 = 68$ A, corresponding to two kinds of pleated sheets, with opposite orientations. A pleated sheet is polar: all of the C=O groups point in one direction, and the N—H groups in the opposite direction, and in addition the side chains on one side of the sheet are arranged differently with respect to the residues than are those on the other side of the sheet, so that an isolated sheet would be curved. It is interesting to speculate that this curvature of the pleated sheets may be related to the natural curvature of the feather rachis. An example of a polar sheet in the inorganic field is the kaolin sheet.[5] Curved crystals of the clay minerals have been recently observed with use of the electron microscope, and their curvature has been assumed to result from the polar nature of the kaolin sheets.[7, 8]

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THE STRUCTURE OF HAIR, MUSCLE, AND RELATED PROTEINS

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It is thirty years since x-ray photographs were first made of hair, muscle, nerve, and sinew, by Herzog and Jancke.[1] During this period, despite the efforts of many investigators, the photographs have eluded detailed in-