THE PLEATED SHEET, A NEW LAYER CONFIGURATION OF POLYPEPTIDE CHAINS

BY LINUS PAULING AND ROBERT B. COREY

GATES AND CRELLIN LABORATORIES OF CHEMISTRY,* CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

Communicated March 31, 1951

For many years it has been assumed that in silk fibroin, stretched hair and muscle, and other proteins with the β-keratin structure the polypeptide chains are extended to nearly their maximum length, about 3.6 A per residue, and during the last decade it has been assumed also that the chains form lateral hydrogen bonds with adjacent chains, which have the opposite orientation. A hydrogen-bonded layer of this sort is represented diagrammatically in figure 1.[1-4]

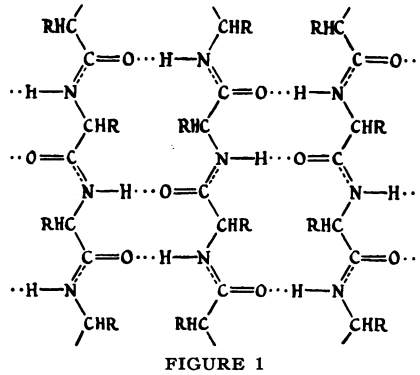


FIGURE 1

Diagrammatic representation of a hydrogen-bonded layer structure of polypeptide chains with alternate chains oppositely oriented.

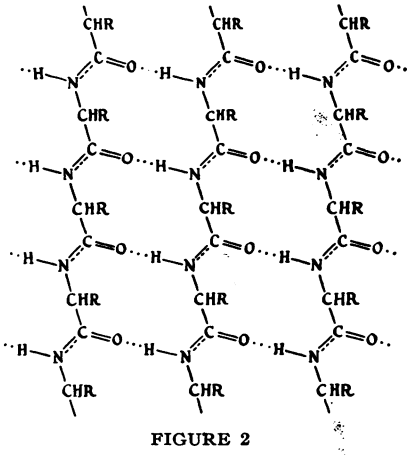


FIGURE 2

Diagrammatic representation of a hydrogen-bonded layer structure of polypeptide chains with all chains similarly oriented (the pleated sheet).

We have now discovered that there is another, rather similar hydrogen-bonded layer configuration of polypeptide chains, which differs from that of figure 1 in several ways. In the new configuration, which we shall call the pleated-sheet configuration, the plane formed by the two chain bonds of the α carbon atom is perpendicular to the plane of the sheet, as shown in figures 2 and 3, rather than being coincident with it. In this structure the successive residues in a chain are similarly oriented, directing their carbonyl groups in one direction and their imino groups in the opposite direction, and all of the chains are oriented in the same way, instead of adjacent chains being opposed in direction.

Let us assume that a polypeptide chain with the configuration indicated diagrammatically in figure 2 is bent in such a way that the planes of the successive amide groups form dihedral angles whose edges are perpendicular to the plane formed by the axes of the groups (the lines connecting successive α carbon atoms). It is found that if the bond distances and bond angles are given the values that we have used in our recent considerations of protein configurations the dihedral angle has the value 106.5°, and the vertical component of the axis of each residue is 3.07 A. It is also found that the carbonyl and imino groups are oriented in such a way that they can form satisfactory hydrogen bonds with corresponding groups in chains

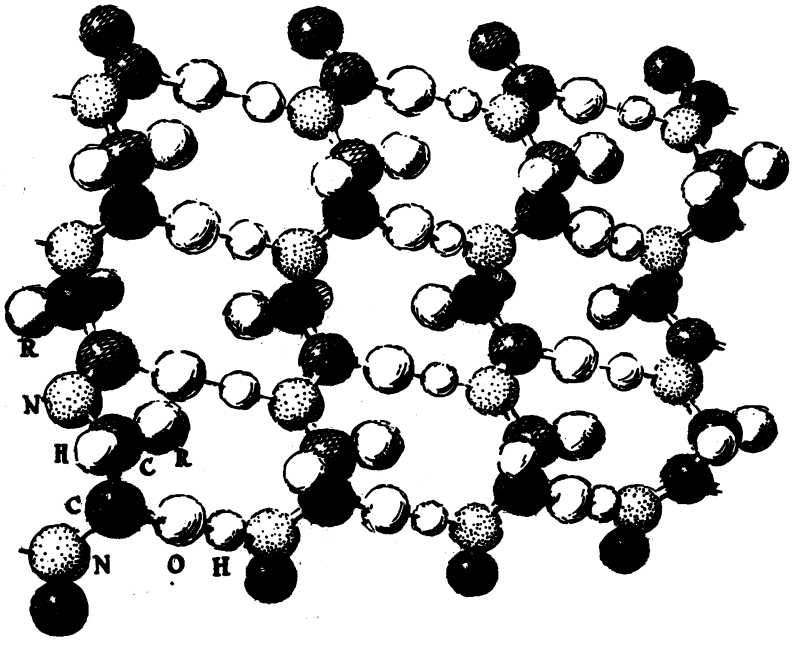


FIGURE 3

Drawing representing the pleated-sheet configuration of polypeptide chains.

obtained by lateral translation. If the lateral translation is given the value 4.75 A the N—H···O distance is 2.75 A; this is a normal hydrogen-bond distance. The N—H axis lies within 6° of the N···O axis, indicating that a stable hydrogen bond should be formed. The coordinates of atoms for the pleated-sheet configuration are given in table 1, and a drawing of the configuration is shown as figure 3.

It is to be noted that each amide group in the chain (neglecting the side chains) may be described as obtained from the preceding one by the operation of a glide plane of symmetry. Because of this, side chains of L-amino

acid residues are related differently to the structure when attached to one α carbon atom than when attached to the α carbon atom of an adjacent residue. The pleated-sheet configuration can accordingly be described as involving only one kind of glycine residue, in case that it were to be assumed by a polyglycine, but two kinds of residues for all optically active amino-acid polymers. These two kinds differ in that, for the L configuration, a residue of one kind points its β carbon atom in the C=O direction, and a residue of the other kind points its β carbon atom in the N—H direction.

We have found some evidence to support the belief that the pleated-sheet configuration is present in stretched muscle, stretched hair, feather keratin, and some other fibrous proteins that have been assigned the β-keratin structure. These proteins give x-ray diagrams on which there is a strong meridional reflection corresponding to spacing about 3.3 A, which is a few per cent larger than the fiber-axis distance per residue for the undistorted

TABLE 1

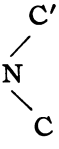
COORDINATES OF ATOMS IN THE POLYPEPTIDE PLEATED-SHEET CONFIGURATION (IN A)

	UNROTATED			7° ROTATION			20° ROTATION		
ATOM	x	y	z	x	y	z	x	y	z
C_1	0.00	1.15	0.00	0.00	1.09	0.00	0.00	0.96	0.00
N_1	−0.36	0.30	1.14	−0.36	0.46	1.17	−0.36	0.35	1.29
C_1'	0.53	−0.28	1.91	0.53	−0.31	1.96	0.50	−0.40	1.98
O_1	1.74	−0.14	1.73	1.72	−0.31	1.75	1.63	−0.64	1.58
C_2	0.00	−1.15	3.07	0.00	−1.09	3.15	0.00	−0.96	3.32
N_2	−0.36	−0.30	4.21	−0.36	−0.39	4.31	−0.34	−0.14	4.49
C_2'	0.53	0.28	4.98	0.53	0.22	5.12	0.50	0.08	5.49
O_2	1.74	0.14	4.80	1.72	−0.04	4.95	1.63	−0.39	5.50
C_1*	0.00	1.15	6.14	0.00	1.09	6.30	0.00	0.96	6.64

pleated sheet, but much smaller than the value 3.6 A for fully extended polypeptide chains. We have noticed that the pleated sheet can be subjected, without rupturing the hydrogen bonds, to a considerable distortion, in such a way as to increase the fiber-axis distance. This distortion is effected by rotating each amide group about its C—C* axis through a small angle. The rotation moves one of the two β positions of each carbon atom farther from the median plane and the other nearer, and the effective rotations for the two non-equivalent kinds of optically active residues are such as to permit each to be an L residue with its side chain farther from the median plane than in the undistorted structure. Presumably the van der Waals repulsion of the side chain atoms and the main chain atoms would be operating in proteins of normal chemical composition with the pleated-sheet configuration, and this would cause some distortion of the chain-lengthening sort. (It is to be noted that two kinds of pleated sheets can be constructed of L-amino-acid residues, of which for one the deformation that

relieves the strain of side chain van der Waals repulsion increases the fiber-axis length, and for the other it decreases it.) It might well occur that the magnitude of the deformation would be such as to give the fiber-axis residue length observed for the β-keratin proteins, about 3.3 A. This deformation results from a 20° rotation of the amide groups, which gives 3.32 A as the residue length. Coordinates for the structure with 20° rotation and also for a less deformed structure, with 7° rotation, are given in table 1.

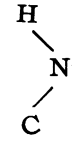
The deformed structures require some distortion of the hydrogen bonds, in that if the hydrogen atom is kept coplanar with the amide group the N—H direction deviates from the N···O axis by an angle somewhat greater than the distorting angle of rotation. The nature of the distortion is such, however, as to suggest that not much strain energy is involved. Let us consider the effect on the stability of the amide group of moving the hydrogen atom onto (or nearly onto) the N···O axis. This motion would keep



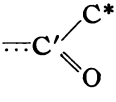
the hydrogen atom nearly in a plane normal to the N plane; that is, it involves moving the hydrogen atom toward one of the tetrahedral corners of the nitrogen atom. If the nitrogen atom were forming a pure double bond with the carbonyl carbon C′ there would be strong resistance to this motion of the hydrogen atom. However, it forms a bond with about one-half double-bond character and one-half single-bond character, corresponding to the resonance

and for the second of the structures the tetrahedral position for the hydrogen atom would be the normal one, whereas for the first the planar position is stable. According we would predict that this rotational distortion of the pleated sheet would not involve so much strain as if the bonds were double bonds.

We may now ask to what extent distortion of the amide group from the planar configuration, through rotation of the two ends



and



in opposite directions about the N···C′ axis, might be expected

to occur. The strain energy of this distortion, which is essentially also the strain energy of distortion of the hydrogen atom out of the plane, can be estimated in the following way. With δ the dihedral angle formed by the planes of the two end groups, the amide resonance energy may be taken equal to $-A \sin^2 (\delta - \pi/2)$, and the strain energy to $A \sin^2 \delta$. The factor A is the amide resonance energy for the planar configuration. This may be estimated as about 30 kcal mole^{-1}. (The experimental value for the carboxylate ion, in which each of the two C···O bonds has 50 per cent double-bond character, is 36 kcal mole^{-1}, and somewhat smaller values are found for gas molecules of amides, esters, and related substances.[5]) We thus find about 0.9 kcal mole^{-1} strain energy for 10° distortion of the amide group, 3.5 kcal mole^{-1} for 20° distortion, and so on, and we may predict that distortions as large as 20° might well occur in structures in

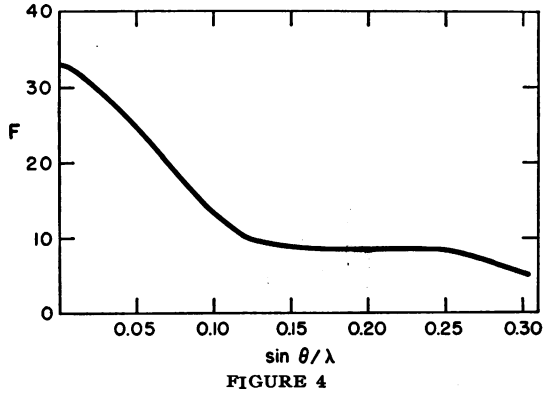


FIGURE 4

Calculated x-ray form factors for the pleated sheet, for planes parallel to the plane of the sheet.

which these distortions would relieve a larger strain, but that in general the polypeptide chain would avoid structures involving such strains. In any case, we would expect the distortion to be divided between the amide residue and the hydrogen bond. In calculating the coordinates of table 1 we have not taken account of these distortions.

The discussion of the pleated sheet in β keratin and other proteins will be presented in following papers. In this discussion we make use of the x-ray scattering form factor for the sheet. The form factor, calculated for reflections from planes parallel to the median plane of the undistorted sheet, is for convenient later reference given here, in figure 4, as calculated from the equation $F = \sum_i f_i \cos (2\pi y_i \sin \theta/\lambda)$, with f_i values as given in the International Tables for the Determination of Crystal Structures. The sum has been taken over the atoms of one residue of the undistorted structure, including also a β carbon atom, with $y = 2.04$.

This investigation was aided by grants from The Rockefeller Foundation, The National Foundation for Infantile Paralysis, and The United States Public Health Service.

* Contribution No. 1552.

[1] Astbury, W. T., *Trans. Faraday Soc.*, **29**, 193 (1933).

[2] Huggins, M. L., *J. Org. Chem.*, **1**, 407 (1936); *Chem. Rev.*, **32**, 195 (1943).

[3] Pauling, L., *J. Am. Chem. Soc.*, **62**, 2643 (1940).

[4] Lotmar, W., and Picken, L. E. R., *Helv. Chim. Acta*, **25**, 538 (1942).

[5] Pauling, L., *The Nature of the Chemical Bond*, Cornell University Press, Ithaca, N. Y., 1939.

THE STRUCTURE OF FEATHER RACHIS KERATIN

BY LINUS PAULING AND ROBERT B. COREY

GATES AND CRELLIN LABORATORIES OF CHEMISTRY,* CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

Communicated March 31, 1951

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