Macromolecules

Volume 13, Number 3 May–June 1980

Structure of β-Keratin

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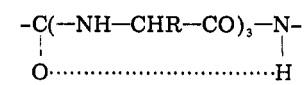
ABSTRACT: It is proposed that when α-keratin is changed on stretching to β-keratin, the parallel-chain polypeptide helices are extended until each has just one amino acid residue per helix turn. The NH and CO groups then extend laterally from each chain axis, in sheets. The angle between the NH and CO sheets is about 120°. NHO hydrogen bonds then connect each chain to two others. If all the helices so connected have the same rotation sense, a structure that is an assemblage of hexagonal prism "6-stacks" is formed. If half of the helices are right handed and half left handed, "wavy sheets" or "pleated sheets" can be formed. The 6-stacks or sheets can be packed together in ways that provide suitable locations for disulfide cross-linking. Hybrid structures containing zones of different types may also exist. Experimental X-ray data from the literature are compared with the results of calculations based on the (simplified) theoretical models. Both sheet structures seem satisfactory, but further work is necessary before 6-stack structures are definitely eliminated.

Structure of α-Keratin

α-Keratin, the crystalline component of normal hair, consists of polypeptide chains in a spiral (= helical) conformation, with NHO hydrogen bonds connecting successive turns of each helix.[1-3] The hydrogen bonds are so located[4,5] as to form 13-atom rings, as indicated by the formula



This helical structure is of the type called "α helix" by Pauling and Corey, but the helix dimensions and atomic coordinates in α-keratin may differ somewhat from those calculated by them.[6]

Attempting to explain a meridional X-ray reflection corresponding to an interplanar spacing of about 5.15 Å, Crick[7] and Pauling and Corey[8] proposed that the polypeptide helices are spirally coiled, in groups of two of three or more, like strands in a rope.

Such coiled-coil structures for α-keratin have not seemed reasonable to me, because of the difficulty in explaining the transformation to β-keratin (for which a structure composed of non-rope-like extended polypeptide chains is generally assumed) when hair is stretched.

Reexamining the α-keratin problem, I have deduced a non-rope-like structure that accounts reasonably well for all the experimental data of which I am aware.[9-11]

In this structure the molecular helices are in groups of three ("3-stacks"), with their axes mutually parallel. Each molecular helix is shifted axially a distance of about 5.15 Å relative to its neighbors. Like residues in the 3-stack are thus placed helically around the 3-stack axis very simply accounting for the 5.15 Å reflection.

The 3-stacks are also arranged with parallel axes to give a structure with a crystallographic unit containing 81 chains.

One way of arranging the molecular chains (at three levels) to give the 81-chain unit has been described.[9-11] Another simpler way is shown in Figure 1.

Following Astbury's ideas,[12,13] I assume some disulfide cross-links between neighboring chains. I suggest that (usually at least) they are between pairs of chains having like residues at the same level, designated by the same letter in Figure 1. More specific determination of the locations of these or other cross-links must await further knowledge of the residue sequences.

Old Models for β-Keratin

For the β-keratin structure, Astbury[12,13] proposed a sheet-like structure, with the –NH–CHR–CO→ directions of adjacent chains in each sheet oppositely oriented: an "antiparallel structure". Introducing the concept of hydrogen bonding between the NH and CO groups in adjacent chains in each sheet[14] and making use of the then-available knowledge of bond lengths, bond angles, and approximate minimum distances between nonbonded atoms, I modified Astbury's proposal and described[2,3] two sheet-like structures (one a parallel-chain structure and the other an antiparallel-chain structure) that seemed theoretically possible. Pauling and Corey[15] later discussed several types of sheet structures, differing in details from those I had proposed.

More recently, Fraser and co-workers,[16,17] on the basis of quite extensive X-ray and infrared data, have concluded that the β-keratin structure is of an antiparallel-chain sheet

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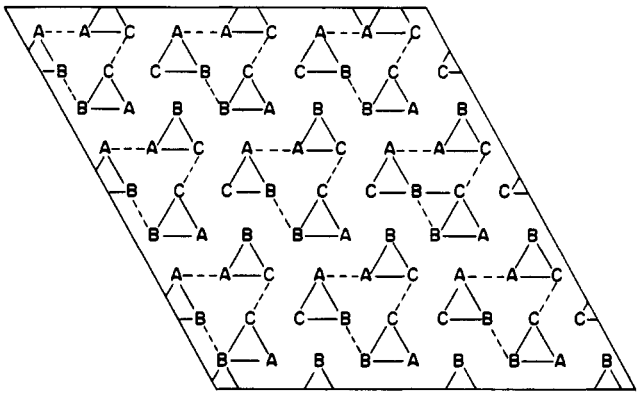


Figure 1. A possible arrangement of the polypeptide chains in the unit cell of α-keratin. A, B, and C designate chains with corresponding residues at different heights (from ref 19).

type, with some randomness in the relative positions of adjacent sheets.

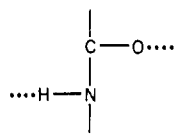
New Models for β-Keratin

If, as I have concluded, the chains in α-keratin all have *parallel* orientation, it does not seem reasonable that mere stretching (with breakage of hydrogen bonds and disulfide cross-links) could produce a structure in which each chain has an orientation opposite to that of its two closest neighbors. I have therefore reexamined the β-keratin structure problem, with results (previously reported briefly[18,19]) that I shall now describe.

I propose that, in the $\alpha \rightarrow \beta$ transformation, the polypeptide helices are extended—not all the way to the planar zigzag structure, but only until there is just one amino acid residue per helix turn. All of the C–O centerlines in any one polypeptide chain then extend laterally in one plane passing through the chain axis and all of the N–H centerlines extend laterally in another plane, making a dihedral angle of about 120° with the plane containing the C–O centerlines. The bisectors of the C–H and C–R bonds of the CHR groups are in a third lateral plane, making angles of about 120° with each of the other two planes.

NHO hydrogen bond formation between the chains obviously produces "6-stacks", each having a shape approximating that of a hexagonal prism; see Figure 2.

The CO··· hydrogen bond extending laterally from each peptide unit is shifted in the axial direction about one-third of the residue height relative to the adjacent NH··· hydrogen bond:



It follows that like residues must be in a spiral arrangement around the 6-stack axis, with an axial shift of two residues per turn of the spiral. This agrees with the X-ray data.

Because the interatomic distances between adjacent chain atoms are not accurately the same and the angles between the bonds connecting these atoms are not all the same and because the hydrogen bond systems connecting the chains are not likely to be strictly linear, there must be slight departures from the idealized structure pictured. These departures may lead to small departures from strict parallelism of the chain axes and the 6-stack axis. For isolated 6-stacks one might expect a slight helical twisting. I suggest, however, that such twisting is obviated or minimized by cross-linking between neighboring 6-stacks.

One reasonable arrangement of 6-stacks (structure A) is shown in Figure 3. If the closest edges of adjacent

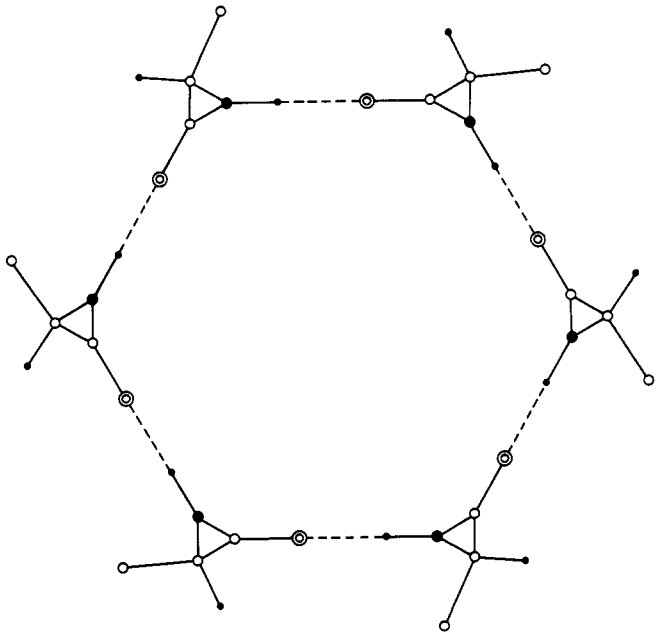


Figure 2. Idealized projection of a hypothetical 6-stack arrangement of polypeptide chains in β-keratin (from ref 19). (This projection represents right-handed polypeptide helices, with dextro orientation of the bonds at each CHR group. For right-handed polypeptide helices with levo orientation of the bonds at each CHR group, the C–H and C–R bonds should be interchanged.)

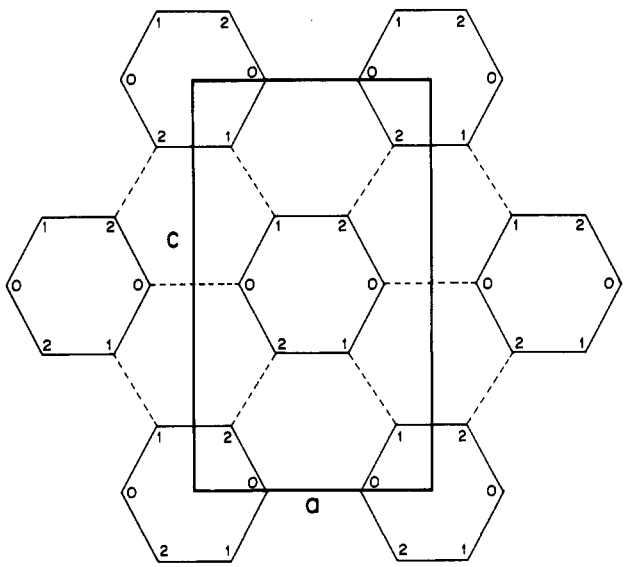


Figure 3. Projection of a portion of the (idealized) hypothetical structure A. The chain axes are at the hexagon corners. Interchain hydrogen bonds are indicated by full lines and cross-links by dashed lines. The orthogonal pseudounit is outlined by heavy lines; a = 16.32 Å; b = 6.66 Å; c = 28.27 Å. Relative heights of like residue centers (in units of $b/6$) are shown by numbers (0, 1, 2).

6-stacks have polypeptide chains with like residues at the same height and if the distances between the closest edges of adjacent 6-stacks are appropriate for disulfide cross-linking, a projected pattern of hydrogen bonds and cross-links like that shown seems reasonable.

This idealized structure could obviously be represented by a pseudohexagonal unit, but I use a pseudo-orthorhombic unit for better comparison with the other structures that I shall discuss. An actual structure of this type would certainly depart from the idealized one represented in various ways. It would probably have triclinic or pseudomonoclinic symmetry.

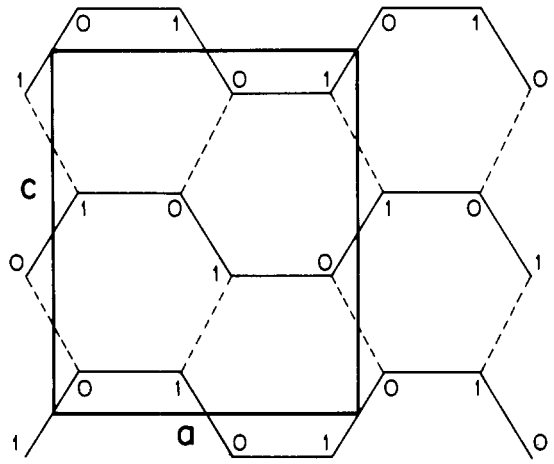


Figure 4. Projection of a portion of the (idealized) hypothetical structure B. The chain axes are at the bends in the wavy lines; a = 16.1 Å; b = 6.66 Å; c = 19.4 Å.

I have considered several other ways of assembling 6-stacks, but have not found any that conform better with the X-ray evidence.

Because of possible departures from strict linearity of the N–H · · · O–C system and small modifications of the geometry of the 1-residue-per-turn polymers it appears possible that 1-residue-per-turn helices might hydrogen bond together to form 3-stacks, instead of the 6-stacks I have assumed. Although I once proposed[20] a 3-stack structure for β-keratin, I now consider it impossible. With the axial shift of one-third residue at each triangular prism edge, the repeat distance in the structure would remain at about 3.33 Å, instead of twice that, as required by the X-ray data.

A 9-stack prism structure is similarly ruled out, since it would require an axial repeat of about 10 Å.

In the 6-, 3-, and 9-stack structures, the helix rotation sense for all the chains in each stack must be the same. Assuming, as I have, that each polypeptide helix is right handed in α-keratin, the β-keratin helices produced from α-keratin might be expected to also be right handed. This is by no means certain, however, since, when the chains are stretched to or beyond the 1-residue-per-turn state, transformation to left-handed helices should be very easy. If such transformations would produce a more stable structure, they might well occur.

It is also possible that extension of chains in the amorphous part of the keratin structure might produce a mixture of right- and left-handed helices.

Because of these considerations, I have considered crystal structures, for β-keratin, containing both right- and left-handed molecular helices.

I have dealt primarily with two such structure types. In what I designate structure B, the polypeptide chains are joined by hydrogen bonds to form "wavy sheets", with the rotations (R,L) of the chains in each sheet following the pattern –RRLLRRLL–. Figure 4 shows how the wavy sheets might be arranged to give good locations for disulfide cross-links.

Structure C is an assemblage of "pleated sheets", with right- and left-handed rotations alternating: –RLRLRL–. These sheets can be packed to accommodate cross-links, as shown in Figure 5.

The new sheet structures differ fundamentally from any of the sheet structures that have previously been proposed, by me or others.

It is conceivable that a β-keratin "crystal" might be twinned, containing zones of two or more types. The primary requirement, I believe, is that the interchain

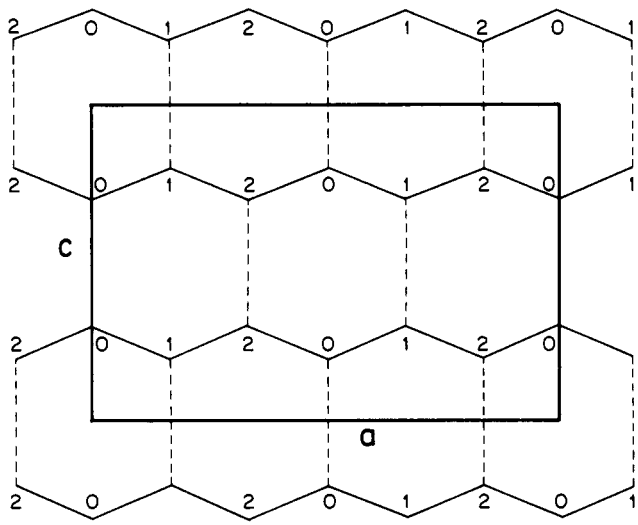


Figure 5. Projection of a portion of the (idealized) hypothetical structure C. The chain axes are at the bends in the zigzag lines. Interchain hydrogen bonds are indicated by full lines and cross-links by dashed lines; a = 28.2 Å; b = 6.66 Å; c = 19.4 Å.

connections by hydrogen bond and disulfide cross-links, at roughly 120° angles, must be satisfied—even at the boundaries between zones. In another paper[19] I have shown how this result can be accomplished.

Comparison with Experimental Data

As a test of structures A, B, and C, I have compared the observed X-ray spacings, d_{hkl}, reported by Fraser and MacRae,[21] and the spacings calculated by them for a pseudocell with a = 9.46 Å, b = 6.68 Å, and c = 9.7 Å with the spacings calculated for the idealized pseudocells for structures A, B, and C; see Table I.

For comparison with the experimental intensities, I have calculated rough (relative) theoretical intensities for the new structures in the following way.

I assume that the relative intensity of a reflection having indices hkl can be approximated by the equations

$$I_{hkl} = f_d F^2_{hkl} \tag{1}$$

$$F_{hkl} = \sum_i \cos 2\pi(hx_i + ky_i + lz_i) \tag{2}$$

Here x_i, y_i, and z_i are the coordinates of the residue centers in the pseudocell (with the origin at a center of symmetry) and f_d is a function that decreases rapidly as d_{hkl} decreases. This function can be considered as the square of a hypothetical "residue structure factor", corresponding to an "atomic structure factor", times other corrections normally applied in crystal structure analysis. For my present purposes I neglect multiplicity factors.

If two or more hkl sets give calculated spacings so close together that they would be expected to contribute to the same observed spot, I calculate the expected theoretical intensity by

$$I = f_d(F^2_{h_1k_1l_1} + F^2_{h_2k_2l_2} + \cdots) \tag{3}$$

The function f_d depends greatly on the (largely unknown) distribution of atomic scattering centers around the residue centers (on the molecular axes). As a crude approximation I use the empirical relation

$$f_d = d^3_{hkl}/1000 \tag{4}$$

The calculated spacings and intensities for the A, B, and C structures all give as good agreement with the observed data as could be expected, considering the approximations

Table I
Comparison of Observed and Calculated Data

obsd					calcd											
Fraser and MacRae					structure A				structure B				structure C			
int		hkl	d_{hkl}	I	hkl	d_{hkl}	F_{hkl}	I	hkl	d_{hkl}	F_{hkl}	I	hkl	d_{hkl}	F_{hkl}	I
									Layer Line 0							
s[a]	9.7	001	9.7						002	9.70	40.7	1513	002	9.70	−29	773
vvs	4.68	200	4.73	136	303	4.71	−9.8	949	302	4.70	22.5	85	600	4.70	36	133
									004	4.85	12.3					
									104	4.64	13.1					
m[a]	3.85				305	3.92	12.6	9	005	3.88	0	0	105	3.84	2	11
													005	3.88	0	
													701	3.94	12	
													404	3.97	7	
m	2.37	400	2.37	20	0,0,12	2.36	24.6	12	604	2.35	4.8	2	12,0,0	2.35	36	17
					606	2.36	17.4		008	2.43	−10.1		806	2.38	0	
mw	1.57	600	1.58	2	8,0,12	1.54	−23.5	4	906	1.57	2.6	4	18,0,0	1.57	36	12
					7,0,13	1.59	22.1		10,0,2	1.59	25.5		18,0,2	1.55	−29	
									808	1.55	−12.4		15,0,7	1.56	29	
									6,0,10	1.57	−17.3					
									Layer Line 1							
w	5.43	110	5.46	30	013	5.44	0	0	012	5.49	0	0	310	5.43	0	0
s	3.80	210	3.86	18	215	3.81	0	0	312	3.84	0	0	611	3.77	0	0
m	2.23	410	2.23	6	518	2.26	0	0	614	2.21	0	0	318	2.22	0	0
					1,1,12	2.20	0						12,1,1	2.20	0	
									Layer Line 2							
s	3.33	020	3.34	11	020	3.33	0	0	020	3.33	27.3	28	020	3.33	0	0
m	2.68	220	2.73	16	323	2.72	0	0	322	2.72	−12.1	8	224	2.69	0	0
					026	2.72	0		124	2.71	−16.3					
w	2.25	320	2.29	8	523	2.26	0	0	226	2.23	−23.1	9	921	2.27	0	0
					426	2.26	0		424	2.27	−15.5					
vw	1.92	420	1.93	1	626	1.92	0	0	624	1.92	−4.0	0.1	826	1.92	0	0
					4,2,10	1.91	0									
					3,2,11	1.91	0									
									Layer Line 3							
m	2.15	130	2.17	16	231	2.14	0	0	132	2.15	0	0	330	2.16	0	0
					033	2.16	0		231	2.13	0		132	2.16	0	
s	2.00	230	2.02	1	136	1.99	0	0	332	2.01	0	0	631	2.00	0	0
					332	2.03	0		134	2.00	0		433	2.01	0	
													134	2.01	0	
													532	2.02	0	
w	1.81	330	1.82	0	039	1.81	0	0	532	1.80	0	0	930	1.81	0	0
					532	1.82	0		136	1.82	0		833	1.80	0	
									530	1.83	0		734	1.81	0	
													136	1.83	0	
vw	1.27	630	1.29	0	10,3,5	1.28	0	0	936	1.28	0	0	11,3,10	1.27	0	0
					7,3,14	1.26	0		10,3,3	1.28	0		5,3,12	1.27	0	
									3,3,12	1.27	0		18,3,3	1.26	0	
													18,3,1	1.28	0	
													17,3,4	1.28	0	
													8,3,11	1.29	0	

								Layer Line 4								
w	1.67	040	1.67	8	040	1.67	0	0	040	1.67	−2.1	0.3	040	1.67	0	0
					141	1.65	0		141	1.65	0		141	1.65	0	
					042	1.65	0		140	1.66	−7.3		042	1.65	0	
									041	1.66	0					
w	1.58	240	1.58	3	046	1.57	0	0	342	1.57	−14.6	2	044	1.58	0	0
					343	1.57	0		044	1.58	−17.2		640	1.57	0	
					244	1.59	0						541	1.59	0	
					145	1.59	0						343	1.59	0	
					341	1.59	0									
vvw	1.50	340	1.48	3	444	1.51	0	0	541	1.48	0	1	743	1.50	0	0
					347	1.48	0		444	1.47	−10.1		840	1.51	0	
					248	1.48	0		442	1.52	−11.0		345	1.51	0	
									145	1.52	0		842	1.49	0	
													644	1.49	0	
								Layer Line 5								
vw	1.29	250	1.29	0	350	1.29	0	0	352	1.28	0	0	552	1.29	0	0
					352	1.29	0		154	1.28	0		550	1.30	0	
					253	1.30	0		350	1.29	0		253	1.30	0	
					055	1.30	0		253	1.29	0		453	1.28	0	
													154	1.28	0	
													651	1.28	0	
								Layer Line 6								
m	1.11	060	1.11	5	060	1.11	36	2	060	1.11	−1.9	0.03	060	1.11	36	2
		160	1.11						360	1.09	−1.7		161	1.11	0	
		260	1.08						160	1.11	−2.1		062	1.10	−29	
									260	1.11	−2.0					
									161	1.11	−2.2					

[a] Diffuse.

resulting from the idealization assumptions—such as the placing of all the scattering centers *on* the molecular axes, with all residues treated as equivalent. These approximations lead directly to the calculated zero intensities for the reflections on all of the layer lines except 0 and 6 for structures A and C and for the reflections on the odd layer lines for structure B.

The absence of a calculated spacing for structure A, corresponding to the strong diffuse equatorial reflection at $d = 9.7$, results from the assumption that the ideal distribution of scattering centers has hexagonal symmetry, with dimensions to give agreement for the other equatorial reflections. Reasonable departures from the ideal structure dimensions could lead to agreement with the 9.7 Å reflection, but I have not discovered any 6-stack structure that would explain a *strong* one and also give reasonable distances for interchain hydrogen bonding and disulfide cross-linking.

It is conceivable that crystalline β-keratin is a composite of a 6-stack structure and a sheet-like structure (such as B or C) produced by extension and crystallization of the chains in the amorphous part of the original keratin. If so, the diffuse 9.7 and 3.85 Å reflections would be well accounted for.

Concluding Remarks

More research obviously needs to be done before the β-keratin structure can be considered known and thoroughly understood. In addition to the X-ray data, evidence obtained by infrared spectroscopy and other techniques needs to be reexamined and reinterpreted. I suggest also that there must be some unambiguous experimental technique for determining the –NH–CHR–CO→ direction (or directions) and the screw sense (or senses) of the helices in crystalline polypeptide structures.

I predict that various other natural and synthetic polypeptides that appear to be structurally related to β-keratin will be found to contain one-residue-per-turn helices, in arrangements similar to those I have discussed, but often (if there are few or no disulfide cross-links) differing with regard to the structural relationships that are not determined by hydrogen bonding requirements.

Although I am much interested in these problems, I doubt if I shall have the time or facilities to deal with them properly. I hope that others—especially those who have already worked in this field—will carry on.

Acknowledgment. I gratefully acknowledge my indebtedness to Astbury, the pioneer in keratin structure research, and to those—especially Fraser and coworkers—whose research results I have found very helpful.

In conclusion, I take pleasure in dedicating this paper to my friend, Professor Paul J. Flory, on the occasion of his 70th birthday.

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Calculation of the Structures of Collagen Models. Role of Interchain Interactions in Determining the Triple-Helical Coiled-Coil Conformation. 2. Poly(glycyl-prolyl-hydroxyprolyl)[1a]

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Received December 3, 1979

ABSTRACT: The conformational space of regularly repeating structures of poly(glycyl-prolyl-hydroxyprolyl), $(GPH)_n$, was examined for stable triple-stranded complexes. The three strands were assumed to be equivalent. The structures generated included (a) coiled coils with either screw or rotational symmetry and (b) parallel-chain complexes with either screw or rotational symmetry. The dihedral angles for rotation about the single bonds of the three residues in the GPH unit were treated as the independent variables which repeated in each tripeptide unit. The interchain distance also was an independent variable in structures with rotational symmetry and in parallel-chain structures, with interchain orientation being an additional variable in the latter. Some coiled-coil complexes with screw symmetry were found to have much lower energies than the other structures. Many of the low-energy structures, including the most stable triple-helical coiled-coil complex with screw symmetry, were identical with the analogous structures in $(GPP)_n$ computed in earlier work [M. H. Miller and H. A. Scheraga, *J. Polym. Sci., Polym. Symp.*, **54**, 171 (1976)]. The most stable triple-stranded structure of the two molecules is identical with that which had been proposed for collagen. The hydroxyl group of hydroxyproline does not form any hydrogen bonds in this structure, and its nonbonded and electrostatic interactions with the rest of the molecule are very weak. They do not contribute to the stability of the collagen-like triple helix. Therefore, the observed increased stability of this type of triple helix with the sequence $(GPH)_n$, compared to that with the sequence $(GPP)_n$, must be attributed to interactions with the solvent. There is no difference in the interactions involving the hydroxyl group when hydroxyproline is placed in either position X or Y in the chain. Therefore, the preference of hydroxyproline for position Y in the GXY repeating units in collagen is not due to energetic factors within the triple-stranded structure.

I. Introduction

This paper is part of a series of studies on the conformational properties of collagen-like regular-sequence poly(tripeptide)s.[2-4] In the first paper of the series,[2] dealing with poly(glycyl-prolyl-prolyl), $(GPP)_n$, the complete conformational space was examined for both the single-chain polymer and the triple-stranded complex. Coiled coils with screw symmetry and parallel-chain complexes with either screw symmetry or rotational symmetry were considered. A coiled-coil triple-stranded complex has the lowest potential energy. The dihedral angles of its repeating tripeptide unit are (ϕ_{Gly}, ψ_{Gly}, ω_{Gly}, ϕ_{Pro}, ψ_{Pro}, ω_{Pro}, ϕ_{Pro}, ψ_{Pro}, ω_{Pro}) = (-74°, 170°, 180°, -75°, 168°, 180°, -75°, 153°, 180°). This conformation was used as the reference state in this paper. It is the only minimum-energy structure found which is similar to the models which were proposed for collagen on the basis of X-ray diffraction measurements of fibers.[5-8] The helical parameters (to be defined below) of this structure agree within 22% or better with those of proposed models of collagen,[5-7] with those obtained from X-ray crystallographic measurements of a $(Pro\text{-}Pro\text{-}Gly)_{10}$ crystal,[9] and with those obtained in a recent X-ray diffraction study of stretched tendon collagen.[8]

In the present study, a similar analysis is carried out for poly(glycyl-prolyl-hydroxyprolyl), $(GPH)_n$. The repeating unit of this copolymer is an important structural element of collagen. In the known sequence of the triple-helical part of the α1(I) chain of mammalian skin collagen,[10,11] consisting of 1014 residues (338 tripeptides), the Gly-Pro-Hyp sequence occurs 39 times and is the most frequently occurring Gly-X-Y tripeptide sequence. Considering the two imino acid residues individually, Pro is the residue found most frequently in position X, and Hyp is the most frequent in position Y. Also, the γ-hydroxyprolyl residue is found only in position Y. The enzymatic hydroxylation of prolyl residues in position Y takes place after the synthesis of the polypeptide chain.[12]

Poly(Gly-Pro-Hyp) is among the poly(tripepetide)s which have been studied experimentally as models of collagen.[13-15] Its X-ray fiber diffraction pattern is very similar to that of collagen.[15] Other physical properties, such as optical rotatory parameters, are also very close to those seen for collagen.[16] The triple helix formed by $(GPH)_n$, however, is more stable than that formed by $(GPP)_n$; i.e., the melting transition, corresponding to a triple-helix to random-coil transition, occurs at a higher temperature. The transition temperature is T_m = 297 K for $(Pro\text{-}Pro\text{-}Gly)_{10}$, but T_m = 331 K for $(Pro\text{-}Hyp\text{-}Gly)_{10}$ in aqueous acetic acid.[17-19] An increase of 18 K of T_m is observed in a 1,2-propanediol–acetic acid mixture.[19-21]

A similar effect is seen in natural collagen. The denaturation temperature of chick tendon procollagen before hydroxylation is 297 K. It increases to 311 K upon complete hydroxylation,[22] with no change in the sharpness of the transition curve.[23] Similarly, there is an 8 K difference in T_m of the 36-residue proteolytic fragments α1CB2 obtained from rat skin collagen and rat-tail tendon collagen.[24] The degree of hydroxylation and T_m are higher in the

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