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A Triple-Helical Model for (Gly-Pro-Hyp)$_n$ with *cis* Peptide Units*

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Synopsis

Synthetic regular polytripeptides of the type (Gly-R_2-R_3) where R_2, R_3, or both, are imino acids have been widely studied as model compounds for collagen. One such polytripeptide is poly(Gly-Pro-Hyp), since triplets with this sequence constitute about 10% of collagen. Recently, a new model has been proposed for this polytripeptide in which one of the three peptide bonds in the tripeptide unit is in the *cis* conformation, and the γ-hydroxyl group of hydroxyproline forms a direct interchain hydrogen bond within the triple helix. We have confirmed this structure by model building using computer techniques, and the helical parameters obtained by us are close to the experimentally observed values. The model is also found to be comparable in stability with other models from energy considerations.

INTRODUCTION

Synthetic regular polytripeptides of the type (Gly-R_2-R_3)$_n$, where R_2, R_3, or both, are imino acids, have been widely studied as model compounds for obtaining information about the molecular structure of collagen (see review by Traub and Piez[1]). Their structure is now accepted as corresponding closely to the coiled-coil triple-helical model.[2–4] The most stable collagenlike conformation is obtained for polytripeptides of the type (glycine-imino acid-imino acid)$_n$[1] and, even among these, the polypeptide with the imino acid hydroxyproline in the third position of the triplet has been shown to be more stable than the one having proline in that position.[5] Recent experimental evidence has also conclusively demonstrated that hydroxylated proline in the third position in the repeating triplets (Gly-R_2-R_3) lends additional stability to the triple-helical structure of collagen.[6–8] Hence the hydroxyproline residues, in addition to stabilizing the triple-helical structure due to the stereochemical properties of the pyrrolidine ring (restricting the range of the appropriate dihedral angle ϕ), also lend added stability by virtue of their γ-hydroxyl group.

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We recently put forward a hypothesis on the role of hydroxyproline in stabilizing the collagen structure by forming additional hydrogen bonds through their γ-hydroxyl groups.[9,10] However, in this model, the stabilizing intramolecular hydrogen bond cannot be formed if the hydroxyproline has the sequence (Gly-Pro-R_2) adjacent to it in the neighboring chain. Hence the model cannot account for the experimental observation that the melting temperature of the polytripeptide (Pro-Hyp-Gly)$_{10}$ is considerably higher than that of (Pro-Pro-Gly)$_{10}$.[5] Taking into consideration these data and the fact that proton magnetic resonance studies on (Pro-Hyp-Gly)$_{10}$ indicate the presence of *cis* peptide units (quoted by Berg et al.[11]), a new model involving a *cis* peptide unit has been proposed for poly(Gly-Pro-Hyp)$_n$ by Berg et al.[11]

It has been learned from a referee of this paper that the above evidence for the presence of *cis* peptide units is not fully confirmed, and that it has to be taken to be tentative. However, we have also been studying poly(Gly-Pro-Hyp) as a model for collagen, and, in that connection, have considered the possibility of the occurrence of *cis* peptide units. Among the various possibilities that were tried, the one satisfactory structure that was obtained has the same stereochemistry and topology as the one put forward by Berg et al.[11] From purely structural considerations, we have computed a stereochemically satisfactory triple-helical model for poly(Gly-Pro-Hyp), in which the Gly-Pro peptide bond is in the *cis* conformation, and the γ-hydroxyl group of hydroxyproline forms a direct hydrogen bond with the carbonyl oxygen in a neighboring chain of the triple helix. Since our study confirms that the stabilizing energy of poly(Gly-Pro-Hyp) with the *cis* peptide unit has the same order of magnitude as the collagenlike structures (Table IV), we have considered it worthwhile to present this information in this paper. It should be mentioned that no claim is made that any part of the collagen structure itself has the structure worked out here for poly(Gly-Pro-Hyp). The attempt is essentially to work out, and verify from stereochemical considerations, that the structure with the *cis* peptide units is tenable and is a reasonably satisfactory structure for one of the modifications of this polypeptide.

METHOD

The coiled-coil triple-helical structure has a tripeptide as the basic repeating unit and hence the polypeptide chain conformation can be completely described by a set of three pairs of dihedral angles (ϕ_i, ψ_i), i = 1, 2, 3, if we consider only planar peptide units and fixed values for the bond angles at the C^α atoms.[12] To obtain the range of allowed values for these dihedral angles at each of the C^α atoms, we examined the dipeptide maps, which show that nearly the same range of values is allowed for the dihedral angles (ϕ, ψ) when one of the peptide units is in the *cis* conformation as when both the peptide units are in the *trans*

conformation. Since scanning through the whole range of allowed values for all six parameters involves an immense amount of computation, the approximate ranges of these parameters corresponding to a suitable triple-helical structure were first obtained using skeletal models. The criteria used for selecting the ranges of these parameters were (1) the values of the helical parameters should be close to the experimentally observed values and (2) each pair of dihedral angles (ϕ, ψ) should be sterically allowed. The stereochemical restrictions imposed by the geometry of the pyrrolidine rings at two of the three C^α atoms were also taken into consideration. Models showed that a suitable triple-helical structure can be obtained only when the Gly-Pro peptide bond is in the *cis* conformation. This is in agreement with the recent observation, made from ^{13}C nmr studies on the pentapeptide (Gly-Gly-Pro-Gly-Gly), that *trans-cis* isomerism occurs only with respect to the Gly-Pro peptide bond.[13] The approximate range for the dihedral angles ϕ and ψ at the glycine C^α atom is between 50° to 70°, for ϕ and −150° to −130° for ψ while the values of ϕ and ψ at the other two C^α atoms are restricted to the range between −80° to −60° for ϕ and 160° to 180° for ψ.

The whole range of possible structures was then scanned, using a computer program, by systematically varying the dihedral angles over the above ranges at intervals of 10°. The bond angles at the C^α atoms were also varied over the range 105° to 115° at intervals of 5°.

The criteria now used for selecting suitable triple-helical structures were (1) the values of the helical parameters and (2) the possible formation of interchain hydrogen bonds between the backbone atoms. In poly(Gly-Pro-Hyp), only the glycyl amino group is free to act as a donor for such hydrogen bonds. However various different schemes of hydrogen bonding are possible depending on which one of the carbonyl oxygens on the two neighboring chains acts as the acceptor. Preliminary calculations indicated that, in all the structures with suitable helical parameters, the best hydrogen bond is formed between the glycyl amino group of the A chain and the carbonyl oxygen of the second residue in the B chain. This scheme of hydrogen-bond formation is identical to that in the other one-bonded models of collagen, which have all the peptide units in the *trans* conformation.[1,3,4]

Both these criteria were deliberately made very broad-based at this stage, so as not to miss any good structures that may have dihedral angle values lying in between the sets of values scanned. Hence, we selected all those structures for which 1) the helical parameters lay within the range 2.65–2.95 Å for unit height h and −100°–−120° for unit twist t; and 2) an interchain hydrogen bond of the type N_4H_4(chain A)··· O_2(chain B) was formed. The hydrogen-bond parameters were allowed to vary over the range 2.4 Å–3.4 Å for the hydrogen-bond length l and a maximum value of 60° for the hydrogen-bond angle θ (NHΛN··· O).

However, in spite of the relaxed criteria used, out of the 3^9 conforma-

TABLE I
Polypeptide Chain Conformations Having Suitable Helical Parameters h,t and an Interchain Hydrogen Bond l,θ

No.	τ_1	ϕ_1	ψ_1	τ_2	ϕ_2	ψ_2	τ_3	ϕ_3	ψ_3	ρ_1[a](Å)	h(Å)	t(deg)	l(Å)	θ(deg)
1	105	250	30	105	110	340	115	100	360	1.99	2.67	−107.4	3.26	41.3
2	105	250	30	105	110	350	115	100	360	2.06	2.67	−104.9	2.91	56.3
3	110	240	30	105	110	340	105	100	360	2.53	2.66	−111.7	3.33	59.3
4	110	240	40	105	110	340	110	100	350	2.02	2.67	−114.4	2.57	47.2
5	110	250	30	105	110	350	115	100	360	1.83	2.68	−105.4	2.78	38.2
6	110	250	30	110	110	340	110	100	350	1.73	2.70	−110.2	2.81	52.5
7	110	240	30	110	110	340	110	110	360	1.93	2.66	−107.8	2.51	58.4
8	110	240	40	110	110	340	115	110	350	1.82	2.66	−110.5	2.53	32.5
9[b]	110	240	30	115	110	340	110	100	350	2.28	2.73	−112.3	3.25	28.2
10	110	230	30	115	110	340	110	100	360	1.82	2.76	−112.5	3.16	56.6
11	110	240	30	115	120	350	115	100	360	1.65	2.66	−104.1	2.87	47.8
12	110	240	30	115	120	360	115	100	360	1.43	2.66	−101.6	2.43	58.7
13	115	250	30	105	110	360	115	100	360	1.75	2.67	−103.3	2.73	46.2
14	115	240	30	110	110	340	110	110	360	1.96	2.66	−108.2	3.10	40.2
15	115	250	30	110	110	350	110	100	350	2.00	2.68	−108.1	2.87	31.3
16	115	240	30	110	110	350	110	100	360	1.66	2.72	−108.3	2.41	41.3
17	115	250	30	110	100	340	115	110	350	2.03	2.75	−109.7	2.95	28.9
18	115	240	30	110	100	340	115	110	360	1.61	2.79	−109.9	2.60	44.1
19	115	250	30	110	100	340	115	120	350	2.33	2.68	−106.8	3.20	44.5
20	115	240	30	110	100	340	115	120	360	2.04	2.72	−107.1	2.67	57.2
21	115	230	40	110	110	350	115	110	360	1.67	2.69	−108.3	2.48	52.0
22	115	240	40	115	110	340	110	110	340	2.17	2.65	−112.9	3.02	24.9
23	115	240	30	115	120	360	115	100	360	1.60	2.65	−101.9	2.69	45.3

[a] Radial distance of atom C_1^α from the helix axis.

[b] This is the only structure allowed by stereochemical contacts being satisfactory.

tions examined, only 23 combinations passed the above two tests. They are listed in Table I along with the relevant parameters.

These structures were then checked to see whether the triple-helical structure was allowed according to the "extreme limit" criteria, both from the point of view of intrachain, as well as interchain, contacts. Taking into consideration only the backbone atoms, interchain short contact indicated that all except one of the conformations were sterically impossible. Even this one conformation (footnote b in Table I) has a $C_3^\beta \cdots O_2$ short contact. However, we used this conformation as a starting point for obtaining a stereochemically allowed structure, by using a slightly modified version of the method introduced by Ramachandran et al.,[14] wherein, having fixed the dimensions of the peptide units and the helical parameters, it is possible to describe the conformation of the chain uniquely in terms of its eight parameters h_1, h_2, h_3, μ, ν, δ_1, δ_2, and δ_3. We have only replaced the parameters μ and ν by ρ_1 and ρ_2, the radial distances of atoms C_1^α and C_2^α from the helix axis. The parameters h_1, h_2, and h_3 are the vertical components of the virtual bonds C_1^α C_2^α, C_2^α C_3^α, and C_3^α C_4^α, respectively, and δ_1, δ_2, and δ_3 define the tilts of the three peptide units about these bonds. Each δ was taken to be zero when the plane of the peptide group was parallel to the helix axis and C=O pointed downwards; δ increases with clockwise rotation of the peptide group, when viewed from C_i^α to C_{i+1}^α. At this stage, we introduced the dimensions of the standard *cis* unit[12] in place of the *cis* unit considered so far, which had the Pauling-Corey dimensions.

We then examined a series of structures with unit height varying between 2.73 and 2.82 Å, and unit twist between −110° and −115°. It was found that, while stereochemically allowed structures could be obtained with the value of the unit height lying anywhere between 2.79 and 2.82 Å, the actually observed range for this parameter,[15,16] the value of unit twist was restricted to the range −112° to −114°, due to the combined restrictions imposed by the "contact criteria" and the necessity for the formation of a good interchain hydrogen bond.

From the stereochemically allowed structures thus obtained, we have selected the best one that simultaneously satisfies the following conditions:

1) the helical parameters are close to the experimentally observed ones;

2) a direct interchain hydrogen bond is formed;

3) the bond angles at C^α atoms have the most reasonable values, i.e., a value less than 115° at the $C_1{}^\alpha$ atom and values of less than 113° at the other two imino acid C^α atoms;

4) the values of the dihedral angles ϕ_2 and ϕ_3 are within the range −60°–−80°, allowed by the stereochemical requirements of the pyrrolidine ring geometry.

The pyrrolidine ring atoms were then attached to the α carbon atoms $C_2{}^\alpha$ and $C_3{}^\alpha$. From the orientation of the hydroxyproline ring, it was evident that the only possible interchain hydrogen bond involving its

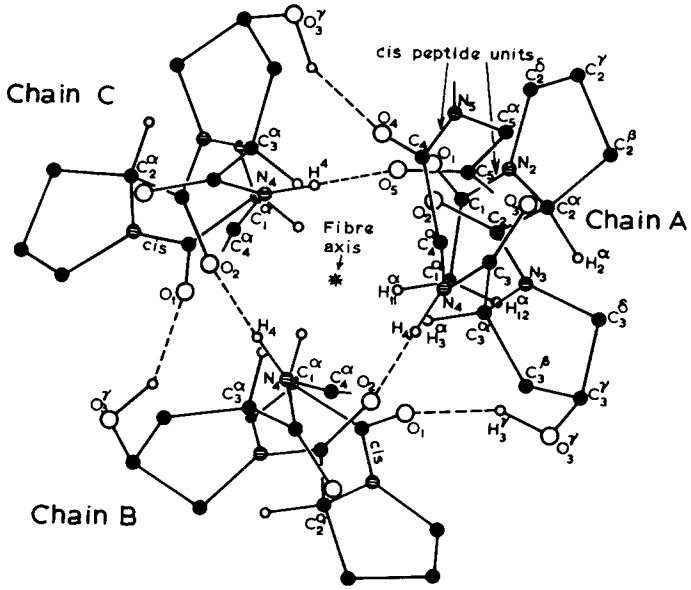

Fig. 1. A projection down the helical axis, showing the atomic arrangement in the triple-helical structure of (Gly-Pro-Hyp)$_n$ with *cis* peptide units in the structure. The hydrogen bonds are shown by dotted lines.

γ-hydroxyl groups must have the hydroxyproline of the A chain as the donor, and the carbonyl oxygen of the glycine residue of the B chain as the acceptor. The proline and the hydroxyproline rings were then puckered, within the theoretically allowed ranges,[17] so as to relieve short contacts involving the ring atoms, and the γ-hydroxyl group of hydroxyproline was suitably oriented so as to form a direct hydrogen bond with the oxygen of the carbonyl group of the glycine residue in the neighboring chain.

THE MODEL

We report here a model for poly(Gly-Pro-Hyp), which satisfies all the above conditions. The unit height is 2.80 Å and the unit twist is −113.0°. The structure is stabilized by two direct interchain hydrogen bonds per tripeptide unit. The projection of the structure down the helical axis is shown in Figure 1, and the bond lengths and bond angles in one tripeptide unit of (Gly-Pro-Hyp) are shown in Figure 2. The cylindrical polar coordinates of the atoms in one tripeptide unit are given in Table II, and the dihedral angles and hydrogen-bond parameters are given in Table III along with the values reported by Berg et al.[11] for the dihedral angles.

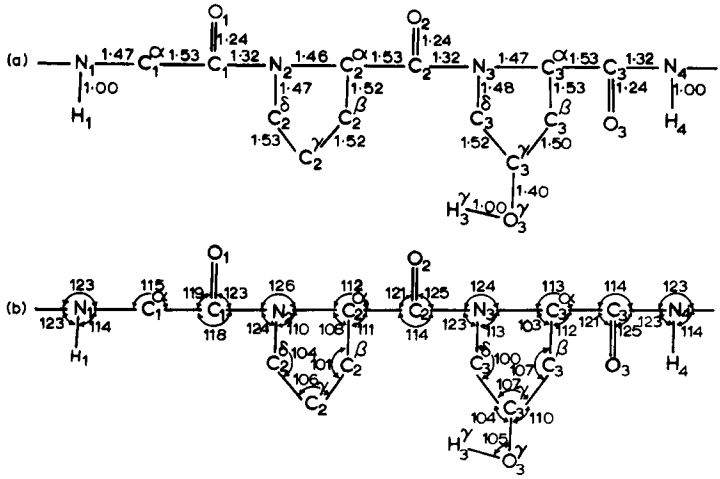

Fig. 2. A schematic diagram of one tripeptide unit of $(Gly\text{-}Pro\text{-}Hyp)_n$. (a) Bond lengths. (b) Bond angles.

DISCUSSION

The triple-helical structure reported here, with the Gly-Pro peptide unit having a *cis* conformation, seems to be a reasonably satisfactory structure for the compound poly(Gly-Pro-Hyp). It is stereochemically satisfactory and the helical parameters are close to the observed ones.

TABLE II
Coordinates of the Atoms in a Set of Three Residues of the Proposed Model for (Gly-Pro-Hyp) with a *cis* Peptide Unit

Atom	r(Å)	ϕ(deg)	Z(Å)
N_1	1.98	−21.3	−1.27
H_1	1.67	−51.5	−1.32
C_1^α	2.00	0.0	0.0
H_{11}^α	2.89	−8.6	0.53
H_{12}^α	1.10	−8.3	0.60
C_1	2.69	34.1	−0.13
O_1	2.69	52.5	−1.02
N_2	3.70	34.2	0.73
C_2^δ	4.89	45.9	0.72
C_2^α	4.05	20.3	1.80
C_2^β	5.41	26.0	2.30
C_2^γ	5.61	41.4	2.00
H_2^α	4.33	6.8	1.38
C_2	3.04	18.5	2.94
O_2	2.27	39.7	3.07
N_3	3.42	1.0	3.74
C_3^δ	4.75	−7.0	3.49
C_3^α	2.76	−10.5	4.90
C_3^β	3.89	−27.6	5.15
C_3^γ	4.86	−24.0	4.05
O_3^γ	4.72	−34.4	2.97
H_3^γ	3.47	−37.1	2.86
H_3^α	1.78	−21.8	4.62
C_3	2.77	8.5	6.13
O_3	3.72	22.8	6.14

The triple helix is stabilized by two hydrogen bonds per tripeptide unit—one of which is through the γ-hydroxyl group of hydroxyproline. It can hence account for the greater thermal stability of (Pro-Hyp-Gly)$_{10}$ as compared to that of (Pro-Pro-Gly)$_{10}$[5] and also the observation that the entropy and enthalpy changes occurring during the transition from triple helix to single chains indicate the presence of two hydrogen bonds per tripeptide for this compound.[11] It is, however, not the suggestion of the authors that (Pro-Pro-Gly)$_{10}$ has a *cis* peptide unit in its structure. This polymer may conceivably have an all-*trans* structure, in which case its energy would correspond to the value given in Table IV for the first three structures. If the *cis* peptide unit occurs in (Pro-Hyp-Gly)$_{10}$, then its energy per tripeptide, as given in Table IV, is about 2 kcal/mol lower than that of the other types of structures. It should be emphasized that the calculations presented in this paper have no relevance to the collagen structure *per se*, and that the considerations presented here deal essentially with the structure of (Gly-Pro-Hyp)$_n$.

The helical parameters for our model are close to those reported for poly(Gly-Pro-Hyp) from X-ray diffraction studies.[15,16] The value of

TABLE III
Dihedral Angles and Other Parameters Related to the Triple-Helical Structure of poly(Gly-Pro-Hyp)$_n$ with a *cis* Peptide Unit

Unit height = 2.80 Å; unit twist = −113.0°						
	Our Calculations				Model of Berg et al.[11]	
Atom	τ(deg)	ϕ(deg)	ψ(deg)	ω(deg)[a]	ϕ(deg)	ψ(deg)
C_1^α	115	51	−143	0	40	−120
C_2^α	112	−71	152	180	−80	180
C_3^α	113	−80	173	180	−80	180

Hydrogen-Bond Parameters		
Nature of Hydrogen Bond	l(Å)	θ(deg)
N_4H_4(chain A) · · · O_2(chain B)	2.83	23.8
$O_3\gamma H_3\gamma$(chain A) · · · O_1(chain B)	2.86	23.9

[a] Same in our calculations and in the model of Berg et al.[11]

TABLE IV
Energy (in kcal/mol) of Various One-Bonded Triple-Helical Models With the Sequence (Glycine — Imino Acid — Imino Acid)$_n$

Model	V_{nb} (van der Waals energy)	V_{hb} (hydrogen-bond energy)	V_{others} (electrostatic, torsional etc.)	V_{total} (per tripeptide unit)
Rich and Crick[3]	−27.8	−4.2	7.4	−24.6
Ramachandran[4]	−27.4	−2.2	7.3	−22.3
Yonath and Traub[c]	−26.7	−4.4	7.9	−23.2
Present study	−27.9	−2.8[a] +(−2.5)[b]	7.7	−25.5

[a] Hydrogen-bond energy for the N-H · · · O bond.
[b] Hydrogen-bond energy for the O-H · · · O bond.
[c] As given by Traub and Piez[1].

unit height 2.80 Å lies within the observed range of values, which is between 2.79 and 2.82 Å for poly(Gly-Pro-Hyp), while the number of units per turn n for our model is 3.20, a value only slightly less than 3.27, the reported lower limit for this parameter from experiment.[15] However, it is well known that the accuracy of measurement of helical parameters, from the poor fiber diffraction photographs exhibited by this material, is not very good.

A comparison of the Fourier transform of this model structure with the Fourier transforms of other triple-helical models shows a fairly good similarity. However, the layer lines have to be assigned indices different from those for collagen and other polymers. Details will be reported elsewhere.

Energy calculations also show that, if the energy difference due to the substitution of a *cis* unit in place of a *trans* peptide unit is ignored, then

the potential energy for the model with a *cis* unit is of the same order as that of the other triple-helical models, as can be seen from Table IV. The value of the nonbonded energy was calculated using the six-exponent potential function,[12] and the hydrogen-bond energy was calculated using the appropriate energy functions for the N-H ··· O and O-H ···O hydrogen bonds,[18,19] and are listed separately in Table IV. The electrostatic, torsional, and distortional energy contributions[12] are put together as V_{others}. Hence, even if the contribution due to the additional hydrogen bond is ignored, the van der Waals energy by itself is comparable to that for the other model structures. Hence these calculations also lend good support to the proposed triple-helical model, involving a *cis* unit, for the polytripeptide(Gly-Pro-Hyp)$_n$. However the possibility of this structure occurring in local regions of the collagen triple helix seems rather remote since 1) it requires that the sequence (Gly-Pro-R$_3$) be present in *all the three* chains, in the same region along their length, for a regular set of direct interchain hydrogen bonds to be formed (collagen is generally known to have the same sequence in only two of the chains of the triple helix[1]); and 2) the helical parameters differ appreciably from the accepted value for collagen. Hence the polypeptide chains will have to undergo distortion in order to accommodate small regions having the *cis* unit structure.

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