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Calculation of the Structures of Collagen Models. Role of Interchain Interactions in Determining the Triple-Helical Coiled-Coil Conformation. 3. Poly(glycylprolylalanyl)[1a]

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ABSTRACT: The conformational space of regularly repeating structures of poly(glycylprolylalanyl), (GPA)$_n$, 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 GPA unit were treated as the independent variables which repeated in each tripeptide unit. In parallel-chain structures, the interchain distance and orientation also were independent variables. Some coiled-coil complexes with screw symmetry were found to have much lower energies than the other structures. Some of the lowest 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. The most stable triple-stranded structure is very close to that which had been proposed for collagen. It is stabilized by interchain interactions because its conformation differs from the most stable single-stranded (GPA)$_n$ conformation. Alanine can easily be substituted in place of proline in position Y of the GXY repeating unit in a collagen-like structure. More single-stranded and triple-stranded conformations, however, were found for (GPA)$_n$ than for (GPP)$_n$. The energies of complexes with different types of symmetry were closer to each other than in (GPP)$_n$ because of the greater flexibility of the polypeptide chain containing alanine in place of proline.

I. Introduction

The two previous papers of this series[2,3] reported the computed conformational properties of the regular-sequence poly(tripeptide)s poly(glycylprolylprolyl) and poly(glycylprolylhydroxyprolyl), abbreviated (GPP)$_n$ and (GPH)$_n$, respectively. In this paper and the subsequent one,[4] the effect of substituting alanine in place of one of the prolyl residues of (GPP)$_n$ is investigated. This paper describes studies on poly(glycylprolylalanyl), abbreviated as (GPA)$_n$, in which the L-alanyl residue is substituted in position Y of the repeating unit GXY. The next paper[4] deals with the substitution of alanine in position X, i.e., with the poly(tripeptide) (GAP)$_n$. The substitution of other amino acids in place of proline in triple-helical conformations will be discussed elsewhere.[5]

All of the poly(tripeptide)s discussed here have been studied experimentally as models of collagen[6,7] because some of them form a collagen-like triple-helical structure under certain conditions and also exhibit the collagen-like triple helix-to-random coil transition. After proline and hydroxyproline, alanine is the most frequently occurring amino acid in positions X and Y of collagen.[8,9] The Gly-Pro-Ala sequence occurs 31 times, the Gly-Ala-Pro sequence once, and the Gly-Ala-Hyp sequence 20 times in the $\alpha 1(\mathrm{I})$ chain of mammalian skin collagen,[8,9] as compared with 39 occurrences of Gly-Pro-Hyp. In addition, (GPA)$_n$ and (GAP)$_n$ can be considered as prototypes for many structural properties of Gly-Pro-Y and Gly-X-Hyp sequences in which X or Y can be any amino acid. In the solid state, (GPA)$_n$ can exist in a form closely resembling collagen,[10] although other forms of the fiber exist as well.[11] In aqueous solution, no triple-helical structure of (GPA)$_n$ was found for low-molecular-weight polymers ($n \leq 60$) at room temperature,[12,13] although circular dichroic spectra indicated that such a structure exists at low temperatures (161 K) in an ethylene glycol–water (2:1) mixture[13] for $n = 60$ and at room temperature in water for a higher polymer[14] ($n > 100$). The latter molecule exhibited a sharp thermal transition[15] near 315 K. In a similar manner, collagen-like triple-helical structures and solvent-dependent transitions have been observed for poly(Gly-Pro-Ser),[16] poly(Gly-Pro-Leu),[17] and poly(Gly-Pro-Phe).[18] The presence of a collagen-like triple helix in these four poly(tripeptide)s with the sequence (GPY)$_n$ is in marked contrast with the behavior of the corresponding polymers with sequence (GXP)$_n$, to be discussed in the next paper.[4] The decreased thermal stability of triple-helical (GPA)$_n$ and (GAP)$_n$, as compared with (GPP)$_n$, might be explained on entropic grounds, in terms of the increased flexibility of the randomly coiled polypeptide chain containing alanine in place of proline. It is paralleled by the observed decrease in the thermal stability of various collagens with decreasing imino acid content.[19–21]

The study reported in this paper was carried out in the same manner described in the two earlier papers of this series.[2,3] Conformational space was explored both for the single-stranded polymer and for triple-stranded coiled-coil and parallel-chain complexes. Minimum-energy conformations were computed and were compared with those obtained earlier[2] for (GPP)$_n$.

II. Computational Procedure

The assumptions and the computational procedures were those reported in the first two papers of the series.[2,3] The same selection of geometrical and energy parameters was made.[2,3,22,23] The procedures described earlier were used for the generation of chain conformations and for function minimization. Six variable dihedral angles were considered, instead of the four used in the work on (GPP)$_n$, because ϕ and χ^1 of alanine also are variables. Trans and cis conformations (with ω fixed at 180 or 0°) were considered only for the peptide bond preceding Pro. In the other two peptide bonds, preceding Gly and Ala, ω was fixed only at 180°. The repeating unit GPA is shown in Figure 1.

Selection of Starting Conformations and Energy Minimization. Coiled-Coil Structures with Screw Symmetry. As the first step, minimum-energy conformations of single-stranded (GPA)$_4$ chains were determined as in the first paper.[2] The starting conformations for this minimization were obtained from stable conformations of small molecules. All conformational energy minima obtained earlier[24] for *N*-acetyl-*N*′-methylglycyl-L-prolinamide and *N*-acetyl-*N*′-methyl-L-prolyl-L-alaninamide were com-

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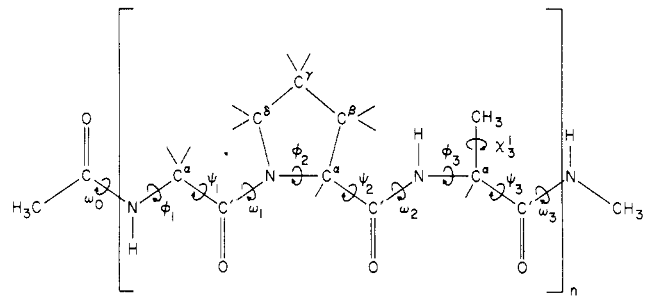

Figure 1. Structure of $(GPA)_n$, indicating the dihedral angles. The end groups used in the computation are shown.

bined and used as starting points for the minimization of the energy of single-stranded terminally blocked $(GPA)_4$. The *N*-acetyl- and *N'*-methylamide terminal blocking groups were used for this polypeptide. The minima of the terminally blocked L-Ala-Gly dipeptide did not have to be considered separately because they are all included in the set selected above, except for those which are incompatible with a Gly-Pro sequence. This computation gave 147 minimum-energy conformations for single-stranded $(GPA)_4$, with an energy spread of 37.8 kcal/mol of $(GPA)_4$. In the first paper,[2] only 82 minima were found for single-stranded $(GPP)_4$. The increase in number is due to the greater flexibility of $(GPA)_4$, caused by the presence of the additional variable backbone dihedral angle ϕ_3 of Ala.

These single-strand minimum-energy conformations were packed into triple-stranded coiled-coil complexes with screw symmetry and examined by a grid search with respect to all five variable dihedral angles of the backbone in the manner described in the first paper.[2] The dihedral angles ϕ_1, ψ_1, ψ_2, ϕ_3, and ψ_3 were fixed either at the values in the single-strand minima or at values differing from the latter by ±30°. The results of the analogous procedure in the first paper[2] showed that this interval was sufficient in examining conformational space for coiled coils. All points representing minima on the grid were used as starting points for energy minimization. In the latter procedure, all six dihedral angles (including χ_3^1) were variables. In addition, all minimum-energy coiled-coil structures found earlier for $(GPP)_4$ (Tables V and VI of ref 2) also were used as starting points in this minimization.

Parallel-Chain Structures with Screw Symmetry. As in the two previous papers,[2,3] the only structures with this symmetry that were tested were those having a polypeptide conformation with $\Theta = 0°$. The gridding procedure described[3] in paper 2 was used to generate triple-stranded structures. In this case, $3^5 = 243$ conformations were generated because there were five variable backbone dihedral angles; χ_3 was kept fixed at 60°. Triple-stranded complexes were generated from these 243 conformations on a grid with interchain separation and interchain orientation as variables, using grid intervals of 1.0 Å and 30°, respectively, with 10 Å as the maximal separation. The energies of all structures generated at the grid minima were at least 33 kcal/mol of $(GPA)_4$ higher than the most stable coiled-coil structure. The energy is a slowly varying function of positions in the grid. Therefore, no energy minimization was carried out on these parallel-chain structures.

Coiled-Coil and Parallel-Chain Structures with Rotational Symmetry. No grid search with respect to interchain separation and orientation was carried out because it would have required prohibitive computer time. Instead, the 50 minimum-energy conformations found for $(GPP)_4$ (Table XI of ref 2) were used as starting points. Energy minimization was carried out with respect to the five backbone dihedral angles and the interchain separation as variables. All ω's were fixed as in Table XI of ref 2, and χ_3 was fixed at 60°.

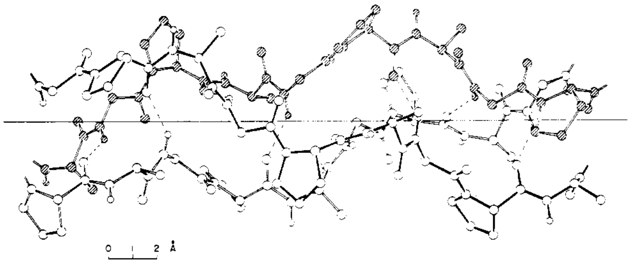

Figure 2. Triple-stranded coiled-coil structure with screw symmetry of $(GPA)_n$ of lowest potential energy. The structure is very similar to the coiled-coil structure of lowest energy for $(GPP)_n$ (see Figure 5 of ref 2). The chains form right-handed helices around the *z* axis ($\Theta > 0$), and the three strands are arranged in a counterclockwise disposition. Strand 1 is shown with dark bonds, strand 2 is shown with shaded atoms, and strand 3 is shown with open bonds and atoms. The axis of symmetry is the *z* axis, shown as a horizontal line, with coordinates increasing to the right. The *x* axis is in the plane of the figure, pointing to the top; the *y* axis points up from the plane of the figure. The $N_1H_1 \cdots O_2C_2$ hydrogen bonds linking the strands are shown with dashed lines. (See Figure 5 of ref 2 for the explanation of the notation.)

III. Results

A. Triple-Stranded Structures. Energy minimization of the coiled-coil structures with screw symmetry resulted in 53 minimum-energy structures with counterclockwise disposition of the chains and 55 structures with clockwise disposition of the chains, with energies ranging from −63.1 to 15.8 kcal/mol of $(GPA)_4$. The dihedral angles for the minima with the two kinds of chain dispositions are given in Tables I and II, respectively, with conformations listed in order of increasing energy. Tables III and IV, respectively, list the helical parameters and the inter- and intramolecular energy components for all of these conformations.

All triple-stranded packings of parallel chains with screw symmetry had energies higher than −30 kcal/mol of $(GPA)_4$. Twenty-nine minima were found for triple-stranded coiled-coil and parallel-chain structures with rotational symmetry. Their energies ranged from −48.8 to −15.7 kcal/mol of $(GPA)_4$.

The lowest energy conformation of $(GPA)_4$ has an energy of −63.1 kcal/mol of $(GPA)_4$. Its dihedral angles are (ϕ_1, ψ_1, ω_1, ψ_2, ϕ_3, ψ_3, χ_3^1) = (−72, −178, 180, 168, −63, 148, 62°) (first line of Tables I and III). Its coordinates are given in Table V. The structure is shown in Figure 2. It is similar to the lowest energy structure of $(GPP)_4$. It, too, is a deformed polyproline II-type coiled coil, with right-handed major helical sense and a counterclockwise disposition of the chains. The same hydrogen-bond arrangement occurs in the two structures, viz., an $N_1H_1 \cdots O_2C_2$ hydrogen bond connecting the glycyl NH of a given chain and the prolyl CO located in the counterclockwise neighboring chain, looking from the N to the C terminus (cf. Figure 5 of ref 2). In terms of the details of the conformation, there are some differences in the two structures. Some dihedral angles differ by as much as 12°. The helical parameters are $\Theta = 62°$ and $D = 8.4$ Å for $(GPA)_n$, as compared with $\Theta = 44°$ and $D = 8.95$ Å for $(GPP)_n$. On the basis of the coordinates of the common backbone atoms (including the C^β), the root-mean-square deviation between the two structures is 0.45 Å. The comparison of the two structures indicates that alanine can be substituted for proline in position Y in the triple-helical coiled-coil conformation without significant distortion of the structure

Table I. Dihedral angles and energies of triple-stranded coiled-coil structures of $(GPA)_4$, with screw symmetry and counterclockwise disposition of the chains

Dihedral angle (degrees)[a]							Energy [kcal/mol of $(GPA)_4$]
ϕ_1	ψ_1	ω_1	ψ_2	ϕ_3	ψ_3	χ_3^1	
-72	-178	180	168	-63	148	62	-63.1
145	-67	180	63	-160	140	61	-53.2
166	79	180	72	-160	122	58	-48.8
164	-173	180	-62	-167	-71	45	-46.4
-88	-159	180	75	-90	147	-57	-43.6
-152	62	180	175	-61	129	60	-43.2
-161	77	180	63	-58	-62	54	-42.3
-65	-63	180	154	-163	131	56	-41.4
162	-176	0	164	-82	137	-57	-41.4
162	-176	0	157	-75	163	63	-41.1
-93	-64	180	152	-161	140	57	-41.0
-154	168	0	151	-150	120	60	-39.9
123	-175	180	177	-63	-46	60	-38.9
170	179	180	92	-60	-40	-59	-38.4
74	180	180	140	-139	49	-53	-38.0
71	180	180	139	-129	52	-52	-37.9
-79	-178	180	75	-108	129	-57	-37.8
-92	-170	180	146	-158	165	60	-37.3
-74	-169	180	65	-82	55	57	-37.1
-167	173	180	102	-147	-50	57	-36.9
173	61	180	67	-165	148	56	-36.2
-86	-171	180	176	-66	112	59	-35.8
157	-177	180	161	-87	-64	64	-35.7
-162	157	0	155	-156	58	-59	-35.7
-27	-60	180	70	-155	45	59	-35.5
-95	153	0	159	-159	49	53	-35.5
-77	-176	180	153	-146	47	57	-34.7
125	-167	180	163	-77	-50	62	-34.5
-80	-119	180	124	-119	143	61	-34.1
166	177	0	177	-81	74	62	-33.4
-151	-175	180	155	-154	171	-55	-32.9
-79	174	180	157	-81	79	55	-32.6
105	-174	0	162	-120	57	57	-31.1
-99	169	0	167	-153	-50	57	-30.7
-171	74	180	171	-156	-59	-61	-30.4
-79	-169	180	123	-124	45	49	-29.9
106	179	0	154	-64	50	61	-28.5
-168	-168	180	124	-75	170	65	-28.3
-109	-172	180	135	-74	112	58	-26.9
77	178	0	136	-161	146	58	-26.8
70	107	180	155	-96	74	61	-25.2
66	107	180	147	-90	77	61	-24.7
144	-161	0	-180	-79	79	59	-24.3
-71	-169	0	169	-74	-30	62	-21.4
96	90	180	165	-71	-42	61	-20.8
-124	176	0	157	-83	-46	-58	-18.4
66	88	180	-179	-64	-49	60	-17.4
155	164	180	168	-75	-62	57	-16.9
157	158	180	171	-73	-52	59	-15.5
-90	175	0	145	-77	-53	-60	-10.3
-134	-63	180	162	-89	164	65	-6.4
-151	-61	0	176	-76	-59	54	-5.6
121	176	0	174	-76	111	62	14.5

[a] Only ϕ_1, ψ_1, ψ_2, ϕ_3, ψ_3, and χ_3^1 were varied in the energy minimization; ω_1 was kept fixed at the values indicated; $\omega_2 = \omega_3 = 180°$ for all conformations.

Table II. Dihedral angles and energies of triple-stranded coiled-coil structures of $(GPA)_4$, with screw symmetry and clockwise disposition of the chains

Dihedral angle (degrees)[a]							Energy [kcal/mol of $(GPA)_4$]
ϕ_1	ψ_1	ω_1	ψ_2	ϕ_3	ψ_3	χ_3^1	
-83	-164	180	78	-97	152	-53	-57.0
-87	-170	180	78	-95	157	-62	-56.8
-176	75	180	60	-55	-56	54	-48.3
-167	173	180	-178	-154	147	62	-46.4
135	179	180	-52	-159	-66	49	-42.7
-83	-88	180	156	-156	151	65	-41.9
-94	177	180	-53	-159	153	59	-40.1
-105	-176	180	-53	-160	161	59	-39.9
-82	178	180	160	-69	71	60	-37.5
-87	-154	180	83	-77	84	61	-37.0
-165	-178	180	129	-70	156	61	-36.6
-91	-179	180	147	-63	80	57	-36.4
-173	71	180	179	-157	-60	54	-36.1
174	180	0	164	-162	151	51	-35.5
70	-168	0	161	-121	124	57	-34.7
-176	-173	0	137	-154	60	55	-34.7
-83	-152	180	168	-63	139	62	-34.5
-149	-166	0	128	-90	84	66	-33.9
123	-165	180	151	-75	-50	-55	-33.6
57	-160	180	83	-59	-65	51	-33.4
109	179	0	151	-86	73	62	-32.6
-163	78	180	-47	-152	135	59	-32.6
163	166	180	164	-78	-52	60	-32.1
-148	170	0	139	-143	92	55	-32.0
174	174	0	166	-76	90	62	-31.9
-129	-178	0	145	-118	77	-57	-31.6
74	177	180	154	-143	40	-55	-31.4
-79	-169	180	160	-75	156	64	-31.1
-139	80	180	-34	-64	91	58	-30.6
-58	-72	180	142	-160	89	58	-30.5
177	90	180	-56	-73	170	-173	-29.5
-132	62	180	175	-75	161	63	-29.1
57	84	180	159	-120	37	63	-28.9
-67	167	0	147	-162	-54	54	-28.5
158	179	0	173	-82	77	61	-28.3
166	84	180	159	-69	-61	57	-28.1
176	172	180	155	-134	-59	56	-27.2
-92	178	180	160	-155	114	56	-27.1
164	103	180	167	-64	160	62	-27.0
-78	-136	180	101	-75	-180	70	-26.5
85	92	180	100	-151	175	-56	-26.2
-157	-75	180	166	-148	171	59	-26.1
87	-178	0	132	-161	160	59	-25.7
-152	178	180	131	-99	147	-58	-25.3
-122	157	0	134	-87	-45	-59	-24.5
122	171	180	164	-76	-43	61	-22.3
-69	154	180	168	-84	165	63	-22.3
132	-96	0	180	-77	85	59	-21.5
137	176	0	152	-76	134	61	-21.5
137	-102	0	161	-71	92	60	-19.3
-118	89	180	160	-69	158	69	-18.9
72	109	180	154	-88	60	53	-18.5
118	-101	0	175	-76	89	60	-11.7
-156	-64	0	170	-69	-38	60	-10.3
115	-101	0	170	-81	75	62	-9.6

[a] Only ϕ_1, ψ_1, ψ_2, ϕ_3, ψ_3, and χ_3^1 were varied in the energy minimization; ω_1 was kept fixed at the values indicated; $\omega_2 = \omega_3 = 180°$ for all conformations.

Table III. Helical Parameters, Interchain Energy, and Intrachain Energy of Triple-Stranded Coiled-Coil Structures of $(GPA)_4$, with Screw Symmetry and Counterclockwise Disposition of the Chains[a]

Helical Parameters			Energy[b] [kcal/mol of $(GPA)_4$]				
			Interchain		Intrachain		Total
D (Å)	θ[c] (degrees)	ρ_1[d] (Å)	E_{ES}	E_{NB}	E_{ES}	E_{NB}	E_{tot}
8.4	61.7	1.6	-7.3	-39.0	-0.7	-16.1	-63.1
6.6	-67.0	3.7	-5.1	-31.1	-5.6	-11.4	-53.2
8.1	89.9	2.8	-2.7	-32.3	-3.8	-10.1	-48.8
7.2	76.9	2.7	-2.9	-32.9	-0.9	-9.8	-46.4
3.2	-76.4	6.1	-3.3	-23.8	-4.5	-12.2	-43.6
7.6	-93.3	2.8	2.4	-31.6	-2.4	-11.5	-43.2
3.9	-73.6	6.3	0.1	-26.3	-3.1	-13.1	-42.3
4.4	69.2	5.6	-2.4	-30.0	-0.3	-8.6	-41.4
7.1	55.3	2.8	-1.3	-24.7	-1.2	-14.2	-41.4
7.1	53.2	2.8	-1.6	-24.6	-1.0	-13.6	-41.1
4.4	89.7	5.6	-2.5	-29.7	-1.3	-7.5	-41.0
4.7	66.7	4.8	1.0	-27.5	-1.0	-12.4	-39.9
7.5	59.5	3.8	-6.0	-21.1	1.3	-13.1	-38.9
4.8	72.2	5.7	1.6	-20.2	-4.2	-15.7	-38.4
4.6	74.1	6.5	-1.0	-22.2	0.1	-14.9	-38.0
4.5	73.3	6.5	-1.6	-22.9	-0.4	-12.6	-37.9
5.9	-87.2	4.3	-1.6	-17.8	-5.3	-13.1	-37.8
3.3	-72.9	7.5	-1.5	-18.6	-3.0	-14.3	-37.3
6.6	-73.1	4.1	-0.7	-15.3	-6.4	-14.6	-37.1
4.5	81.3	6.1	1.8	-23.5	-2.4	-12.8	-36.9
7.3	89.5	3.0	1.0	-23.8	-5.1	-8.3	-36.2
4.3	69.7	5.7	-2.3	-16.9	-2.3	-14.2	-35.8
8.9	44.3	3.4	-0.7	-22.2	-0.3	-12.4	-35.7
4.3	80.4	5.0	-3.7	-26.7	3.9	-9.1	-35.7
5.2	-63.2	4.5	-1.2	-19.8	-3.6	-10.9	-35.5
4.0	78.9	5.1	-4.7	-29.1	5.1	-6.8	-35.5
8.3	-73.4	6.6	1.3	-19.7	-1.1	-15.2	-34.7
8.5	45.1	3.1	7.7	-29.3	0.7	-13.6	-34.5
3.5	79.4	6.3	-8.5	-33.3	-0.8	8.4	-34.1
4.0	56.5	6.3	-0.3	-16.9	-2.4	-13.8	-33.4
7.8	-94.4	3.3	0.1	-15.9	-5.1	-11.9	-32.9
4.0	-63.8	6.8	-0.5	-12.6	-4.6	-14.9	-32.6
7.8	-40.1	3.2	-0.5	-20.4	-0.7	-9.5	-31.1
6.9	-50.6	3.2	-4.2	-18.0	4.1	-12.5	-30.7
3.9	-82.6	6.5	4.7	-25.4	1.2	-10.9	-30.4
7.5	-76.4	3.9	0.3	-17.5	-2.3	-10.4	-29.9
4.5	-57.9	6.1	0.5	-12.9	-1.6	-14.5	-28.5
3.8	-64.7	7.6	-0.8	-12.2	-3.6	-11.5	-28.3
3.9	-62.6	7.2	-0.4	-11.7	-4.4	-10.3	-26.9
6.3	-77.3	3.4	-0.1	-15.4	1.3	-12.5	-26.8
3.9	69.6	6.9	-0.9	-20.5	0.4	-4.2	-25.2
3.9	68.4	7.0	-1.8	-19.5	-0.2	-3.1	-24.7
3.9	51.3	7.1	0.2	-20.9	-4.0	0.3	-24.3
6.5	51.0	5.0	-0.6	-21.3	11.6	-11.0	-21.4
7.2	-64.4	4.5	0.8	-13.4	3.7	-11.9	-20.8
4.9	-52.4	7.0	0.5	-9.5	4.5	-14.0	-18.4
6.7	-65.3	4.7	-0.9	-10.1	5.7	-12.2	-17.4
8.3	42.0	5.4	0.1	-8.0	0.9	-9.8	-16.9
7.4	52.8	5.7	0.2	-8.1	0.6	-8.1	-15.5
2.2	-31.2	13.8	0.2	-2.4	8.7	-16.8	-10.3
7.2	-63.6	3.1	-6.9	-24.4	2.0	20.9	-6.4
6.1	-46.0	5.5	3.6	-7.6	6.2	-7.6	-5.6
3.6	58.5	6.4	0.1	23.8	1.3	-10.6	14.5

[a] The conformations are listed in the same order as in Table I.

[b] The subscript ES refers to the electrostatic energy term, NB refers to the nonbonded energy term. Both of these terms include the contributions to the hydrogen bond energy. In addition to the four energy terms shown here, E_{tot} also contains a torsional energy term E_{TOR}, contributed by the side-chain rotation about the C^α-C^β bond in Ala and a prolyl ring energy which depends on whether ω = 0° or 180° preceding Pro.

[c] The sign of θ determines the handedness of the major helix.[3]

[d] The distance of the glycyl N atom from the z-axis.

Table IV. Helical Parameters, Interchain Energy, and Intrachain Energy of Triple-Stranded Coiled-Coil Structures of $(GPA)_4$, with Screw Symmetry and Clockwise Disposition of the Chains[a]

Helical Parameters			Energy[b] [kcal/mol of $(GPA)_4$]				
			Interchain		Intrachain		Total
D (Å)	θ[c] (degrees)	ρ_1[d] (Å)	E_{ES}	E_{NB}	E_{ES}	E_{NB}	E_{tot}
3.5	-79.6	5.9	-5.4	-33.0	-4.1	-14.5	-57.0
3.5	-79.8	5.9	-5.2	-33.0	-4.3	-14.4	-56.8
4.7	-76.9	5.6	-1.0	-30.4	-3.5	-13.3	-48.3
9.0	-98.4	2.8	4.0	-34.1	-4.2	-12.1	-46.4
5.5	85.3	3.6	-4.0	-27.9	0.2	-11.0	-42.7
5.4	102.6	4.7	-3.4	-28.7	-0.8	-9.0	-41.9
6.4	89.9	2.7	-2.1	-21.1	-2.4	-14.5	-40.1
6.3	91.8	2.8	-2.4	-21.5	-2.8	-13.3	-39.9
4.9	-73.6	5.6	-0.6	-17.6	-3.8	-15.4	-37.5
5.8	-74.1	4.6	-0.4	-19.7	-7.1	-9.9	-37.0
4.6	-74.3	6.3	-1.3	-17.4	-4.3	-13.6	-36.6
4.9	-73.3	5.6	-0.2	-17.0	-4.8	-14.3	-36.4
4.7	-88.3	5.8	2.7	-30.2	0.9	-9.5	-36.1
4.1	86.6	5.2	0.8	-23.6	-2.0	-10.8	-35.5
8.2	-45.9	2.5	-2.8	-25.5	1.1	-7.6	-34.7
3.3	-62.4	6.6	0.7	-22.3	0.1	-13.1	-34.7
7.2	80.1	2.6	-3.3	-20.3	-0.5	-10.5	-34.5
6.6	58.3	4.0	-3.3	-23.5	-1.2	-5.9	-33.9
8.5	40.1	3.5	0.5	-21.2	0.2	-13.1	-33.6
4.8	-74.5	5.3	1.6	-23.7	-1.3	-10.0	-33.4
5.4	-61.0	5.1	1.1	-18.2	-2.2	-13.4	-32.6
4.5	-77.8	4.9	1.2	-18.3	-2.9	-12.5	-32.6
8.0	55.4	4.6	1.4	-19.5	-0.4	-13.5	-32.1
3.4	71.4	6.1	0.5	-20.9	-0.1	-11.5	-32.0
6.1	60.4	3.8	0.0	-15.3	-1.6	-15.0	-31.9
6.6	68.0	4.0	-3.1	-21.0	1.1	-8.7	-31.6
3.8	65.4	7.7	-1.4	-16.6	1.2	-14.6	-31.4
8.4	55.1	2.9	-0.8	-12.7	-0.9	-16.6	-31.1
4.6	76.4	4.6	2.2	-17.5	-5.4	-9.9	-30.6
4.4	81.9	5.8	3.8	-22.6	1.6	-13.3	-30.5
5.0	65.4	4.8	-3.9	-16.2	2.6	-10.1	-29.5
8.2	-69.1	4.0	1.5	-18.1	-2.1	-10.3	-29.1
5.2	-70.0	5.7	1.8	-22.5	2.7	-10.9	-28.9
3.6	-66.4	5.0	-0.4	-22.2	6.8	-12.6	-28.5
3.4	50.5	7.5	-0.2	-11.2	-2.1	-14.8	-28.3
3.6	-68.1	7.3	-0.2	-17.3	2.7	-13.2	-28.1
3.7	67.5	7.6	1.1	-15.5	-2.0	-10.7	-27.2
8.5	-71.3	4.0	-0.2	-11.3	-2.2	-13.4	-27.1
7.9	-103.2	3.2	3.2	-27.1	-0.0	-3.1	-27.0
5.1	91.6	4.7	-5.0	-24.1	0.2	2.4	-26.5
6.8	92.0	4.3	3.2	-20.0	-1.5	-8.0	-26.2
5.5	87.2	5.3	-0.3	-14.5	-3.8	-7.5	-26.1
4.9	-76.9	4.7	0.8	-16.0	0.7	-11.2	-25.7
7.2	-70.6	4.5	-0.8	-9.4	-4.8	-10.4	-25.3
5.0	-60.0	5.3	3.7	-25.3	2.6	-5.3	-24.5
8.5	31.1	4.7	-2.3	-9.2	1.3	-12.1	-22.3
8.0	35.4	5.5	0.4	-14.7	-1.9	-6.1	-22.3
3.4	48.7	7.8	2.0	-16.4	-4.6	-2.5	-21.5
7.1	53.8	3.4	-0.5	-10.6	1.0	-11.5	-21.5
3.8	50.1	7.4	2.6	-17.5	-3.7	-0.7	-19.3
8.1	-62.0	4.5	2.0	-14.6	-3.4	-2.9	-18.9
3.3	64.6	7.4	1.5	-18.6	-0.4	-1.0	-18.5
2.3	36.9	11.1	1.2	-7.8	-5.0	-0.3	-11.7
4.6	-44.7	7.3	2.1	-6.9	5.6	-11.0	-10.3
2.2	-33.0	12.4	1.1	-5.4	-6.0	0.6	-9.6

[a] The conformations are listed in the same order as in Table II.

[b] The subscript ES refers to the electrostatic energy term, NB refers to the nonbonded energy term. Both of these terms include the contributions to the hydrogen bond energy. In addition to the four energy terms shown here, E_{tot} also contains a torsional energy term E_{TOR}, contributed by the side-chain rotation about the C^α-C^β bond in Ala and a prolyl ring energy which depends on whether ω = 0° or 180° preceding Pro.

[c] The sign of θ determines the handedness of the major helix.[3]

[d] The distance of the glycyl N atom from the z-axis.

Table V. Cartesian Coordinates[a] of One Tripeptide Unit[b] of the Minimum-Energy Structure[c] of $(GPA)_n$

Residue	Atom	X	Y (Angstrom units)	Z
GLYCINE	N	1.56	0.0	0.0
	HN	0.84	-0.67	0.19
	CA	1.34	1.31	0.59
	HA[d]	1.53	2.02	-0.08
	HA[d]	0.38	1.41	0.85
	C'	2.23	1.51	1.82
	O	3.02	0.63	2.16
PROLINE	N	2.06	2.69	2.47
	CA	2.83	3.02	3.65
	HA[d]	3.79	2.74	3.53
	CB	2.70	4.52	3.81
	HB	2.57	4.79	4.86
	HB	3.59	5.03	3.46
	CG	1.48	4.92	2.99
	HG	0.65	5.17	3.65
	HG	1.70	5.81	2.40
	CD	1.12	3.75	2.09
	HD	0.09	3.44	2.24
	HD	1.23	4.01	1.04
	C'	2.33	2.24	4.87
	O	1.27	1.62	4.82
ALANINE	N	3.11	2.30	5.94
	HN	3.97	2.81	5.97
	CA	2.76	1.61	7.17
	HA[d]	2.61	0.65	6.94
	CB	3.92	1.71	8.16
	HB	3.67	1.15	9.06
	HB	4.82	1.28	7.71
	HB	4.10	2.75	8.41
	C'	1.46	2.20	7.72
	O	1.16	3.37	7.49

[a] Expressed in a right-handed coordinate system whose z-axis coincides with the major helical axis (the horizontal axis in Fig. 2).

[b] The coordinates of the other tripeptide units in the same and in neighboring strands can be obtained by the use of symmetry operations as described in ref. 2.

[c] This is the first conformation listed in Tables I and III.

[d] See footnote 23.

and with no penalty of potential energy.

A comparison of Table I with the corresponding Table V of the first paper[2] for coiled coils with counterclockwise disposition of the chains reveals only a few similarities of conformations other than the one just discussed. Many coiled-coil structures are listed for $(GPA)_n$ which have no counterparts for $(GPP)_n$ because ϕ_{Ala} takes values very different from that of the fixed ϕ_{Pro}. Even in those cases in which ϕ_{Ala} is near the value of −75°, the other dihedral angles in $(GPA)_n$ often differ widely from any of the combinations found for $(GPP)_n$, indicating that the absence of a prolyl ring in position Y and/or the difference in backbone geometry[22] between Ala and Pro result in differences in the conformation of the triple helix. There are some low-energy coiled-coil conformations, however, which are favorable for both molecules. In fact, each of the five lowest energy conformations of $(GPP)_4$ (see Table V of ref 2) has a corresponding low-energy $(GPA)_4$ conformation (Table I), with dihedral angles which differ only by a few degrees. No such similarities occur for the coiled coils with clockwise dispositions of the strands (Table II of this paper and Table VI of ref 2). However, the energy difference between the lowest energy conformation with clockwise and the one with counterclockwise disposition is much less for $(GPA)_n$ than for $(GPP)_n$, viz., 6.1 kcal/mol of $(GPA)_4$ as against 18.7 kcal/mol of $(GPP)_4$, indicating the greater flexibility of the former molecule.

The effect of changing the puckering of the prolyl ring[3] was tested on the lowest energy conformation of $(GPA)_n$. After substitution of the "up" puckering for proline, as described in the preceding paper,[3] and energy minimization, the energy was higher by 2.14 kcal/mol of GPA units. This is even higher than the energy difference computed[3] for GPP, justifying the use of the "down" puckering. In a similar calculation, using a slightly different ring geometry,[25] an energy difference of 0.96 kcal/mol of GPA units was obtained; the "down" puckering was lower in energy. If the three ω's also are allowed to vary (instead of being fixed at 180°), there is no change in the dihedral angles and in the energy of the lowest energy (collagen II-like) conformation.

B. Comparison with Experiment. An X-ray powder diffraction measurement[10] on $(\text{Pro-Ala-Gly})_n$, with $n \approx 10$, showed collagen-like reflections, with $D \sim 8.7$ Å. No value of Θ was reported. Our computation gave $\Theta = 61.7°$ and $D = 8.38$ Å for the lowest energy conformation.

The agreement between computed and observed values of D is about as good as in the case of $(GPP)_n$ and $(GPH)_n$, discussed in the earlier papers of this series.[2,3]

IV. Conclusions

The most stable conformations of triple-stranded $(GPA)_n$ were found to be coiled coils with screw symmetry, as contrasted with parallel-chain structures. This is the same result as those obtained for triple-stranded $(GPP)_n$ and $(GPH)_n$ in the preceding papers.[2,3] The energies of the computed lowest energy triple-stranded structures with different kinds of symmetry were as follows: −63.1, −30.0, and −48.8 kcal/mol of $(GPA)_4$, for coiled-coil structures with screw symmetry, for parallel chains with screw symmetry, and for structures with rotational symmetry, respectively. The lowest energy structure of $(GPA)_n$ is very close to that of $(GPP)_n$, and its helical parameters are close to that found for $(GPA)_n$ experimentally. The most stable structure of triple-stranded $(GPA)_n$ does not arise from the lowest energy conformation of single-stranded $(GPA)_n$ but from a higher energy one. Thus, the triple-stranded structure is stabilized by interchain interactions.

The similarity of several of the energetically most stable $(GPP)_n$ and $(GPA)_n$ conformations, in addition to the lowest energy conformation, indicates that alanine (or another amino acid) can easily be substituted into position Y in GPP, without large distortions of the conformation. However, the larger number of triple-helical minimum-energy conformations found for $(GPA)_n$, as compared with $(GPP)_n$, is a reflection of the greater flexibility of the poly(GPA) polypeptide chain.

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Miniprint Material Available: Full-size photocopies of Tables I–V (7 pages). Ordering information is given on any current masthead page.

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