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Conformations of Sequential Polypeptides Containing L-Alanyl and Glycyl Residues

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The conformations of four polypeptides containing ordered sequences of L-alanyl(A) and glycyl (G) residues, $(A)_n$, $(A_3G)_n$, $(A_2G)_n$, and $(AG_2)_n$, are investigated in solution. The optical rotatory dispersion and infrared spectra showed that all the polymers assume both an α-helical conformation and a β-conformation in solution. The stability of the α-helix decreases in the oder: $(A)_n$, $(A_2G)_n$, $(A_3G)_n$, and $(AG_2)_n$; this may be explained in terms of the interaction between methyl groups of L-alanyl residues regularly arranged on the surface of the α-helix.

The study of sequential polypeptides (regular copolymers) is very useful in clarifying the role of the residue sequence in determining the conformations of polypeptides and proteins. For instance, poly-glycyl-L-prolyl-L-alanine was synthesized as an analog of collagen.[1] Recently, three series of sequential polypeptides containing L-valyl and γ-methyl-L-glutamyl residues, S-benzyl-L-cysteinyl and γ-ethyl-L-glutamyl residues, and glycyl and γ-ethyl-L-glutamyl residues have been synthesized and influence of the residues sequence on conformation was reported by Fraser *et al.*[2–4]

Poly-L-alanine takes an α-helical conformation in an organic solvent[5] and in water as a part of a block copolymer with D,L-glutamic acid or D,L-lysine.[6–8] In the solid state, however, poly-L-alanine can take both a β-conformation and an α-helical conformation.[9,10] On the other hand, poly-glycine forms a β-conformation in the solid state.[11] Poly-L-alanylglycine was shown to form a β-conformation similar to that found in *Bombyx mori* silk fibroin in an oriented film.[12]

In this paper we intend to investigate the effect of the residue sequence on the conformation of various sequential polypeptides containing L-alanyl and glycyl residues, because these polymers may reveal simpler types of side chain-side chain interactions and side chain-main chain interactions than do the polymers containing other amino acids.

Experimental

Preparation of Polymers. The several sequential polypeptides, poly-(L-alanyl)$_3$-glycine, poly-(L-alanyl)$_2$-glycine, and poly-L-analyl-glycyl-glycine, were obtained from the corresponding tri- and tetrapeptide-*p*-nitrophenyl esters by treatment with triethylamine. The optical purity of each sequential polypeptide was checked by comparing the specific rotation of the acid hydrolyzate of each polymer with that of L-alanine. The racemization of each polymer was less than 5%. The syntheses of these polymers have previously been reported by one of the present authors (S.T).[13]

The poly-L-alanine was prepared by the polymerization of L-alanyl-N-carboxyanhydride in dioxane, using triethylamine as the initiator. The intrinsic viscosity of each polymer in dichloroacetic acid was 1.80 for poly-L-Ala, 0.15 for poly-(L-Ala$_3$-Gly), 0.26 for poly-(L-Ala$_2$-Gly), and 0.22 for poly-(L-Ala-Gly$_2$). These polymers are abbreviated as $(A)_n$, $(A_3G)_n$, $(A_2G)_n$, and $(AG_2)_n$.

Solution Studies. The polymers can dissolve only in dichloroacetic acid (DCA) or in trifluoroacetic acid (TFA), so the solutions were prepared by dissolving the vacuum-dried polymer in DCA and by then adding

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[1] S. M. Bloom, S. K. Dasgupta, R. P. Patel and E. R. Blout, *J. Amer. Chem. Soc.*, **88**, 2035 (1966).

[2] R. D. B. Fraser, B. S. Harrap, T. P. MacRae, F. H. C. Stewart and E. Suzuki, *J. Mol. Biol.*, **12**, 482, (1965).

[3] R. D. B. Fraser, B. S. Harrap, T. P. MacRae, F. H. C. Stewart and E. Suzuki, *ibid.*, **14**, 423, (1965).

[4] R. D. B. Fraser, B. S. Harrap, T. P. MacRae, F. H. C. Stewart and E. Suzuki, *Biopolymers*, **5**, 251, (1967).

[5] A. R. Downie, A. Elliott, W. E. Hanby and B. R. Malcolm, *Proc. Roy. Soc.* (London), **A 242**, 325, (1957).

[6] W. B. Gratzer and P. Doty, *J. Amer. Chem. Soc.*, **85**, 1193, (1963).

[7] N. Lotan, A. Berger, E. Katchalski, R. T. Ingwall and H. A. Scheraga, *Biopolymers*, **4**, 239, (1966).

[8] R. T. Ingwall, H. A. Scharaga, N. Lotan, A. Berger and E. Katchalski, *ibid.*, **6**, 331, (1968).

[9] A. Elliott and B. R. Malcolm, *Proc. Roy. Soc.* (London), **A 249**, 30, (1959).

[10] S. D. Dover and A. Elliott, *J. Mol. Biol.*, **30**, 201, (1967).

[11] A. Elliott, E. M. Bradbury, A. R. Downie and W. E. Hanby, "Polyamino Acids, Polypeptides, and Proteins," ed. by M. A. Stahmann, The University of Wisconsin Press, Madison (1962), p. 255.

[12] R. D. B. Fraser, T. P. MacRae, F. H. C. Stewart and E. Suzuki, *J. Mol. Biol.*, **11**, 706, (1965).

[13] S. Takahashi, This Bulletin, **42**, 521, (1969).

DCA and chloroform($CHCl_3$) or TFA and DCA to give a solution of the required solvent ratio. All these solvents except TFA (Nakarai Chemicals, Analytical Grade) were redistilled before use.

The optical rotatory dispersions over the 260—450 mμ wavelength range were measured with a JASCO ORD/UV 5 apparatus made by the Japan Spectroscopic Co., Ltd., using a 0.2 cm, 1 cm, or 5 cm cell set in a water-jacketed cell holder. The temperature in the cell was controlled with flowing water from a Haake constant-temperature bath. The concentrations of solutions were in the range of 0.2—0.7 g/100 cc. Absorption due to the solvents precluded measurements at shorter wavelength below 260 mμ.

To compute $[m']_\lambda$, the reduced mean residue rotation (in degrees·cm²/dmol) at the λ wavelength from the observed rotation, χ_λ, the following equation was used:

$$[m']_\lambda = \frac{3}{n^2+2}\cdot\frac{\bar{M}}{c}\cdot\frac{\chi_\lambda}{l}$$

Here, n is the refractive index of the solvent calculated by means of Lorentz-Lorenz's equation from the value of the refractive index of each pure solvent; c is the concentration of the polymer in grams per 100 cc; l is the path length in decimeters, and $\bar{M}$ is the mean residue molecular weight of the polymers, that is, 71.08 for $(A)_n$, 67.57 for $(A_3G)_n$, 66.40 for $(A_2G)_n$, and 61.72 for $(AG_2)_n$. We adopted the above values for $\bar{M}$ because it is difficult to estimate the contribution of the glycyl residues to the optical rotations of the sequential polypeptides. The optical rotatory dispersion parameters, a_0 and b_0 were obtained by plotting the original value of the optical rotation in terms of the equation given by Moffitt and Yang:[14]

$$[m']_\lambda = \frac{a_0\lambda_0^2}{\lambda^2-\lambda_0^2}+\frac{b_0\lambda_0^4}{(\lambda^2-\lambda_0^2)^2}$$

Linear relations were obtained between $[m']_\lambda\cdot(\lambda^2\text{-}\lambda_0^2)/\lambda_0^2$ and $\lambda_0^2/(\lambda^2-\lambda_0^2)$ using a value of $\lambda_0=212$ mμ.

The infrared spectra were measured with a model DS 402 G apparatus made by the Japan Spectroscopic Co., Ltd., using a cell with NaCl windows. The concentrations of the solutions ranged from 2 to 3 g/100 cc. The infrared spectrum of the solution and that of the solvent mixture with the same solvent composition were measured separately, the difference in the spectra was obtained by subtracting the optical density of the latter from that of the former.

Results

Optical Rotatory Dispersion. The dispersion curves of four kinds of polymers in various solvent compositions at 24.5°C are shown in Fig. 1. In the TFA-DCA system, all the polymers exhibit negative rotations; the negative rotation of each polymer decreases as the solvent composition is changed from a high TFA content to 100% DCA, and then to a high $CHCl_3$ content. In 66.7% $CHCl_3$—33.3% DCA, the polymers $(A)_n$ and $(A_2G)_n$ exhibit positive rotations above 355 and 340 mμ respectively.

The parameter, b_0, for the polymers is plotted in Fig. 2 as a function of the solvent composition. It will be seen that the magnitude of $-b_0$ for each polypeptide is not zero in a DCA solution, and that it decreases as the proportion of TFA increases in the TFA-DCA system; on the other hand, in the DCA-$CHCl_3$ system, the magnitude of $-b_0$ increases as the proportion of $CHCl_3$ increases. Thus, all the polymers assume an α-helical conformation to some extent, although the maximum magnitudes of $-b_0$ and the stabilities of the helices towards DCA and TFA differ markedly from polymer to polymer.

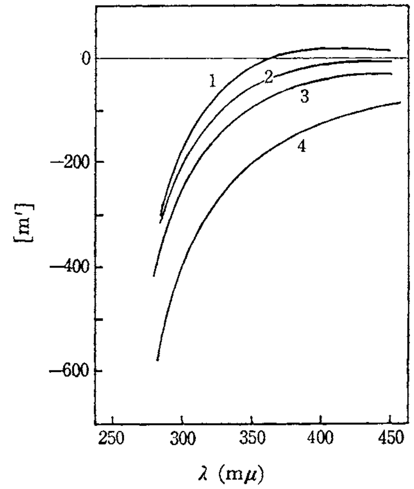

Fig. 1(a). ORD curves of the polymer $(A)_n$ at 24.5°C.
(1): 66.7% $CHCl_3$ - 33.3% DCA, (2); 33.3% TFA - 66.7% DCA, (3); 50% TFA - 50% DCA, (4); 66.7% TFA - 33.3% DCA.

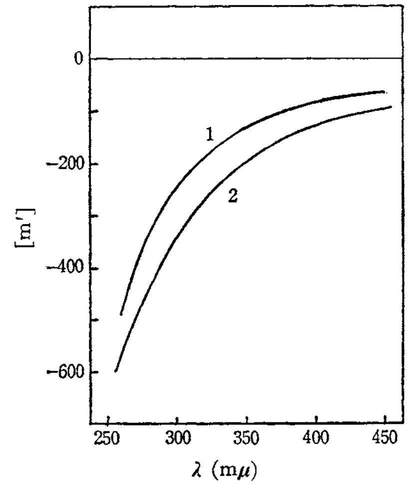

Fig. 1(b). ORD curves of the polymer $(A_3G)_n$ at 24.5°C.
(1); 66.7% $CHCl_3$ - 33.3% DCA, (2); 50% TFA-50% DCA.

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[14] W. Moffitt and J. T. Yang, *Proc. Nat. Acad. Sci. U.S.*, **42**, 596, (1956).

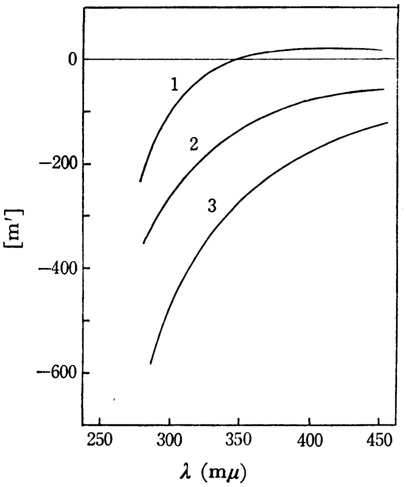

Fig. 1(c). ORD curves of the polymer $(A_2G)_n$ at 24.5°C.
(1); 66.7% $CHCl_3$ - 33.3% DCA, (2); 100% DCA (3); 50% TFA - 50% DCA.

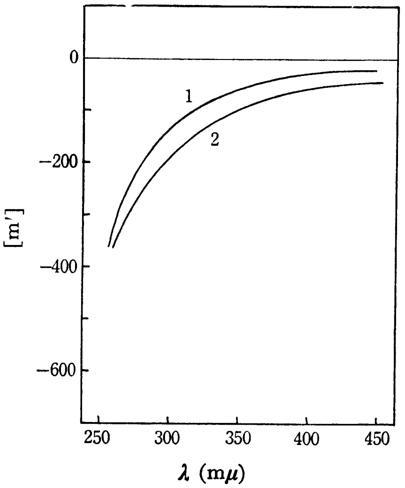

Fig. 1(d). ORD curves of the polymer $(AG_2)_n$.
(1); 66.7% $CHCl_3$ - 33.3% DCA, (2); 100% DCA.

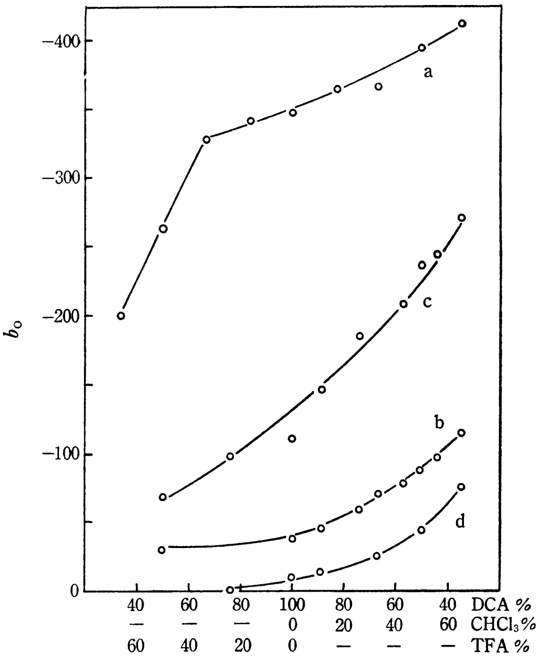

Fig. 2. Variation of the ORD parameter b_0 with solvent composition.
(a); $(A)_n$, (b); $(A_3G)_n$, (c); $(A_2G)_n$, (d); $(AG_2)_n$.

The value of b_0 of the homopolymer $(A)_n$ increases from −200 in 66.7% TFA - 33.3% DCA to −327 in 33.3% TFA - 66.7% DCA; thereafter it increases very slowly to the value of −411 in 66.7% $CHCl_3$ - 33.3% DCA. This behaviour is almost the same as that found by Fasman.[15] Other polymers, having smaller magnitudes of b_0 than $(A)_n$ over the whole range of the solvent composition, exhibit gradual increases in the b_0 values as the solvent composition is changed from a high TFA content to 100% DCA, and then to a high $CHCl_3$ content. However, the magnitude of the value b_0 of each polymer decreases in the order of $(A_2G)_n$, $(A_3G)_n$, and $(AG_2)_n$ over the whole range of the solvent composition.

Each parameter of the polymers in 66.7% $CHCl_3$—33.3% DCA showed only a small change when the temperature of the solution was raised to 57°C, and the values of the parameters returned to the original values when the temperature was

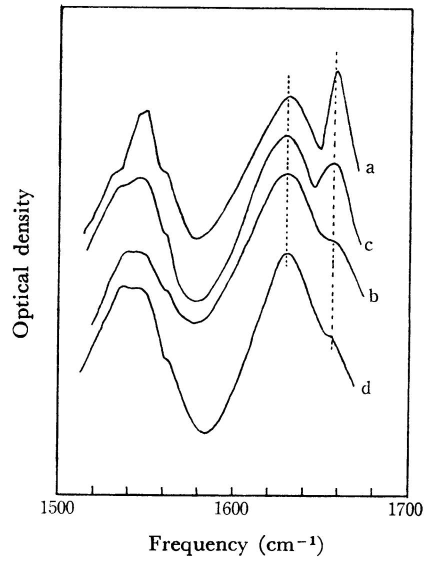

Fig. 3. IR spectra of the polymers.
(a); $(A)_n$, (b); $(A_3G)_n$, (c); $(A_2G)_n$, (d); $(AG_2)_n$.

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[15] G. D. Fasman, "Polyamino Acids, Polypeptides and Proteins," by M. A. Stahmann, The University of Wisconsin Press, Madison (1962), p. 221.

lowered to 24.5°C. In the same solvent proportion, the $[m']_\lambda$ values of all the polymers were independent of the concentration in the range of 0.1—1.0 g/100 cc.

Infrared Spectra. The infrared spectra over the frequency range from 1500 cm^{-1} to 1700 cm^{-1} are shown in Fig. 3. In the 25% DCA - 75% $CHCl_3$ solution, it was found that all the polymers exhibited an amide I band at about 1630 cm^{-1}, which may be attributed to a β-conformation,[16] and another band at about 1655 cm^{-1} due to an α-helical conformation.[16] This 1655 cm^{-1} component reveals a strong absorption band in the polymers $(A)_n$ and $(A_2G)_n$, but only a weak band in $(A_3G)_n$ and $(AG_2)_n$. The 1655 cm^{-1} component, however, may also contain a contribution from a random-coiled conformation of the polypeptides. Thus, all the polymers assume the β-conformation in addition to the α-helical conformation.

Discussion

Judging from the infrared spectra of the polymers, there is a possibility that all the polymers assume not only the α-helix but also a β-conformation in solution. The behaviour of the β-conformation of these polymers in solution can be investigated on the basis of an analysis of the parameters a_0 and b_0. These parameters can be written in the following forms:[17]

$$a_0 = a_0^R + a_0^H f_H + a_0^\beta f_\beta$$
$$b_0 = b_0^H f_H + b_0^\beta f_\beta$$

Here, a_0^R, a_0^H, and a_0^β are the contributions to a_0 from the random, α-helical, and β-conformations; b_0^H and b_0^β have the usual meanings, and f_H and f_β are the fractions of the α-helical conformation and the β-conformation respectively. For the parameters corresponding to the α-helical conformation, we adopt the values of $a_0^H=680$ and $b_0^H=-630$.[17]

On the other hand, for the β-conformation various values of the parameters have been reported.[18-23] It, therefore, might be quite difficult to estimate the values of the parameters for the polymers containing L-alanyl and glycyl residues. In this paper we assume tentatively the values of $a_0^\beta=700$ and $b_0^\beta=0$. We measured the ORD curve of each polymer in 100% TFA, and we adopted the a_0 value obtained from the curve as the a_0^R of each polymer.

We can now calculate the fraction of the β-conformation from the observed values of a_0 and b_0 according to the above equations. However, it should be emphasized that, because of the ambiguity of the numerical values of the parameters assumed above, the content of the β-conformation thus calculated does not represent the absolute values, but, rather, relative values.

The fraction of the β-conformation, f_β, thus obtained is plotted in Fig. 4 as a function of the solvent composition. In the polymer $(AG_2)_n$, f_β is nearly constant over the whole range of the solvent compositions, while in the polymer $(A_3G)_n$, f_β increases as the proportion of $CHCl_3$ increases. In the polymer $(A_2G)_n$, f_β changes greatly, and in $(A)_n$ f_β has a maximum at 100% DCA and decreases as the proportions of both $CHCl_3$ and TFA increase.

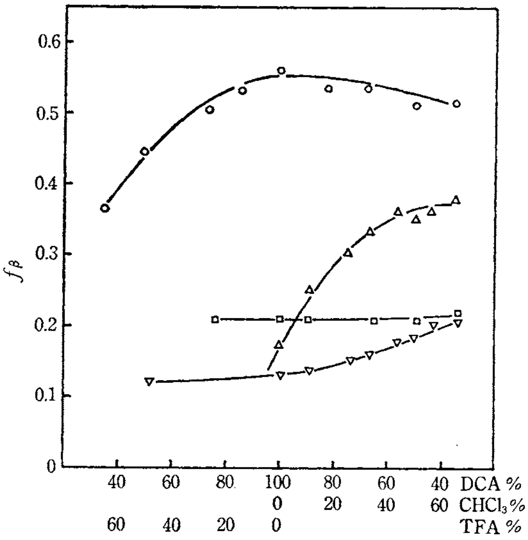

Fig. 4. Variation of the fraction of β-conformation with solvent composition.
-○-; $(A)_n$, -▽-; $(A_3G)_n$, -△-; $(A_2G)_n$, -□-; $(AG_2)_n$.

On the other hand, because of $b_0^\beta=0$, we can see directly the behaviour of the α-helix of each polymer from the b_0 value in Fig. 2.

The contents of the α-helix conformation and the β-conformation in the highest $CHCl_3$ composition for each of the polymers are shown in Table 1. The ratio of f_H to f_β decreases in the order of $(A)_n$, $(A_2G)_n$, $(A_3G)_n$, and $(AG_2)_n$. This order coincides with the observation of the infrared measurements. In the infrared spectra, the optical density of the 1655 cm^{-1} component

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16) T. Miyazawa and E. R. Blout, *J. Amer. Chem. Soc.*, **83**, 712, (1961).

17) P. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 432, (1961).

18) G. D. Fasman and E. R. Blout, *J. Amer. Chem. Soc.*, **82**, 2262, (1960).

19) A. Wada, M. Tsuboi and E. Konishi, *J. Phys. Chem.*, **65**, 1119, (1961).

20) E. M. Bradbury, A. Elliott and W. E. Hanby, *J. Mol. Biol.*, **5**, 487, (1962).

21) K. Imahori and I. Yahara, *Biopolym., Symp.*, **1**, 421, (1964).

22) S. Ikeda, M. Maeda and T. Isemura, *J. Mol. Biol.*, **10**, 223, (1964).

23) S. Ikeda, *Biopolymers*, **5**, 359, (1967).

TABLE 1. COMPARISON OF THE PROPERTIES OF THE POLYMER

Polymer	% Helix*	% β-Conformation**	Conformation***
$(A)_n$	65	52	$\alpha+\beta$
$(A_3G)_n$	18	21	$\alpha+\beta$
$(A_2G)_n$	43	38	$\beta+\alpha$
$(AG_2)_n$	12	22	$\beta+\alpha$

* Percent of α-helix content in 66.7% $CHCl_3$-33.3% DCA solution.

** Percent of β-conformation content in the same solution.

*** Obtained from the IR spectra in Fig. 3.

also decreases in the order of $(A)_n$, $(A_2G)_n$, $(A_3G)_n$, and $(AG_2)_n$ compared to that of the 1630 cm^{-1} component (Fig. 3).

As has been mentioned above, the values of the β-contents of the polymers depend largely on the choice of the values of the parameters, so that further quantitative discussions can not be made concerning the behaviour of the β-conformation of the polymers. In the following discussions, we will discuss the behaviour of the α-helix in solution independently of the β-conformation, on the basis of the observation of the paramter b_0.

It is known that the L-alanyl residue stabilizes the α-helical conformation of polypeptide[5-8,24] and that the glycyl residue markedly reduces the α-helical stability.[4,25] In the γ-benzyl-L-glutamateglycine random copolymers[25] and in the sequential polypeptides containing γ-ethyl-L-glutamyl and glycyl residues,[4] the helical stabilities of the polypeptides are reduced as the content of the glycyl residue increases. In this experiment, however, the stability of the α-helix is reduced in the order of $(A)_n$, $(A_2G)_n$, $(A_3G)_n$, and $(AG_2)_n$, as may be seen in Fig. 2. This order does not coincide with the order of the increment of the glycine content. Therefore, the instabilities of the α-helices of these polymers can not be explained merely by the main-chain flexibility at the position of the glycyl residues.[4] It is necessary to consider also the regularity of the residue sequence and the contribution of the side chain of the L-alanyl residue to the α-helical stability.

The distribution of the glycyl (G) and L-alanyl (A) residues on the surface of a 3.6 residue right-handed-turn α-helix is illustrated schematically in Fig. 5 for each of the four polymers. If the residue in the center of each diagram is referred to as the 0 residue, it is convenient to number successive residues +1, +2, *etc.* in the -CO-C$_\alpha$-NH- direction and −1, −2, *etc.* in the opposite direction. As may be seen in Fig. 5, in the polymer $(A_2G)_n$, if one G is located at the position 0, the 0, ±3, ±6, *etc.* positions are all occupied by G's. In the polymer $(A_3G)_n$, the positions 0, ±4, ±8, *etc.* are all occupied by G's. If the values of b_0 reflect the stabilities of the α-helices, then it should be concluded from the experiments that the introduction of G's decreases the stability of an α-helix, especially in the case of two G's at 0 and 4 as compared with two G's at 0 and 3. The polymer $(AG_2)_n$, where both types of relative arrangements of G's exist, is most unstable.

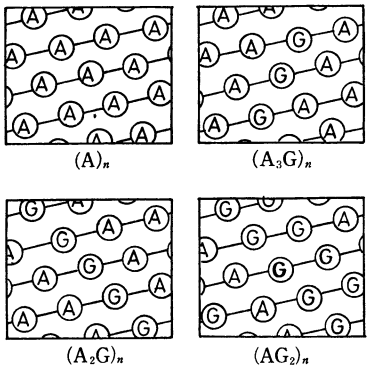
$(A)_n$ $(A_3G)_n$
$(A_2G)_n$ $(AG_2)_n$

Fig. 5. Radial projection showing the distribution of the residues on an α-helix for each of the polymers.

The α-helix of poly-L-alanine in water is considered to be more stabilized by the hydrophobic bond between the methyl group of the residue A(O) and the C$_\alpha$H group of the residue A(3).[7,24] This interaction should operate on the poly-L-alanine in the polar organic solvents as the lyophobic bond. This interaction may exist to some extent between A(0) and G(3), but not between G(0) and A(3). Although we do not have accurate knowledge of the magnitudes of these lyophobic interaction energies, we can understand the variation in the α-helical stabilities of the polymers if the energy of this interaction between A(0) and G(3) is assumed to be a half or less than that between A(0) and A(3). We can calculate, based on the above assumption, the total energy of the lyophobic interaction between the residues at 0 and 3 for each repeating unit of the polymers. Of course, the energy between G(0) and G(3) is zero. In the polymer $(A_3G)_n$, where the repeating unit is G(0)-A(1)-A(2)-A(3), the total energy is a sum of the energies of the interactions between G(0) and A(3), between A(1) and G(4). between A(2) and A(5), and between A(3) and A(6); their relative magnitudes are 0, 0.5, 1, and 1 respectively. Therefore, the sum is given by 2.5, and the energy per residue is 0.62. The interaction energy per residue for the other polymers is calculated by the same procedure, resulting in an energy of 1 for $(A)_n$, of 0.67 for $(A_2G)_n$, and of

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[24] M. Bixon, H. A. Scheraga and S. Lifson, *ibid.*, **1**, 419, (1963).

[25] H. Block and J. A. Kay, *ibid.*, **5**, 243, (1967).

0.33 for $(AG_2)_n$. Thus, the average lyophobic interaction energies per residue of the polymer decrease in the order of $(A)_n$, $(A_2G)_n$, $(A_3G)_n$, and $(AG_2)_n$; this order coincides with that of the instabilities of the α-helices observed in the optical rotatory measurements.

The glycyl residue does not have a methyl group, so the polar hydrogen-bond breakers (DCA or TFA) are more easily accessible to the α-helical backbone at the position of the glycyl residue than at that of the L-alanyl residue. It is, therefore plausible that the interaction between A(0) and G(3) is weaker than that between A(0) and A(3).

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