1240 BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 48(4), 1240—1244 (1975) [Vol. 48, No. 4

Conformations of Sequential Polypeptides of L-Leucine and Glycine in Solution

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(Received May 7, 1974)

With the aid of optical rotatory dispersion, circular dichroism, and infrared spectra, the conformations of the sequential polypeptides of L-leucine and glycine, $(\mathrm{Leu_3Gly})_n$, $(\mathrm{Leu_2Gly})_n$, $(\mathrm{LeuGly})_n$, and $(\mathrm{LeuGly_2})_n$, were studied and compared with the conformation of poly-L-leucine. It was shown that the polymers $(\mathrm{Leu})_n$, $(\mathrm{Leu_3Gly})_n$, $(\mathrm{Leu_2Gly})_n$, and $(\mathrm{LeuGly})_n$ could be α-helical in solution, but $(\mathrm{LeuGly_2})_n$ was not. The order of the stability of the α-helix of these polymers was found to be: $(\mathrm{Leu})_n > (\mathrm{Leu_3Gly})_n \sim (\mathrm{Leu_2Gly})_n > (\mathrm{LeuGly})_n > (\mathrm{LeuGly_2})_n$. This was attributed to interactions between side chains of L-leucines which were regularly arranged on the α-helix.

Poly-L-alanine is known to assume the very stable α-helix conformation in solution[1], on the other hand, glycine destabilizes the α-helix when it is incorporated in a polypeptide chain which otherwise might be α-helical[2]. By studying the sequential polypeptides of L-alanine and glycine we concluded that, although the short range interactions between a side chain and the atoms of the backbone of the same amino acid residue were important, the interactions between the neighboring residues could not be neglected in explaining the stability of the α-helix of the polymers, which was in the order: Ala > $(\mathrm{Ala_2Gly})_n$ > $(\mathrm{Ala_3Gly})_n$ > $(\mathrm{AlaGly_2})_n$ and was not proportional to the alanine content.

Poly-L-leucine also forms the very stable α-helix in water.[4] Because the side chain of leucine is much larger than that of alanine, it is desirable to study the conformational stability of sequential polypeptides of L-leucine and glycine. In this paper we report a conformational study of sequential polypeptides of L-leucine and glycine in solution.

Experimental

Polymers. The sequential polypeptides, poly(L-leucyl$_3$-glycine) (intrinsic viscosity in dichloroacetic acid at 25 °C, $[\eta]=0.158$ dl/g), poly(L-leucyl$_2$glycine)($[\eta]=0.191$), poly(L-leucylglycine) ($[\eta]=0.151$), and poly(L-leucylglycylglycine) ($[\eta]=0.262$), were obtained from the *p*-nitrophenylesters of L-leucyl$_3$glycine, L-leucyl$_2$glycine, (L-leucylglycyl)$_2$, and L-leucylglycylglycine, respectively.[5] Poly-L-leucine ($[\eta]=1.86$ in 80% trifluoroacetic acid/20% chloroform) was prepared via the usual N-carboxyanhydride method by Dr. A. Warashina of the Faculty of Science, Nagoya University. These polymers are abbreviated: $(\mathrm{L_3G})_n$, $(\mathrm{L_2G})_n$, $(\mathrm{LG})_n$, and $(\mathrm{L})_n$, respectively. The fairly large values of the intrinsic viscosity in solvents favoring the random coil conformation should be noted as they indicated that the degrees of polymerization of these polymers were high enough for conformational studies.[6]

Solution Studies. The plymers could only be dissolved in dichloroacetic acid (DCA) or in trifluoroacetic acid (TFA), so their solutions were prepared by dissolving vacuum-dried polymer in TFA and then adding TFA and chloroform or 2,2,2-trifluoroethanol (TFE) to give a solution of the required solvent ratio.

Optical rotatory dispersion (ORD) spectra over the 280—500 nm wavelength range were measured with a JASCO ORD/UV 5 spectrometer using a 1 cm cell. The solution concentrations were in the range 0.8—1.1 g/100 ml.

Circular dichroism (CD) spectra below 250 nm were measured with the same apparatus using a 0.2 cm cell. The concentration of the solution was 0.035 g/100 ml. All measurements were performed at 24 °C. To calculate $[m']$, the reduced mean residue rotation in degree cm^2/dmol, and $[\theta]$, molar ellipticity in the same units, from the observed rotation angle, χ^{ORD} and χ^{CD}, Eqs. (1) and (2) were used.

$$[m'] = \frac{n^2+2}{3}\cdot\frac{M}{c}\cdot\frac{\chi^{\mathrm{ORD}}}{l} \tag{1}$$

$$[\theta] = \frac{45\ln 10}{\pi}\cdot\frac{M}{c}\cdot\frac{\chi^{\mathrm{CD}}}{l} \tag{2}$$

where the refractive index of the composite solvent, n, was calculated from the Lorentz-Lorenz equation using the refractive index of each pure solvent, c is the concentration of a polymer in grams per 100 ml, l is the path length in decimeters, and M is the mean residue molecular weight of polymer. ORD parameters, a_0 and b_0, were obtained by plotting the values of the optical rotation in terms of the Moffitt-Yang equation (3)[7] with a value of $\lambda_0=212$ nm.

$$[m'] = \frac{a_0\lambda_0^2}{\lambda^2-\lambda_0^2} + \frac{b_0\lambda_0^4}{(\lambda^2-\lambda_0^2)^2} \tag{3}$$

Infrared (IR) spectra were recorded with a JASCO IR-G spectrophotometer. The concentration of the solution ranged from 3 to 5 g/100 ml. Films of each polymer were prepared from TFA solution by casting the solution (polymer concentration about 5 g/100 ml) on a KBr plate and by drying the film under a vacuum at 40 °C.

Results

ORD Measurements. a_0 and b_0 values as a function of solvent composition were obtained from ORD spectra measured in a TFA-CHCl$_3$ system. These values are plotted in Fig. 1, which shows that $(\mathrm{L})_n$ had a random coil conformation in TFA, a random coil favoring solvent, and changed to an α-helix when the content of CHCl$_3$, a helix favoring solvent, increased.[1] The values of b_0 for $(\mathrm{LG})_n$ and $(\mathrm{LG_2})_n$ were low and did not change appreciably with solvent composition, indicating that the glycyl residues in these polymers inhibited the transition from the random coil to the helix. Polymers $(\mathrm{L_3G})_n$ and $(\mathrm{L_2G})_n$ showed a transition, but in these cases the helical content, which was considered to reflect the stability of the helix, was reduced. The magnitude of b_0 is slightly larger for $(\mathrm{L_2G})_n$ than for $(\mathrm{L_3G})_n$ throughout the whole range of TFA concentration. The

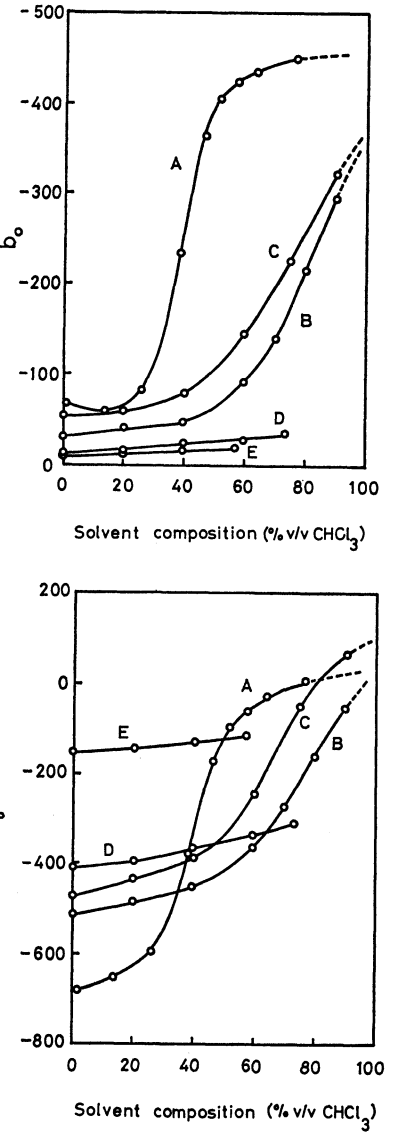

Fig. 1. Variation of the ORD parameters, a_0 and b_0, with solvent composition. A: $(L)_n$, B: $(L_3G)_n$, C: $(L_2G)_n$, D: $(LG)_n$, E: $(LG_2)_n$. Data on $(L)_n$ were obtained by A. Warashina in Ref. 8.

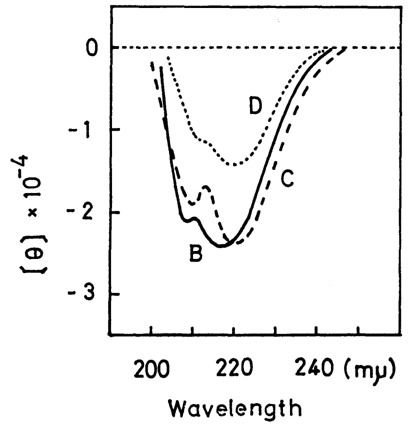

Fig. 2. CD spectra of the polymers in 96.8%TFE/3.2% TFA (v/v).
Letter symbols are the same as used in Fig. 1.

increase in b_0 in each case is accompanied by the corresponding increase in the value of a_0.

CD Measurements. The CD spectra in the far ultraviolet region for $(L_3G)_n$, $(L_2G)_n$, and $(LG)_n$ in 96.8% TFE/3.2% TFA are shown in Fig. 2. The polymers $(L)_n$ and $(LG_2)_n$ did not dissolve in such a solvent having such a large amount of TFE which was required if the solution was to be transparent in the far-ultraviolet region.

The polymer $(L_3G)_n$ exhibited two minima at 209 and 218 nm in the CD spectrum, and $(L_2G)_n$ exhibited minima at 210 and 222 nm. These bands are characteristic of the α-helix.[9] Though the magnitude of the molar ellipticity was reduced, polymer $(LG)_n$ also exhibited a spectrum characteristic of an α-helix. The value of the molar ellipticity of the band near 220 nm was -24000 for $(L_3G)_n$, -23000 for $(L_2G)_n$, and -14000 for $(LG)_n$ (a molar ellipticity of -32000 was reported for a complete α-helix in the 220 nm region[9]). From these observations, we can conclude that an α-helix exists in the polymers $(L_3G)_n$, $(L_2G)_n$, and also exists in $(LG)_n$ in solutions which contain a large amount of a helix-favoring solvent.

IR Measurements. IR spectra of the polymers in 70% $CHCl_3$/30% TFA solutions are shown in Fig. 3. All the polymers exhibited the amide I band at 1650 cm^{-1} and the amide II band around 1540 cm^{-1}, these were considered to originate from either the α-helical or the randomly coiled conformation of the polypeptide chain.[10] In addition to these bands, we observed a strong band around 1620 cm^{-1}. The peak of this band shifted from 1610 cm^{-1} as the glycine content of the polymer increased from $(L)_n$ to $(LG_2)_n$. Although the absorption band near 1630 cm^{-1} had been generally attributed to the β-structure,[10] however, in this case, the band at 1610—1635 cm^{-1} was not ascribed to the β-structure for the following reasons:

(i) The IR spectra of $(L)_n$ in TFA–$CHCl_3$ systems containing different amounts of TFA are shown in Fig. 4. As may be seen in Fig. 1, the b_0 value of $(L)_n$ showed that $(L)_n$ changed from the random coil to the α-helix as the TFA content decreased from 80% to 40%. The randomly coiled conformation of $(L)_n$ in 80% TFA has also been confirmed by NMR spectra.[8] In contrast, in the IR spectra, $(L)_n$ exhibited a band at 1610 cm^{-1} in addition to the amide

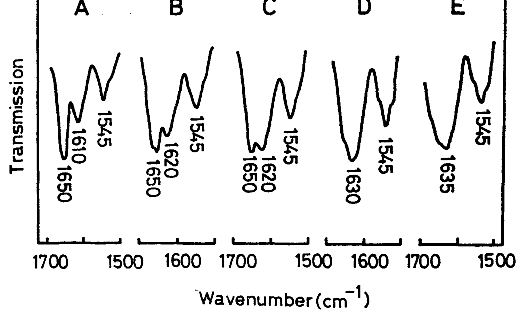

Fig. 3. IR spectra of the polymers in 70% $CHCl_3$/30% TFA (v/v).
Letter symbols, see Fig. 1.

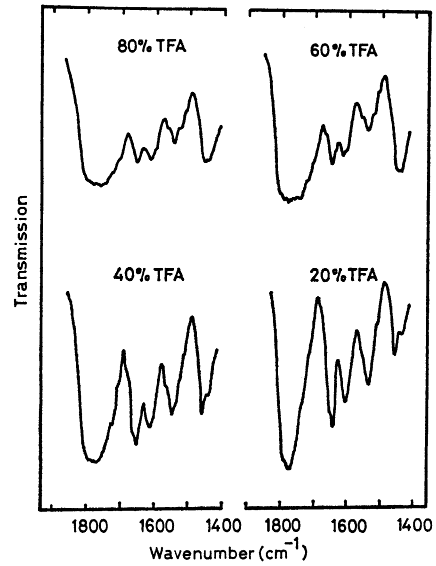

Fig. 4. IR spectra of $(L)_n$ in TFA–$CHCl_3$.

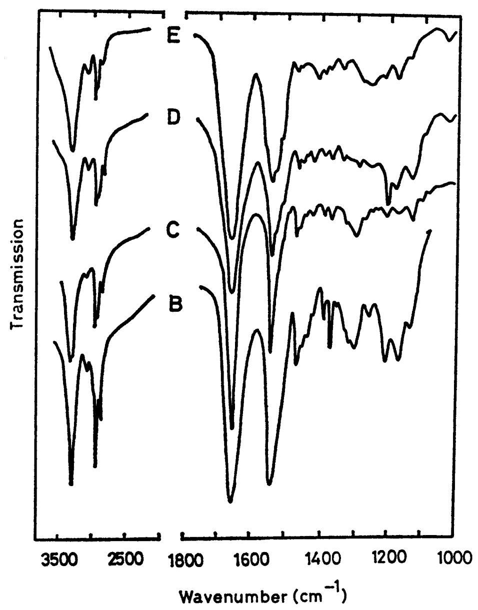

Fig. 5. IR spectra of films of the sequential polypeptides. All films were prepared from TFA solutions. Letter symbols, see Fig. 1.

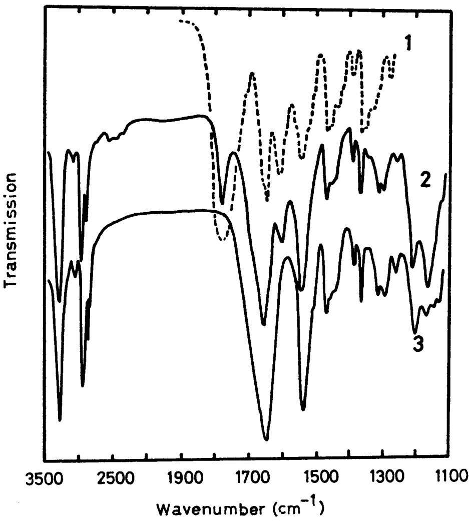

Fig. 6. IR spectra of $(L)_n$. 1: solution (80% $CHCl_3$/20% TFA (v/v)), 2: film from TFA solution (incompletely dried), 3: film from TFA solution (completely dried).

I band at 1650 cm^{-1} and the amide II band at 1545 cm^{-1} for all TFA concentrations between 80 and 20%.

(ii) As was pointed out in the previous section, $(L_2G)_n$ and $(L_3G)_n$ exhibited CD spectra characteristic of the α-helix in a TFA–TFE system. However, IR spectra of the polymers in 50% TFA/50% TFE were the same as those in 70% TFA/30% $CHCl_3$: they showed the band at 1620 cm^{-1} in addition to the usual amide I band at 1650 cm^{-1} and the amide II band at 1545 cm^{-1}.

(iii) In general, the polypeptide chains cast in film have been considered to have the same conformation as they might be assumed to have in a solvent such as $CHCl_3$. IR spectra of the sequential polypeptides cast in films from TFA solutions did not exhibit the band near 1620 cm^{-1}, they exhibited only the amide I band at 1650—1660 cm^{-1} and the amide II band at 1545 cm^{-1} (Fig. 5). The 1610 cm^{-1} band was observed in a film of $(L)_n$ if the film was incompletely dried: this band disappeared as TFA was completely removed (Fig. 6).

All these results strongly suggest that the band near 1620 cm^{-1} which was observed in solutions containing TFA should not be attributed to the β-structure of the polypeptide chain but to interactions between the polymer and the solvent TFA. Recently, Nagayama[11a] attributed the band at 1610 cm^{-1} to solvation of TFA with the polypeptide chain, and Combelas *et al.*[11b] also gave the same conclusion.

Discussion

Our experimental results showed that the sequential polypeptides except for $(LG_2)_n$ were α-helical in solution, although each polymer had a different helicity. The helical content, f_α, was calculated from Eq. (4) for each of the polymers,

$$f_\alpha = \frac{(-455)-(-60)}{b_0-b_0^{\mathrm{TFA}}}, \tag{4}$$

where we assumed that the b_0 values of $(L)_n$ were −455 at 100% helix and −60 at 100% random coil, and that all the sequential polypeptides had random coils in 100% TFA, although the b_0 value obtained with 100% TFA solution for each sequential polypeptide (b_0^{TFA}) was not zero. The results are shown in Fig. 7.

$(L)_n$ changed from a random coil to an α-helix when the $CHCl_3$ content increased from 25% to 55%, the midpoint of the transition was at 40% $CHCl_3$. On the other hand, polymers $(L_2G)_n$ and $(L_3G)_n$ showed a transition at a $CHCl_3$ content higher than 40%. For $CHCl_3$ contents between 40% and 90%, the helicity of $(L_2G)_n$ was slightly higher than that of $(L_3G)_n$. When the $CHCl_3$ content reached 90%, the helix content of $(L_2G)_n$ was the same as that of

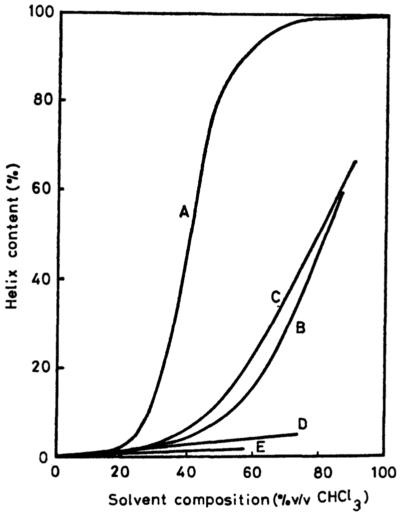

Fig. 7. Variation of the helix content (in %) of the polymers with solvent compositions. Helix content is calculated from equation (4). Letter symbols, see Fig. 1.

(L$_3$G)$_n$, 67%. The helix content of (L$_3$G)$_n$ may be higher than that of (L$_2$G)$_n$ when the CHCl$_3$ content is higher than 90%: this expectation was supported by CD spectra although the solvent system (TFA–TFE) was different from that used in the ORD studies (TFA–CHCl$_3$). Analysis of the CD spectra (Fig. 2) gave helical contents of 75% for (L$_3$G)$_n$ and 72% for (L$_2$G)$_n$. On the other hand (LG)$_n$ and (LG$_2$)$_n$ were hardly α-helical in TFA–CHCl$_3$, although (LG)$_n$ was partially α-helical in 96.8% TFE/3.2% TFA as shown by CD spectra.

These observations show us that the presence of a glycyl residue in an amino acid sequence has an important effect on the conformation of these polypeptides, causing a marked reduction in the stability of the α-helix. The magnitude of this reduction depends not only on the glycine content but also on the residue sequence, as the stability of the α-helix decreased in the order: (L)$_n$>(L$_3$G)$_n$~(L$_2$G)$_n$>(LG)$_n$>(LG$_2$)$_n$. In spite of the helicity of (L$_2$G) being slightly higher than that of (L$_3$G)$_n$, (L$_3$G)$_n$ and (L$_2$G)$_n$ were conservatively considered to have nearly equal helical stability, noting that the degree of polymerization of (L$_2$G)$_n$ might be slightly higher than that of (L$_3$G)$_n$ since the former polymer had a higher intrinsic viscosity in DCA than the latter and that the stability of the α-helix might depend on chain length.[12]

A schematic representation of α-helices, showing the radial projection of the distribution of amino acid residues onto the surface of the α-helix, is presented in Fig. 8 for each of the polymers. Each residue position corresponds to the location of an α-carbon of an amino acid, not of a side chain. The successive residues were numbered 1, 2, 3, *etc.* in the –CO–C$_\alpha$–NH– direction from the C–terminal of the chain.

The α-helix is stabilized by the hydrogen bond between the NH group of the *i*-th residue and the CO group of the (*i*+4)th residue.[13] In addition to the hydrogen bond, non-covalent interaction between aliphatic side chains is the other important factor stabilizing the α-helix in solution.[14] For instance, non-covalent interaction between the *i*-th and the (*i*+3)th residues has been taken into account to explain the remarkable stability of the α-helix of poly-L-alanine in water.[15] In (L)$_n$, the interaction becomes more larger than in poly-L-alanine since leucine has a large aliphatic side chain. This has been actually confirmed in conformational studies on (L)$_n$ in water by Ostroy *et al.*[4a]

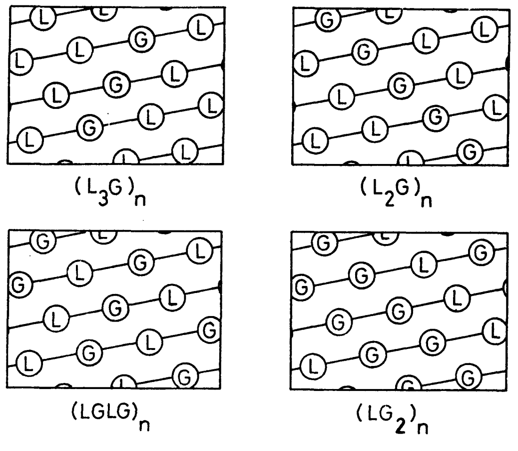

Fig. 8. Radial projection showing the distribution of the residues on the α-helix. L: leucine, G: glycine.

By considering a molecular model, we can see that two types of non-covalent interaction, one between the *i*-th and the (*i*+3)th residues and the other between the *i*-th and the (*i*+4)th residues, are possible in (L)$_n$. The magnitude of the energy of the non-covalent interaction should depend on the distance between the residues—the shorter distance would produce the larger interaction energy. We estimated the interatomic distances between the atoms of L-leucyl residues in the α-helix with the aid of a molecular model and deduced the following conclusion: the interaction between the *i*-th and the (*i*+3)th residues was the most effective, followed by the one between the *i*-th and the (*i*+4)th residues. Such interactions are not expected with polyglycine. The energy of the hydrogen bond would not be affected so much with a change in the side chain of an amino acid, provided that only amino acids which could form the α-helix were considered. However, the energy associated with the interaction between lyophobic side chains would depend significantly on the nature of side chains, and the larger the energy of the interaction, the more stable the α-helix would be. In this respect, eight kinds of side chain–side chain interaction are conceivable in the sequential polypeptides of L-leucine (L) and glycine (G) (see Fig. 8): L(*i*)–L(*i*+3), L(*i*)–G(*i*+3), G(*i*)–G(*i*+3), G(*i*)–L(*i*+3), L(*i*)–L(*i*+4), L(*i*)–G(*i*+4), G(*i*)–G(*i*+4), G(*i*)–L(*i*+4). It was concluded from examination of a molecular model that only three kinds of interaction were significant with the order of the interaction energy being L(*i*)–L(*i*+3)>L(*i*)–G(*i*+3)>L(*i*)–L(*i*+4). If we assume a value of 1.0 for the energy of interaction for L(*i*)–L(*i*+3), 0.4 for L(*i*)–G(*i*+3), and 0.1 for L(*i*)–L(*i*+4), then we could calculate the average interaction energy per residue for each of the polymers to be 1.1 for $(L)_n$, 0.7 for $(L_3G)_n$, 0.7 for $(L_2G)_n$, 0.3 for $(LG)_n$, and 0.3 for $(LG_2)_n$. This order of the interaction energies is in good agreement with the experimentally observed stabilities of the α-helix in the polymers.

Similar studies on the influence of glycine on the stability of the α-helix of other sequential polypeptides have been reported by us³⁾ and by Fraser *et al.*²⁾ Our previous study showed that the stabilities of the α-helix of sequential polypeptides containing L-alanine (A) and glycine were in the order: $(A)_n > (A_2G)_n > (A_3G)_n > (AG_2)_n$. In this case a small amount of the α-helix was observed for $(AG_2)_n$. Fraser *et al.* reported that the stabilities of the α-helix of their sequential polypeptides of γ-ethyl-L-glutamic acid (E) and glycine were in the order: $(E)_n > (E_5G)_n > (E_4G)_n > (E_3G)_n > (E_2G)_n > (EG)_n$.²⁾ These two results and our present results on $(L_xG_y)_n$ apparently contradict each other. The contradiction may, however, be eliminated if we consider that the nature and magnitudes of interaction between side chains of polypeptides are different in three series of sequential polypeptides. As discussed in the previous paper³⁾, the order of the α-helical stability of the polymers $(A_xG_y)_n$ was explained by interactions between the i-th L-alanyl residue and $(i+3)$th residue. Recently, Brack and Spach reported that the α-helical stability of their sequential polypeptides of L-alanine and glycine in solution were in the order: $(A)_n > (A_2G)_n > (A_2G_2)_n > (AG)_n > (A_3G_3)_n > (A_2G_3)_n > (AG_2)_n > (AG_3)_n$.¹⁶⁾ This order can be explained by our calculation with the exception of $(AG_2)_n$. For the polymers $(E_xG_y)_n$, interactions between the i-th and the $(i+3)$th residues and between the i-th and the $(i+4)$th residues are equally important because the γ-ethyl-L-glutamic acid side chain is long enough to make the contribution of the $(i+3)$th and the $(i+4)$th residues on the i-th residue equal. If we assumed a value of 1.0 for the interaction energy for $E(i)-E(i+3)$ and $E(i)-E(i+4)$, and 0.4 for $\mathrm{E}(i)-\mathrm{G}(i+3)$ and for $E(i)-G(i+4)$, we could calculate the average energy of the interaction to be 2.0 for $(E)_n$, 1.5 for $(E_5G)_n$, 1.4 for $(E_4G)_n$, 1.35 for $(E_3G)_n$, 1.1 for $(E_2G)_n$, and 0.7 for $(EG)_n$. This order of the interaction energy is in good agreement with their experimental results.

We wish to thank Professor F. Oosawa of Nagoya University and Professor T. Ooi of Kyoto University for their encouragement and discussions. We are greatly indebted to Dr. A. Warashina for his kind gift of poly-L-leucine. Thanks are also due to Professor K. Yamasaki of Nagoya University who kindly made available an ORD-CD spectrometer for the experiment.

References

1) P. Doty and W. B. Gratzer, "Polyamino Acids, Polypeptides, and Proteins," ed. by M. A. Stahman, University of Wisconsin Press, Madison, Wis. (1962), p. 111.

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

3) T. Iio and S. Takahashi, This Bulletin, **43**, 515 (1970); T. Iio, *Biopolymers*, **10**, 1583 (1971).

4) a) S. E. Ostroy, N. Lotan, R. T. Ingwall, and H. A. Scheraga, *Biopolymers*, **9**, 749 (1970). b) C. R. Snell and G. D. Fasman, *ibid.*, **11**, 1723 (1972). c) P. Y. Chou, M. Wells, and G. D. Fasman, *Biochemistry*, **11**, 3028 (1972).

5) T. Iio and S. Takahashi, This Bulletin, **47**, 2720 (1974).

6) J. H. Bradbury and M. D. Fenn, *J. Mol. Biol.*, **36**, 231 (1968).

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

8) A. Warashina, T. Iio, and T. Isemura, *Biopolymers*, **9**, 1445 (1970).

9) G. Holzwarth and P. Doty, *J. Amer. Chem. Soc.*, **87**, 218 (1965).

10) T. Miyazawa and E. R. Blout, *ibid.*, **83**, 712 (1961).

11) a) K. Nagayama, *Tanpakushitsu Kakusan Koso*, **17**, 653 (1972). b) P. Combelas, G. Garrigou-Lagrange, and J. Lascombe, *Biopolymers*, **12**, 611 (1973).

12) A. Yaron, E. Katchalski, A. Berger, G. D. Fasman, and H. A. Sober, *Biopolymers*, **10**, 1107 (1971).

13) L. Pauling, R. Corey, and H. Branson, *Proc. Nat. Acad. Sci. U.S.A.*, **37**, 205 (1951).

14) G. Nemethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).

15) M. Bixon, H. A. Scheraga, and S. Lifson, *Biopolymers*, **1**, 419 (1963).

16) A. Brack and G. Spach, *ibid.*, **11**, 563 (1972).