Regular Polypeptides of Glycine and L-Alanine

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Our papers related to the regular sequential polypeptides are summarized. The following polypeptides having a repeating sequence of glycine and L-alanine were synthesized and studied: $(Ala)_n$, $(Ala_3Gly)_n$, $(Ala_2Gly)_n$, $(AlaGly_2)_n$, $(D,L-Glu)_n-(Ala_2Gly)_{10}-(D,L-Glu)_n$, $(D,L-Glu)_n-(Ala_2-Gly)_6-(D,L-Glu)_n$, $(D,L-Glu)_n-(Ala_3Gly)_7-(D,L-Glu)_n$, $(D,L-Glu)_n-(Ala)_n-(D,L-Glu)_n$. With the aid of IR and ORD analysis, all the polymer were shown to assume the α-helical structure (partly for some of the polymer) in solution, and the stability of the α-helix of these polymers was in the order: $(Ala)_n>(Ala_2Gly)_n>(Ala_3Gly)_n>(AlaGly_2)_n$. The result was explained in the term of the hydrophobic interaction between side chains of L-alanine or glycine which are regularly arranged on the surface of the α-helix. The reason, why these approaches to understanding the protein conformation are thought desirable, is discussed.

I. INTRODUCTION

Structural and functional expression of a protein is generally governed by the sequence of amino acids in that protein, or in other word, by a primary structure of a protein.[1] Each amino acid in a protein should play a proper role when a protein is folded into a definite conformation. It has been unquestionably one of main problems of protein chemistry to study a role of an amino acid or that of a sequence of amino acids upon the conformation of protein.

Many studies have been made with homopolypeptides, which are polymers containing only one kind of amino acid, to reveal the relation between species of amino acids and the resulted secondary structure. Another attempts with homopolypeptides were to see the nature of stabilizing factor, or factors which governed the stability of secondary structure. Many basic conclusions have been derived in these approaches and proved to be useful for the purpose of investigation of the secondary structure of natural proteins, their application, however, is surely limited to only simple cases.[2]

As the next step following the study of homopolyaminoacids, attention should be pointed toward regular copolypeptides having a repeating sequence of amino acids of more than two kinds. Interactions between side chains of amino acids in polypeptides are so much complicated that such studies of copolypeptides which are still infrequent in numbers at the present time are desired and will be promising. Today, we have only few works directed in this way: poly-(L-Ala-Gly) as a model of silk fibroin,[3] regular copolypeptides of glycine, L-alanine, and L-proline as that of collagen,[4] and regular copolypeptides containing L-gultamic acid and glycine.[5]

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Several years ago, we began to work with regular copolypeptides of glycine and L-alanine and obtained interesting results, which are summarized here, on the relationship between the sequence of these amino acids and the resulting secondary structure of these copolypeptides.[6]

It is well known that poly-L-alanine forms a stable α-helix in various organic solvents as in aqueous media,[7] while polyglycine does not. Furthermore, glycine behaves destructive toward an α-helix when it was incorporated into polypeptides.[5c,8] The study of copolypeptides, $(\text{L-Ala}_x \text{Gly}_y)_n$, which are regularly sequential polymers of these two amino acids, was thus expected to reveal the relationship between an amino acid sequence and a secondary structure, in the simplest manner.

II. $(\text{L-Ala}_x\text{-Gly}_y)_n$

II. 1. Synthetic Works[6a]

Synthesis of a desired repeating unit, $\text{L-Ala}_x\text{-Gly}_y$, was achieved according to the method usually employed in the field of peptide syntheses: an amino acid whose amino group was protected with a benzyloxycarbonyl group was coupled with another amino acid whose carboxyl group was protected by esterification. As a coupling method for peptide bond formation, we chose an azide coupling method[9] since this method was generally accepted as the most probable one that could be used with the slightest danger of racemization of amino acids in concern. In our case, the amount of racemization of L-alanine in the polymers was found to be within 5% even if it had occurred.[*] A repeating unit was then successfully polymerized through a p-nitrophenyl ester method.[5b,10] The whole process for the synthesis of $(\text{L-Ala}_2\text{-Gly})_n$ is illustrated in Fig. 1 as an example.

In these experiments, it will be interesting to note that the ethyl ester of z-L-Ala-Gly$_3$ was found to be extremely resistant to an alkaline hydrolysis in the standard condition. Hydrolysis was achieved only under the most severe condition where already no N-benzyloxycarbonyl group remained without destruction, and we abandoned

Table 1.

| | Amino Acids Analysis | | Elementary Analysis | | | | | | Intrinsic Viscosity*) $[\eta]_{int}$ |
| | | | Calcd. | | | Found | | | |
	Ala	: Gly	C	H	N	C	H	N	
$(A)_n$		—	—	—	—	—	—	—	1.80
$(A_3G)_n$	2.98	: 1.0	48.88	6.71	20.73	47.32	6.80	19.52	0.15
$(A_2G)_n$	2.01	: 1.0	48.23	6.58	21.10	47.21	6.27	19.98	0.294
$(AG_2)_n$	0.99	: 2.0	45.40	5.99	22.69	45.49	5.79	22.67	0.235

*) 25°C, in dichloroacetic acid.

*) Optical rotatory dispersion curve of hydrolysate of a polymer (hydrolysis in 6N HCl, 24 hrs, in refluxing xylene) in 1N HCl was measured in the range of 240-600 mμ and compared with that of a standard solution of L-alanine which was treated under the same condition. Two results coincide well within the limit of experimental error ($\pm$5%).

a hope to obtain (L-Ala-Gly₃)ₙ polymer after several trials though the use of *t*-tert. butyl ester was still remained as a possibility.

The following polymers were synthesized: (L-Ala₃-Gly)ₙ, (L-Ala₂-Gly)ₙ, and (L-Ala-Gly₂)ₙ.[*] Homopolymer, poly-L-alanine, was synthesized by the condensation of *N*-carboxyanhydride of L-alanine.[11] Table 1 listed some of the analytical values of those polymers, where fairly large values of $[\eta]_{int.}$ in dichloroacetic acid should be noticed as they indicated the degree of polymerization of these polymers was high.
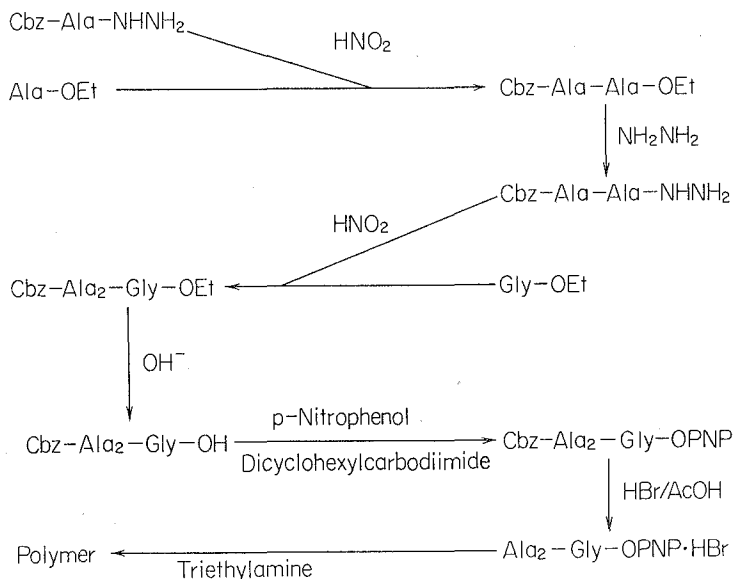

Fig. 1. Synthetic steps of sequential polypeptide, $(A_2G)_n$.

Abbreviations :
Cbz, Benzyl-oxycarbonyl.
PNP, p-Nitrophenyl-.

II. 2. Optical Rotatory Dispersion Studies

Recently, Doyle *et al.*[12] reported that their poly-A₂G could be partially dissolved in aqueous media, our polymers, however, were only soluble in dichloroacetic acid or trifluoroacetic acid. The apparent discrepancy might be explained by the difference in molecular weights of polymers, because the molecular weight of Doyle's polymer which was soluble in water was about 2,200, on the other hand, those of our samples were at least 20,000. Low solubility of polymers in aqueous media forced us to work with exclusively organic solvent systems, polymers dissolved in dichloro- or trifluoro-acetic acid were diluted with an adequate amount of organic solvents (chloroform or methylenechloride) to yield the solution of desired concentration and solvent composition. In the later section, a method to overcome the difficulty is presented.

The values of ORD were represented by an optical rotation per mean molecular wight of residues including glycine (in the case of polymers which contain glycine,

[*] Abbreviations: $(A_3G)_n = (Ala_3Gly)_n$; $(A_2G)_n = (Ala_2Gly)_n$; $(AG_2)_n = (AlaGly_2)_n$.

there are still no agreement among workers how to treat glycine residue that is optically inactive).[13] ORD curve in the range of 260-450 mμ was used to evaluate Moffit's parameters, a_0 and b_0, by means of Moffit-Yang plot for each polymer, where 212 mμ was used as λ_0.[14] If a polypeptide had a regular conformation in space, b_0 is generally expected to be non vanishing,[15] and this is obviously the case with our polymers which showed negative b_0. Variations of b_0 according to a change of solvent composition are shown in Fig. 2.

It is difficult to deduce the helical content in each polymer from only the b_0 value. Each polymer could assume an α-helix, also the possibility of existence of β-structure cannot be ruled out since poly-glycine forms a stable β-structure in a solid state. Thus, methods other than ORD were required.
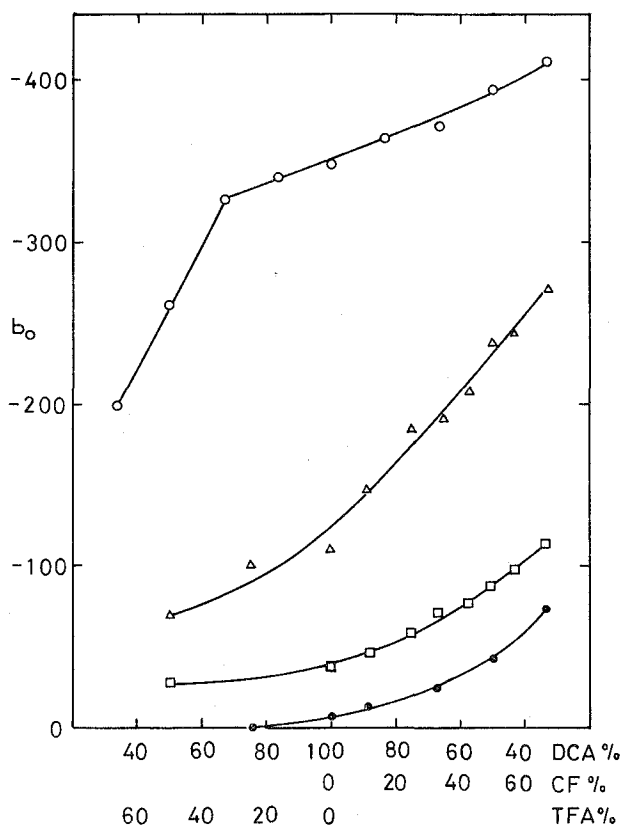

Fig. 2. Variation of ORD parameter b_0 with solvent composition in chloroform (CF)-dichloroacetic acid (DCA)-trifluoroacetic acid(TFA) system.
—○—○ : $(A)_n$,　　—□—□— : $(A_3G)_n$,
—△—△ : $(A_2G)_n$,　　—●—●— : $(AG_2)_n$.

II. 3. Infrared Spectroscopy

IR spectroscopy has provided much evidences on structural assignment of the secondary structure of polypeptides, and was proved also to be helpful to the present study. Figure 3 shows the IR spectra of the sequential polymers in dichloroacetic

acid-chloroform. As clearly seen in Fig. 3, all the polymers exhibited not only a peak at 1650 cm^{-1}, the amide I band assigned to an α-helix, but also a peak at 1630 cm^{-1}, which was usually assigned to a β-structure.[16] Furthermore, it is also evident that the ratio of α-helix to β-structure is different from polymer to polymer: relative content of α-helix to β-structure decreases in the order of $(A)_n$, $(A_2G)_n$, $(A_3G)_n$, and $(AG_2)_n$.[*]
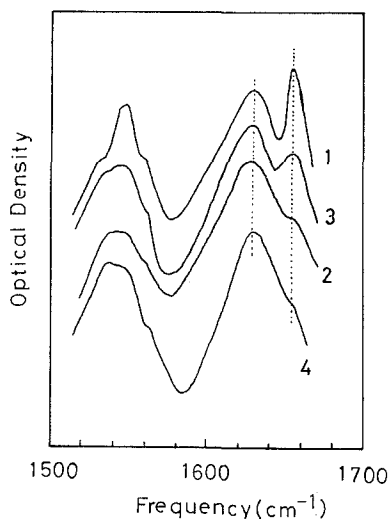

Fig. 3. IR spectra of sequential polypeptides in 75% chloroform-25% dichloroacetic acid system.

(1) : $(A)_n$,　　(2) : $(A_3G)_n$,　　(3) : $(A_2G)_n$,
(4) : $(AG_2)_n$.

II. 4.　Analysis of a_0 and b_0

When Moffit and Yang proposed their ORD theory on the polypeptide conformation, b_0 was considered to be inherent in an α-helix. Recently, however, it was experimentally revealed that some polypeptides, which were proven to be exclusively in the β-form by other means than ORD, had b_0 being not zero.[**] So might we not expect, strictly speaking, to have an unambiguous conclusion on the secondary structure of polypeptides merely on the basis of an apparent value of b_0 in cases where the β-form coexisted with an α-helical region. In such a case, contributions of both α- and β-forms on a_0 and b_0 should be taken into account as in the following expression:

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

where suffix R, H, and β correspond to contributions of random coil, α-helix, and

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[*] Equal intensities have been observed for amide I bands of α-helix and that of β-structure.[17]

[**] Almost all of polypeptides in the β-conformation have b_0 being zero, poly-γ-benzylglutamate of low molecular weight, however, was shown to have large b_0, 420[18] A correlation between a_0 and the content of β-form has not yet been established.

Table 2. Contents of the α-Helix and β-Structure in the Sequential Polypeptides.

	67% CHCl₃-33% DCA		100% DCA	
	f_H	f_β	f_H	f_β
$(A)_n$	65 %	51.9%	55.1%	56.0%
$(A_2G)_n$	18.0	38.0	17.5	16.1
$(A_3G)_n$	43.0	20.9	7.5	33.0
$(AG_2)_n$	12.0	22.1	1.5	20.7

β-form on a_0 and b_0, respectively, f_H is the fraction of α-helix, f_β is that of β-structure. A precise value of helical content for each polymer would then be obtained, in principle, according to the above equation provided that exact values for a_0^R, a_0^H, b_0^H, a_0^β, and b_0^β were established among polypeptides. Although there are still a lot of difficulties to estimate these parameters unambiguously in these days, a reasonable conclusion, being consistent with the results of IR studies described above, was obtained in this approach as follows.

There was no problem to accept the values of 680 and -630 for a_0^H and b_0^H, respectively, since almost all the values reported for α-helical polymers in various solvents fall closely in these values,[15] on the other hand, considerable amounts of discrepancies are found among values of parameters for the β-structure, especially for a_0^β.[18,19] We tentatively assumed 700 for a_0^β, which was the mean value of reported a_0's for polymers being exclusively in β-form, and $b_0^\beta=0$ on the theoretical basis of ORD as predicted by Volkenstein[20] coupled with many experimental results showing $b_0=0$ with the most polypeptides in β form in spite of an exception as mentioned previously. a_0^R was experimentally determined for each polymer as the a_0 value obtained in trifluoroacetic acid as a solvent where b_0 was found to be nearly zero. The values of f_H and f_β calculated on these basis are to be found in Table 2, where some uncertainties might be present particularly with f_β, and it forced us to participate with f_H alone. If we accept an idea of $b_0^\beta=0$, b_0 plots appeared in Fig. 2 should represent the change of contents of α-helix with solvent composition, and should give the relative stabilities of α-helical region in these polymers as in the order of $(A)_n$, $(A_2G)_n$, $(A_3G)_n$, and $(AG_2)_n$, which was the same with the result of IR studies (see Fig. 3). The most interesting observation is concerned with the stability order of α-helices of these polypeptides: the stability of an α-helix is not simply correlated with a content of L-alanine (the stability of an α-helix was greater for $(A_2G)_n$ than for $(A_3G)_n$ whereas the alanine content was higher in the latter) but a sequence of glycine and L-alanine is significantly important to determine the stability of an α-helix.

These results lead us to the conclusion that the stability of an α-helix is much higher in a polymer in which glycine occupied the i-th and the $(i+3)$ th residue positions repeatingly (the case corresponded to $(A_2G)_n$), than a polymer where the relation was the i-th and the $(i+4)$ th (corresponded to $(A_3G)_n$). Both of these two types of glycine-glycine interaction are found in $(AG_2)_n$, the least stable polymer.

II. 5. Discussions

The conclusion on the stability of an α-helix mentioned above was obtained mainly through the inspection of b_0's, where the independency of b_0 on molecular

weight was assumed. It has been questioned on reliability of this assumption since the dependence of b_0 on polymer molecular weight was proved theoretically[21] and experimentally[22] when the polymer molecular weight was "relatively low" (generally, more reduced b_0 value for a polymer of lower molecular weight). Molecular weights of our polymers would be judged sufficiently high in normal sense, and hopefully to be in a region where b_0 might be independent from the influence of molecular weight. But a possibility that our polymers still fall into the range of molecular weights on which b_0 values depend could not be eliminated, as we have no knowledge on the upper limit of molecular weight where b_0 might escape from the influence of molecular weight. Situation may become serious if we call the fact that the molecular weight of $(A_3G)_n$ is quite likely to be lower than that of $(A_2G)_n$.

As the second point of matter, we must take into consideration the fact that rather limited numbers of solvent composition were available in estimation of the value of b_0's. All measurements were carried out exclusively in solvents containing dichloro- or trifluoro-acetic acid owing to insolubility of our polymers in other common organic solvents or in water.

To the third, as seen in IR spectra, our polymers assume the β-conformation in solution as well as α-helix. Unfortunately, our knowledge upon how β-structure contributes to b_0 is quite poor, leading an ambiguous calculation of α-helical content of the polymer from b_0.

The way to find a way out of these problems and to get a deeper idea than that decribed in II. 4 is: (1) to estimate an exact amount of the fraction of each structure in a polymer by some means if possible; (2) to use somewhat new materials to avoid difficulties discussed above. (1) According to the present status of the polymer physical chemistry, structure of a polymer might be established fairly precisely by the analysis of CD or ORD spectrum of a polymer in solution. For such purpose, however, CD or ORD spectrum must be measured until 180mμ, and a proper spectrum of each structure in a polymer must be known. Measurement of CD or ORD spectrum below 260 mμ is extremely difficult for our polymers described above, owing to the reason that dichloro- or trifluoro-acetic acid as a solvent has large extinction coefficient in this region, while an exact spectrum of each structure is remained concealed as long as an experimental condition where the polymer assumed only one conformational structure had not been established. Regular polymers described above were clearly inadequate in these aspects and forced us to leave themselves and consider another type of polymers. (2) If we restart the work with a new type of polymers, two problems should be solved in these polymers: one is related to the homogeneity of molecular weight of polymers, the other to the solubility of polymers in common solvents, desiringly in water. Because technics usually applied for peptide synthesis could not afford polymers whose molecular weights were homogeneous, we chose the so-called solid-phase synthetic method[23] to prepare desired polymers. Solid-phase synthesis of polypeptides, which was developed by Merrifield, is relatively cumbersome method but promised the yield of regular polymers, having a regular sequence and the homogeneous molecular weight provided that the method went well, then we will be able to study the dependence of b_0 on molecular weight. Polymers would be solubilized in water by introducing a block of D,L-glutamic acid to both end of $(L-Ala_xGly_y)_n$ chain, here the solid-phase method would be well suited, too.

The answer to a problem of obtaining a series of polymers consisted from a single conformational structure was not predicted without experiments but eventually was found to be satisfied with our new polymers, which were shown to be free from the β-structure as described below. In such case, discussion on the stability of α-helices could be conducted without perturbation induced by the presence of β-structure. The role of charged portions (poly-D,L-Glu) in a polymer acting as a hindrance toward the formation of inter- or intra-molecularly β-pleated sheets will be discussed in the later section.

III. BLOCK SEQUENTIAL POLYPEPTIDES OF L-ALANINE AND GLYCINE WITH D,L-GLUTAMIC ACID[6c]

III. 1. Preparation of the Polymer

For the syntheses of block sequential polypeptides consisted from a central core, in which glycine and L-alanine were regularly arranged in various manners, and from both wings of poly-D,L-glutamic acid, were achieved by the Merrifield's solid-phase synthetic method[23,24] coupled with the N-carboxyanhydride method.[11]

Polystyrene beads (crossly linked with 2% of divinylbenzene) were reacted with chloromethyl-methyl ether to afford copoly-chloromethylstyrene-divinylbenzene[23] on which L-alanine was fixed with its carboxyl end. When the L-alaninous resin was added to a solution of N-carboxyanhydride of D,L-glutamic acid, an amino group of L-alanine which was fixed on resin by its carboxyl group behaved as an initiating nucleophile to result polymerization of D,L-glutamic acid on it.[22a] Then, L-alanine or glycine was coupled in a desired sequence succeedingly with the aid of dicyclohexylcarbodiimide[24] on an amino terminal of poly-D,L-glutamic acid block, finally N-carboxyanhydride of D,L-glutamic acid to form the N-terminal wing of poly-D,L-glutamic acid. Polypeptides were liberated from the resin by the treatment with hydrobromic acid and dissolved in water to separate from the insoluble materials. Final yields of polypeptides were 12–15% calculating from the first L-alanine. Incorporation of amino acids in each step was quantitatively traced by amino acid analysis which was carried out on a completely acid hydrolyzed sample detouched from resin after each reaction cycle had finished. The results were as satisfactory as shown partly in Fig. 4.

The degree of racemization in the course of polypeptide formation was evaluated as follows. ORD curve of a solution of hydrolyzed polymer, whose L-alanine concentration was determined by amino acid analyzer, was compared with that of the standardized solution of L-alanine itself. The result, both curves agreed well together within maximum error of 7%, means the coupling reaction of L-alanine by dicyclohexylcarbodiimide proceeded without racemization, moreover, the incorporation of glutamic acid into polymers occurred evenly in the respect of D- and L-. Polymers eventually we submitted to further experiments are: (D,L-Glu)$_{23}$-(L-Ala$_2$Gly)$_{10}$-(D,L-Glu)$_9$-(L-Ala), (D,L-Glu)$_{19}$-(L-Ala$_2$Gly)$_6$-(D,L-Glu)$_9$-(L-Ala), (D,L-Glu)$_{59}$-(L-Ala$_3$Gly)$_7$-(D,L-Glu)$_{11}$-(L-Ala), and an additional one, (D,L-Glu)$_n$-(L-Ala)$_{38}$-(D,L-Glu)$_n$[*] which

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[*] They will be denoted as DL(A$_2$G)$_{10}$DL, DL(A$_2$G)$_6$DL, DL(A$_3$G)$_7$DL, and DL(A)DL, respectively.

was obtained via polycondensation of *N*-carboxyanhydride.[7] The reason why the polymerization degree of the central core of these block polypeptides was low in each case came from the relatively small size of net works in copoly-styrene-divinylbenzene resin, which restricted the number of polymerization nearly 100 at the largest. The differences in the number of D,L-glutamic acid contained in both wings of a polymer will be attributed to the possibility that the resin gradually loses its portion by its mechanical breakage to result lesser amount of the reactive center in the later steps of synthesis and the content of glutamic acid incorporated in *N*-terminal side of the polymer is necessarily higher as a consequence. In any way, wings of D,L-glutamic
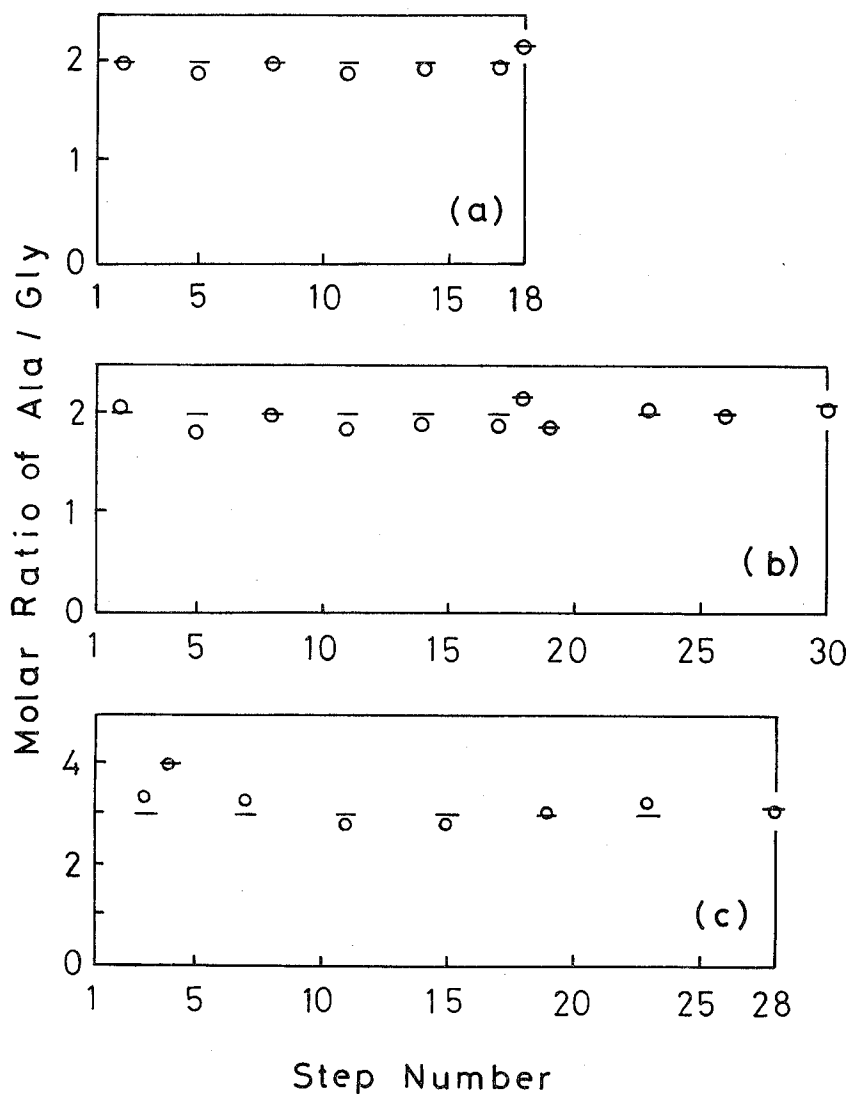

Fig. 4. Analytical data for synthesis of block sequential polypeptides. Each short horizontal line indicates calculated value of molar ratio of alanine to glycin in each step.
(a) : DL(A$_2$G)$_6$DL,   (b) : DL(A$_2$G)$_{10}$DL,   (c) : DL(A$_3$G)$_7$DL.

acid have no meanings other than a solubilizer of polymers in water and contributed nothing on the secondary structure of a central core.

Another good alternative route to synthesize regular polymers should be mentioned: the use of repeating unit of oligopeptides in the solid-phase method. Recently the method is successfully applied in some cases,[4d] but the syntheses of repeating units, oligopeptides, are too much laborious to work with as we experienced in the preceding study and was abandoned.

III. 2. Solution Studies

The following studies of the four block polymers were carried out mainly in water-methanol, water-ethanol, or in dichloroacetic acid-chloroform, with an expectation that ORD measurements might be extended until 200 mμ in these solvents except that containing dichloroacetic acid. To be regrettably, optical rotations of polymers were too small and large extinction coefficient of peptide groups in the shorter wave-
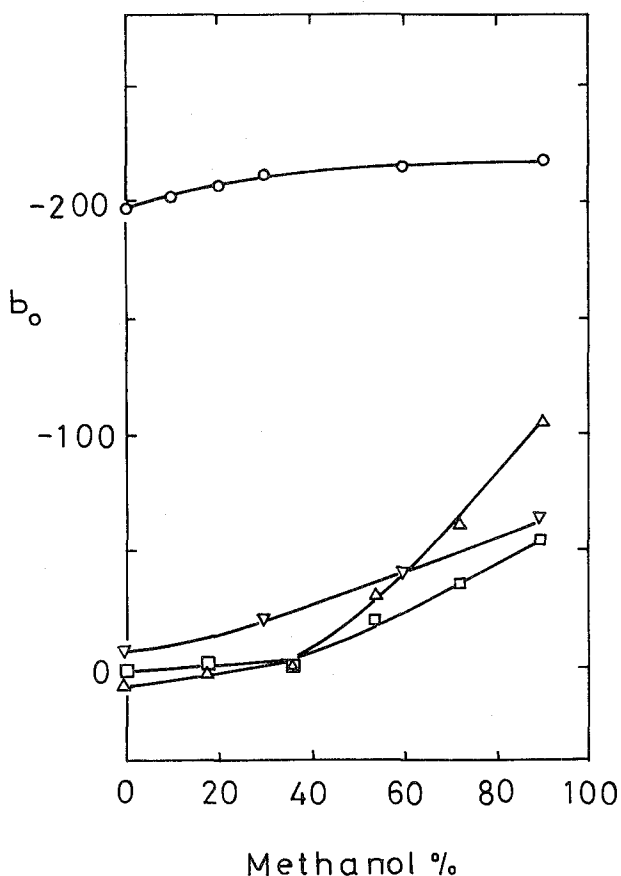

Fig. 5. Variation of ORD parameter b_0 with methanol content in water-methanol system.

—◯—◯— : DL(A)DL,   —□—□— : DL(A₃G)₇DL,
—△—△— : DL(A₂G)₁₀DL, —▽—▽— : DL(A₂G)₆DL.

(89)

length region inhibited observation of well defined curves.[*] It leads us to work again with b_0

In the study of solutions in water or water-methanol, b_0 value was found to be independent from the change of degree of dissociation of γ-carboxyl group in glutamic acid, which was caused by changing pH of the solution, leading to a conclusion that blocks of D,L-glutamic acid did not affect the conformation of the polymer. The degree of dissociation was changed from 0.1 to 1.0 in water, from 0.35 to 1.0 in 90% methanol. Dependence of b_0 values on solvent compositions are shown in Fig. 5 for water-methanol system. b_0 of DL(A)DL remained almost constant throughout the solvent condition, whereas b_0's of the other block regularly sequential polymers not. b_0 values for the latter are nearly zero in water, increasing of their absolute value with increasing content of methanol which is a helical solvent. In a solvent
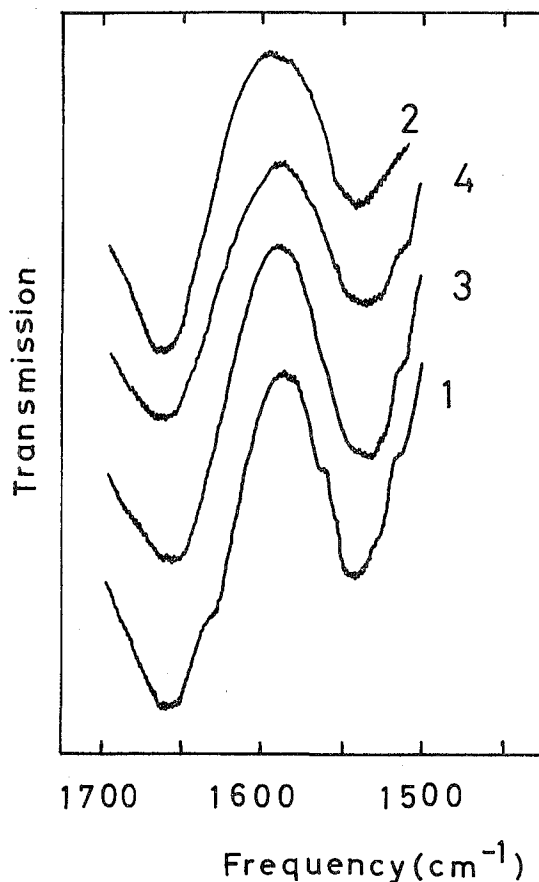

Fig. 6. IR spectra of block sequential polypeptides in 95% chloroform-5% trifluoroacetic acid system.

　　(1) : DL(A)DL, (2) : DL(A$_3$G)$_7$DL, (3) : DL(A$_2$G)$_{10}$DL,
　　(4) : DL(A$_2$G)$_6$DL.

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[*] For example, molecular rotation was -1400 at 230 mμ for the 37.3 mmole/l. Solution of DL(A$_2$G)$_6$DL, on the other hand, optical density was high as 1.35 (optical path length 0.2 mm).

which contains a large fraction of methanol, absolute values of b_0 are in the decreasing order as DL(A)DL, DL(A$_2$G)$_{10}$DL, DL(A$_2$G)$_6$DL, and DL(A$_3$G)$_7$DL.

The regular polymer of (II. 1) showed a large contribution of β-structure as discussed in (II. 3). On the contrary, IR studies of block sequential polymers in chloroform-trifluoroacetic acid (95:5 in volume) which was considered to be a helical solvent revealed an important result of almost no β-structure in these polymers but DL(A)DL. Even the polymer DL(A)DL, an absorption at 1630 cm^{-1} assigned to the β-structure was very low. This observation coupled with the evidence of the β-structure in poly-L-alanine suggested that a small amount of the polymer DL(A)DL might lack one of two blocks of D,L-glutamic acid.

All block polymers showed large absorption at 1650–1660 cm^{-1} as in Fig. 6 which were assigned to that originated[16] from an α-helix of the sequential central core and that caused by random coils of D,L-glutamic acid blocks.[25]

As the existence of β-structure in these polymers was excluded, b_0 change appeared in Fig.5 should be attributed to a change of α-helical content in these polymers with a change of solvent composition. DL(A)DL has been shown to assume an α-helix in water by many workers. Smaller b_0 value of our sample to reported one might be ascribed to a low degree of polymerization, among literatures, we can find an example of Ingwall,[22a] b_0 was -525 for poly-L-alanine of DP (degree of polymerization) =450, -400 for that of DP=160. Such dependency of b_0's on molecular weight is shown theoretically, too,[21] and will explain the lower value with DL(A$_2$G)$_6$DL than that of the higher homologue, DL(A$_2$G)$_{10}$DL.

It is quite interesting to note all of our regular sequential polypeptides do not form an α-helix in water. This might be attributed to an integration of glycine residues in a polypeptide chain, introducing much flexibility in a peptide backbone.[13b] Increasing amount of methanol, a solvent which could stabilize an α-helical conformation, increased a fraction of α-helix in polymers, and the differences in α-helical contents for each of polymers were considered to be reflecting the stability of an α-helix in a respective polymer. As is seen in Table 3 which listed b_0's in several solvent systems, the stability of α-helix decreases in the order of DL(A)DL, DL(A$_2$G)$_{10}$DL, DL(A$_2$G)$_6$DL, and DL(A$_3$G)$_7$DL, the same order as found for polymers without blocks of D,L-glutamic

Table 3. b_0 Values of the Sequential Polypeptides[a].

	water	90% methanol	60% ethanol	DCA	70% chloroform
DL(A)DL	−197	−218	−210	−133	−195
DL(A$_2$G)$_{10}$DL	5	−106[b]	−75	−29	−122[c]
DL(A$_2$G)$_6$DL	−6	−64[b]	−47	0	−53
DL(A$_3$G)$_7$DL	1	−55[b]	−19	0	−54
(A)$_n$	—	—	—	−347	−408
(A$_2$G)$_n$	—	—	—	−109	−270
(A$_3$G$_n$	—	—	—	−37	−115

a) b_0 values per mean residue.
b) b_0 values per L-alanyl residue.
c) in 53% chloroform.

acid. For both of two types of polymers the stability of α-helix is not parallely related with L-alanine content: polymer DL(A_3G)$_7$DL or (A_3G)$_n$ having much abundant L–alanine has less stable α-helix than DL(A_2G)DL or (A_2G)$_n$. Furthermore, the fact that DL(A_2G)$_6$DL had higher b_0 than DL(A_3G)$_7$DL, even DP was lower for the former, confirmed the above discussion.

III. 3. Amino Acid Sequence and Stability of an α-Helix

The preceding experimental results assured us a significant influence of an amino acid sequence played on the stability of an α-helix. In this section, we are going to discuss how L-alanine and glycine sequence affect the stability of an α-helix in the polymer which was composed from these two amino acids.

A representation of α-helices showing the radial projection of the distribution of the residues on the surface of α-helices is schematically illustrated in Fig. 7 for each of the polymers. Each residue position corresponds to the location of an α-carbon of an amino acid, not of side chain. If the residue in the center of each diagram is referred to as the 0 residue, it is convenient to number the successive residues, $+1$, $+2$, etc. in the -CO-C$_\alpha$-NH- direction, and -1, -2, etc. in the opposite one. In the
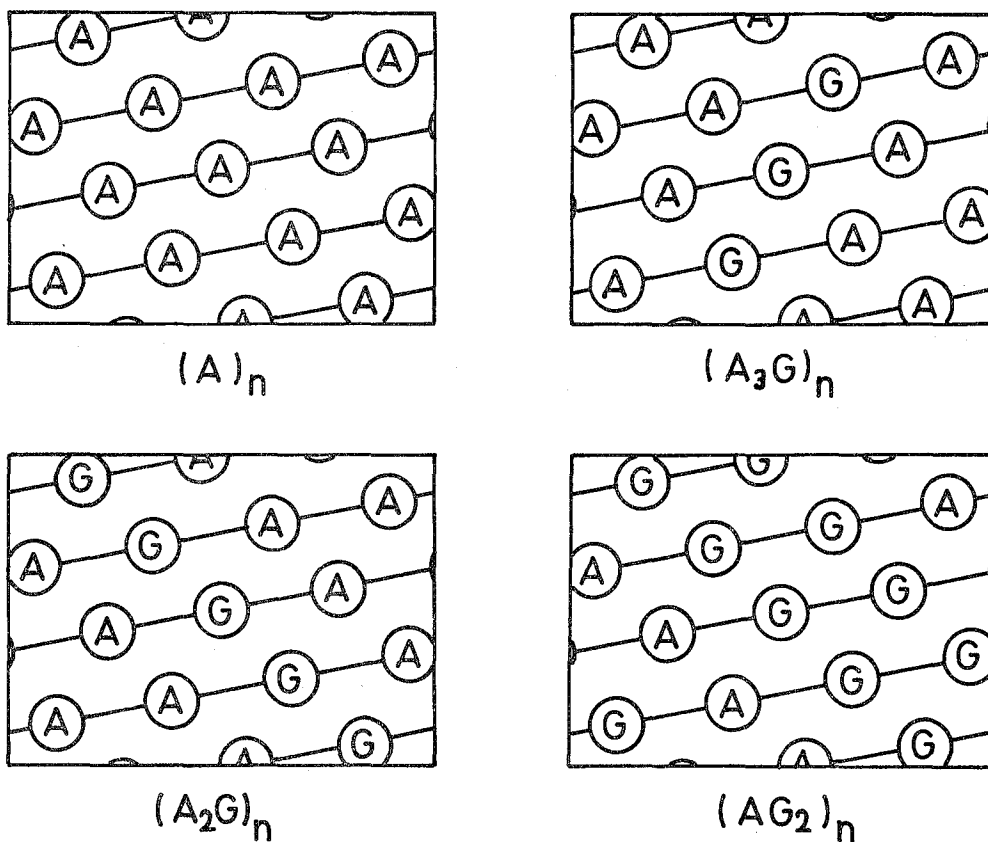

Fig. 7. Radial projections showing distributions of L-alanyl (A) and glycyl(G) residues on α-helices of sequential polypeptides.

(A₃G) polymer glycine residues are related as 0, ±4, ±8, *etc.* each other, while in the (A₂G) polymer, as 0, ±3, ±6, *etc.*

In addition to a hydrogen bond between the i-th and the $(i+4)$ th residue, a hydrophobic interaction between i-th and $(i+3)$ th residue has been taken into account to explain the remarkable stability of α-helix of poly-L-alanine.[26] Such hydrophobic interactions are not expected with poly-glycine. The energy of a hydrogen bond would not vary so much with changes of side chains of amino acids, provided that amino acids which could form an α-helix were concerned, on the contrary, the energy of a hydrophobic interaction produced by a hydrophobic nature of a side chain would depend significantly on side chains themselves, and larger the energy of hydrophobic interactions, the more stable α-helix would follow. In this respect, four kinds of side chain-side chain interactions are conceivable: A(0)-A(3), A(0)-G(3), G(0)-A(3), and G(0)-G(3). The first one would produce as much large interaction energy as in poly-L-alanine, the second one to some extent (the energy clearly smaller than the first), but the latter two would not (A(0)-G(3) and G(0)-A(3) are not in the same relationship, as will be seen by the molecular model). If we assume a value of 1.0 as the energy of a hydrophobic bond of a type A(0)-A(3), and 0.5 for A(0)-G(3), then we could calculate the average energy of hydrophobic interactions for each of the polymer as 1.0 for (A)ₙ, 0.62 for (A₃G)ₙ, 0.67 for (A₂G)ₙ, and 0.33 for (AG₂ₙ), respectively. The difference of values between (A₂G)ₙ and (A₃G)ₙ will become larger if we take a smaller value than 0.5 as the interaction energy of A(0)-G(3). The order of the interaction energy is in good agreement with that of the α-helical content of the polymer, noticing the study of regularly sequential polypeptides could reveal the nature and magnitudes of interaction between side chains of polypeptide.


IV. REGULAR POLYPEPTIDES OF γ-ETHYL-L-GLUTAMATE AND GLYCINE

In the study of polypeptides containing γ-ethylglutamate and glycine, Fraser *et al.*, who worked systematically with regular polypeptides for recent years,[5] reported the result being superficially contradicted to our conclusion.[5c] They synthesized the same type of polymers of us, viz. (Glu)ₙ, (Glu₃Gly)ₙ, (Glu₂Gly)ₙ, and (GluGly₂)ₙ, and analyzed the stability of α-helices of these polymers to deduce the order of the stability being directly related to the glycine content, namely the more stable α-helix with the lesser amount of glycine in a polymer. We believe the contradiction between their result and ours, however, could be eliminated in the following way. In the Glu-Gly polymers, the effective hydrophobic interactions between i-th and $(i+4)$ th residues are larger than that between i-th and $(i+3)$ th as suggested by Nemethy and Scheraga,[27] and this is also supported from an inspection of a molecular model. The situation is thus just the reverse of our alanine-glycine polymers which have the larger interaction between i-th and $(i+3)$ th. The same calculation of the interaction energy of side chains such as given in the preceding section delivered the value of 1.0 for (Glu)ₙ, 0.75 for (Glu₃Gly)ₙ, 0.33 for (Glu₂Gly)ₙ, and 0 for (GluGly₂)ₙ: the order is consistent with experimental results.

It will be adequate to comment on the influence of methanol (and probably of

ethanol, too) upon polymer conformation. Methanol has been recognized as a solvent which stabilized the α-helices of various polypeptides.[28] For instance, poly-L-valine incorporated between poly-D,L-lysine blocks assumed the β-conformation in water and transformed into the α-helix when methanol content increased.[28] In the case of poly-L-lysine, methanol stabilized the α-helix at higher temperature, preventing the conversion to the β-conformation.[29] In the present study, methanol also stabilized the α-helices of the sequential polypeptides. Epand and Scheraga suggested in their paper[28] that methanol destabilizes the side chain hydrophobic bonding in the β-structure of poly-L-valine. We believe that, in the cases of poly-L-valine and poly-L-lysine, methanol molecules cover the surface of the side chain arranging their OH groups so as to contact to another solvent molecules such as water and destabilize the side chain hydrophobic interaction which plays the role stabilizing the β-structure in aqueous solutions.

In the present case, the effect of methanol on stabilizing the α-helix of the sequential polypeptides may be due to the following manner: methanol expels water molecules bound to C=O and N-H groups of the polypeptide chain in randomly coiled conformation and shifts the following equilibrium to the right side,

$$\text{C=O}\cdots\cdots\text{H-O-H} + H_2O\cdots\cdots\text{H-N} \rightleftharpoons \text{C=O}\cdots\cdots\text{H-N} + 2H_2O,$$
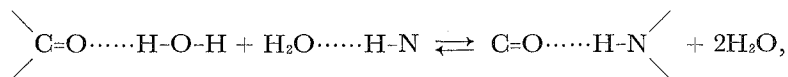
resulting the formation of the α-helix.

V. CONCLUDING REMARKS

Our studies clearly established the fact that the regularly sequential polypeptides behaved essentially different from homopolymers or random co-polymers, and the difference was ascribed to the regular interaction between amino acids which were incorporated into the polypeptide in the regularly repeating manner. We believe these studies would be very useful to reveal the way how an amino acid sequence of protein determines the conformation of protein and finally biological functions of protein.

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