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Monomer Reactivity Ratios in NCA Copolymerization of γ-Benzyl-L-glutamate with L-Leucine, L-Alanine, and L-Phenylalanine

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Copolypeptides composed of γ-benzyl-L-glutamate and L-leucine, L-alanine, or L-phenylalanine, covering the whole range of copolymer composition were synthesized by the N-carboxyanhydride (NCA) method initiated by triethylamine (TEA) in a 1:1 (v/v) mixture of dioxane and methylene dichloride, and the randomness of the sequential distribution of the resulting copolypeptides has been studied. In addition to the Kelen-Tüdös procedure, a differential method, a newer evaluation method using the original equation of Skeist has been used for determination of the monomer reactivity ratios. Since the reactivity ratios are known to be very sensitive to small variations in copolymer composition, four analytical methods have been employed for such determinations to minimize these variations. The results obtained by amino acid analysis, elemental analysis, ultraviolet and infrared spectroscopies are in good agreement with each other. Calculation of these monomer reactivity ratios by the Skeist equation gives values: r_1(γ-BLG)=2.65 and r_2(Leu)=0.38 for copoly(γ-benzyl-L-glutamate/L-leucine); r_1(γ-BLG)=2.36 and r_2(Ala)=0.50 for copoly(γ-benzyl-L-glutamate/L-alanine); and r_1(γ-BLG)=1.64 and r_2(Phe)=0.59 for copoly (γ-benzyl-L-glutamate/L-phenylalanine). This suggests that the copolymers are block-like rather than random. The change of the degree of the sequential distributions, *i.e.*, the heterogeneity, in these copolymer chains with increasing conversion has been studied.

INTRODUCTION

It is well known that copolymer properties can be profoundly influenced by the sequential distribution of each of the comonomer in copolymer chains. To estimate the monomer reactivity ratios, r_1 and r_2, the polymerization should be stopped generally at very low degree of conversion (approximately less than 5%) where the composition of the comonomer feed is not largely different from its initial value. Recently, Kelen and Tüdös[1] have proposed a simple graphically evaluable linear method for the determination of r_1 and r_2 by plotting the instantaneous copolymer composition versus the comonomer feed composition, *i.e.*, they determined r_1 and r_2 by fitting theoretical curve to the experimental data by trial-and-error selections of r_1 and r_2. This method not only provides accurate r values in many cases unobtainable by previous procedures, *e.g.*, Fineman-Ross method,[2] a method of intersections,[3] but also holds important clues as to the copolymerization mechanism.[4] On the other hand, to prepare copolymers of sufficiently high molecular weight by higher efficiency, it is desirable to allow copolymerization until higher conversion. For all copolymerizations except azeotropic copolymerizations, the comonomer feed composition is different from copolymer composition; *i.e.*, when two (or more)

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monomers are copolymerized, the product is a mixture of polymer molecules which vary in composition and sequential distributions as well as in chain length. The composition of comonomer feed changes, since one of the monomers preferentially enters into the copolymer. As regards high conversion copolymerization data, values of r_1 and r_2 have recently been determined from analysis of copolymer composition—conversion data. In order to determine the instantaneous copolymer composition as a function of conversion for any given comonomer feed, one must resort to an integrated form of the copolymerization equation. The most generally useful method is that developed by Skeist.[5] By using computational procedures, one determines the best values of r_1 and r_2 for the experimental data on the variation of copolymer composition with conversion.

In this paper, we concern with copolypeptides composed of γ-benzyl-L-glutamate(G) and L-leucine(L), L-alanine(A), or L-phenylalanine(P), each of these L-amino acids is α-helix former. It is very important to understand that the amino acid composition and sequence should play an important role in determining the conformational properties of proteins, especially, application of the concept of the effect of the aliphatic hydrocarbon side chains on the stability of the α-helical conformation in copolypeptide chains will aid in understanding the conformational behavior of proteins. Copolymers covering the whole range of copolymer composition were synthesized by the N-carboxyanhydride (NCA) method initiated by triethylamine (TEA) in a 1:1 (v/v) mixture of dioxane and methylene dichloride, and the randomness of the sequential distribution of the resulting copolypeptides has been studied. Both the Kelen-Tüdös procedure and the Skeist equation have been used for the determination of the monomer reactivity ratios. The calculation of sequential distribution in copolymer chains was performed by using the computational procedure as a function of the conversion of copolymerization.

EXPERIMENTAL

Materials

The monomers, N-carboxy-γ-benzyl-L-glutamate anhydride (G-NCA), N-carboxy-L-leucine anhydride (L-NCA), N-carboxy-L-alanine anhydride (A-NCA), and N-carboxy-L-phenylalanine anhydride (P-NCA) were prepared according to the method proposed by Blout and Karlson,[6] and purified by repeated recrystallizations from an ethyl acetate solution with the addition of petroleum ether. Each of G-NCA and L-NCA, A-NCA, and P-NCA, in desired mole ratio, was dissolved in a 1:1 (v/v) mixture of dioxane (DO) and methylene dichloride (MC). The total concentration of each NCA at the starting point of copolymerization was kept at 3 wt-%. The copolymerization was initiated with triethylamine (TEA) at an NCA-to-TEA molar ratio ([M]/[I]) of 50. The polymerization was stopped at about 25 mol-% conversion, and the course of the copolymerization was monitored by the titration of the carbon dioxide evolved. All solvents used for synthesis and the initiator were purified more than three times by the usual methods described in the literature. The copolypeptides formed were precipitated in a large amount of cold

Table I. Copolymerization Data

Sample No.	Initial monomer ratio (G mol-%)	Polymer composition (G mol-%)	Conversion (%)	$[\eta]$ (DCA, 25°C) (dl/g)
GL-1	80	90	25	0.88
GL-2	65	82	23	0.95
GL-3	50	70	25	1.05
GL-4	35	55	24	0.90
GL-5	20	36	23	0.88
GA-1	80	89	26	1.11
GA-2	65	79	25	0.98
GA-3	50	67	27	1.28
GA-4	35	51	25	0.85
GA-5	20	32	26	0.90
GA-6	10	16	25	0.78
GP-1	80	86	30	1.45
GP-2	67	76	28	1.32
GP-3	50	60	30	1.40
GP-4	33	43	29	1.67
GP-5	20	28	28	——
G	100	100	82	2.16
L	0	0	58	0.88*
A	0	0	65	0.75*
P	0	0	76	0.65*

* TFA, 25°C

methanol and dried under reduced pressure at 50°C. The composition of these copolypeptides was determined by four analytical methods; amino acid analysis, elemental analysis of C, H, and N-atom, ultraviolet and infrared spectroscopies. Results on all the copolymerizations are listed in Table I.

Measurements

The limiting viscosity number, $[\eta]$ (dl/g) of copolypeptides was determined in dichloroacetic acid (DCA) at 25°C using Ubbelohde type capillary viscometers. The experimentally determined average values of monomer composition in the copolymer and the composition of the initial monomer mixture, together with the limiting viscosity number, $[\eta]$, are summarized in Table I. Infrared spectra (IR) of solid films of the samples cast from chloroform (CF) or CF-trifluoroacetic acid (TFA) (80/20; v/v) mixture were measured with a Perkin-Elmer Model 521 Spectrophotometer in the region of 400 to 4000 cm^{-1}.

RESULTS AND DISCUSSION

Experimental Evaluation of Monomer Reactivity Ratios with Kelen-Tüdös Equation

Figure 1 illustrates the copolymer composition curves for the copolymerization

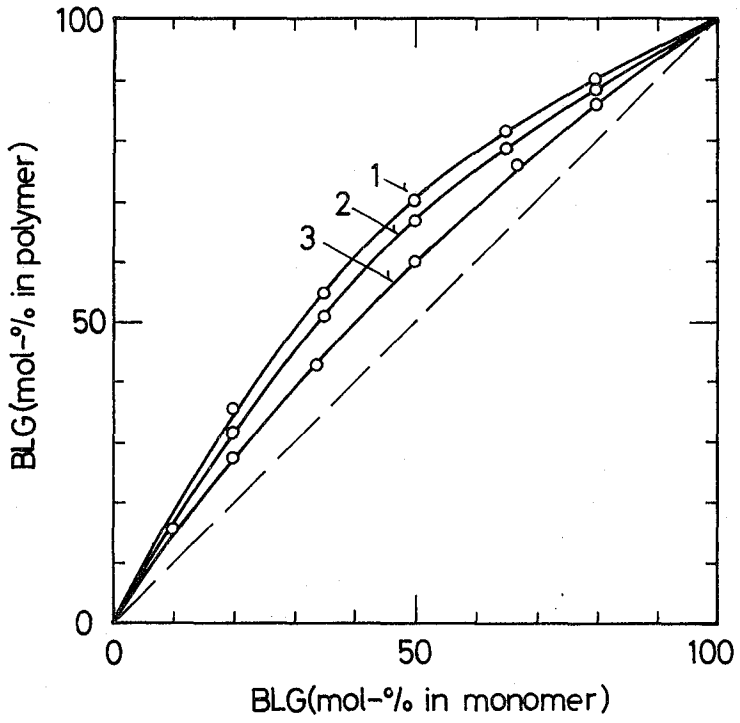

Fig. 1. Copolymer composition curves for: (1) copoly(γ-benzyl-L-glutamate/L-leucine); (2) copoly(γ-benzyl-L-glutamate/L-alanine); and (3) copoly(γ-benzyl-L-glutamate /L-phenylalanine) at about 25% conversion. Data are taken from Table I.

of G with L, A, or P at a conversion level around 23 to 30 mole-%, taken from the data in Table I. At first, to obtain roughly evaluated values of these monomer reactivity ratios and to test its applicability, the Kelen-Tüdös equation (1) was employed:

$$\eta=\left(r_1+\frac{r_2}{\alpha}\right)\xi-\frac{r_2}{\alpha} \tag{1}$$

where α is a suitably chosen parameter, and η and ξ are variables obtained from the charge and copolymer composition in accordance with Eqs. (2) and (3).

$$\eta=Y/(\alpha+X) \tag{2}$$

$$\xi=X/(\alpha+X) \tag{3}$$

where $X=F^2/f$, $Y=F(f-1)/f$, $F=M_1/M_2$, and $f=m_1/m_2$. M_1 and M_2 denote the molar concentrations in the monomer feed, and m_1 and m_2 the same in the final copolymer. Indeed if the η versus ξ plot is linear for a binary copolymerization, the well known copolymerization composition equation holds and the simple two-parameter model is a satisfactory approximation to describe the system quantitatively. However, if the plot is curved a more complicated situation arises, and the simple two-parameter model which provides the foundation of the copolymerization com-

position equation can not hold. A uniform spread of the experimental data may be attained by proper choice of the α value, and the parameter α is suitably obtained by:

$$\alpha=\sqrt{F_m \times F_M} \tag{4}$$

where F_m and F_M are the lowest and highest F values, respectively, in the experimental data. By plotting η versus ξ according to the linear Eq. (1), we can obtain directly r_2 (*i.e.*, r_2/α) and r_1 from the intercepts at $\xi=0$ and $\xi=1$, respectively. The intercepts can be readily obtained either graphically or by computation using the least squares method.[1] The η versus ξ plot gives a straight line between 0 and 1 provided that the system can be adequately described by the conventional copolymerization composition equation:

$$f=F \cdot \frac{1+r_1 F}{r_2+F} \tag{5}$$

Kennedy, *et al.*[4], defined three classes; symbols I, II, and III, to indicate the measure of reliability and applicability of the copolymerization systems to the conventional copolymerization equation (5).

Symbol I indicates that the data yield a well-defined straight line in the η versus ξ plot and that the calculated r values are satisfactory for quantitative studies. Importantly, the simplifying assumptions implicit in the Eq. (5) used to derive the reactivity ratios are valid for these systems, *i.e.*, the two-parameter model adequately describes the experimental results. While, symbols II and III indicate that the η versus ξ plot exhibits a curvature or completely unacceptable scattered data. In such cases, the simple two-parameter model is unsuitable for the description of the

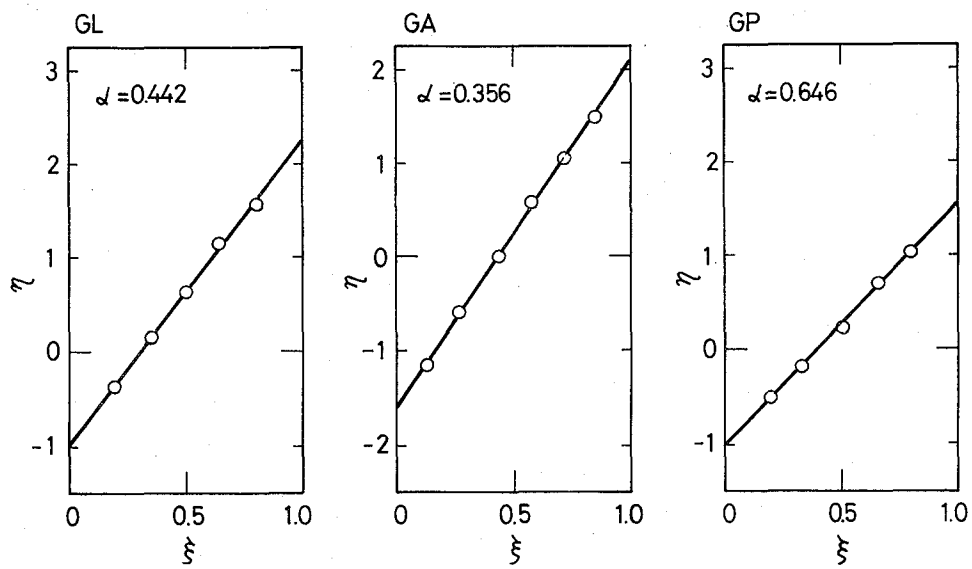

Fig. 2. Data of the copolymer systems plotted according to the Kelen-Tüdös equation for: (GL) copoly(γ-benzyl-L-glutamate/L-leucine); (GA) copoly(γ-genzyl-L-glutamate/L-alanine); and (GP) copoly(γ-benzyl-L-glutamate/L-phenylalanine).

copolymer system, and the r values derived by the Eq. (5) are apt to be erroneous and misleading.

Figure 2 illustrates the calculated result obtained for our experimental system by Eq. (1). As obvious from Fig. 2, each of the η versus ξ plots is regarded as linear (symbol I), thus the two-parameter model seems to be suitable for the description of this copolymer system, even though the real polymerization mechanism of the NCA is essentially different from the simple terminal model for radical polymerization. The numerical values obtained were listed in Table II.

Table II. Values of r_1 and r_2 Computed by the Kelen-Tüdös Method and by The Skeist Equation

System	Conv. (%)	Kelen-Tüdös Method		Skeist Equation	
		r_1	r_2	r_1	r_2
GL	23–25	2.3	0.46	2.65	0.38
GA	25–27	2.1	0.56	2.36	0.50
GP	28–30	1.6	0.67	1.64	0.59

Monomer Reactivity Ratios Evaluation from Conversion—Composition Data

Next, to get more precise values of r_1 and r_2, we used computational procedure to the integrated form of the copolymerization equation derived by Skeist, taking into consideration the change in monomer composition with conversion. The instantaneous mole fractions of G-monomer were calculated according to the differential equation of Skeist indicated as:[5]

$$\frac{dF_G}{dP_G}=\frac{F_G-f_G}{1-P} \tag{6}$$

where P is the apparent molar conversion. In Figs. 3, 4, and 5, the instantaneous mole fractions (F_G) of G-monomer together with the cumulative copolymer compositions (cf_G) and the instantaneous copolymer compositions (f_G) of G-monomer residue, are indicated as functions of conversion for GL, GA, and GP systems at the initial G-monomer feed of 50 mol-%, respectively. The process of the calculation was reported elsewhere in detail.[7]

Finally, computer procedure was used to get best fit to the experimental data by trial-and-error selections of r_1 and r_2. The numerical values obtained were summarized in Table II, together with those obtained by the Kelen-Tüdös method.

It is shown that the numerical values of r calculated by the Kelen-Tüdös equation for each system of these copolymers are slightly different from those evaluated from the integrated calculation method; *i.e.*, the former is lower for r_1, as well as higher for r_2, than the latter. It means that the r values, especially r value for the less reactive comonomer, seems to be rather sensitive to the change in the conversion of polymerization.

For example, when the monomers started to copolymerize in equimolar amounts,

it is shown that the polymer analyzed at low conversion contained significantly more *G*-comonomer than the other. Since *G*-NCA reacts with the growing peptide chain considerably faster than the other, the relative amount of free *G*-NCA monomer will decrease during the course of the copolymerization. This variation in copolymer composition, as the conversion increases, results in a higher concentration of the less reactive monomer.

Figures 3, 4, and 5, illustrate variations of the instantaneous mole fractions (F_G), together with the instantaneous copolymer compositions (f_G) and the cumulative copolymer compositions (cf_G), of *G*-monomer residue with conversion, for copoly (γ-benzyl-L-glutamate/L-leucine) (GL) (Fig. 3), copoly (γ-benzyl-L-glutamate/L-alanine) (GA) (Fig. 4), and copoly (γ-benzyl-L-glutamate/L-phenylalanine) (GP) (Fig. 5) of the initial G-monomer feeds of 50 mol-%, calculated by means of computational procedure with r values estimated from the Skeist equation. In these Figures, it is indicated that the change in monomer composition (F_G) with conversion is relatively significant even at lower conversion, and that the change becomes more significant with increasing the value of the ratio of r_1 to r_2. As expected from mentioned above, it is shown that there is a drift in the instantaneous copolymer composition (f_G) as well as the cumulative one (cf_G) toward the less reactive monomer,

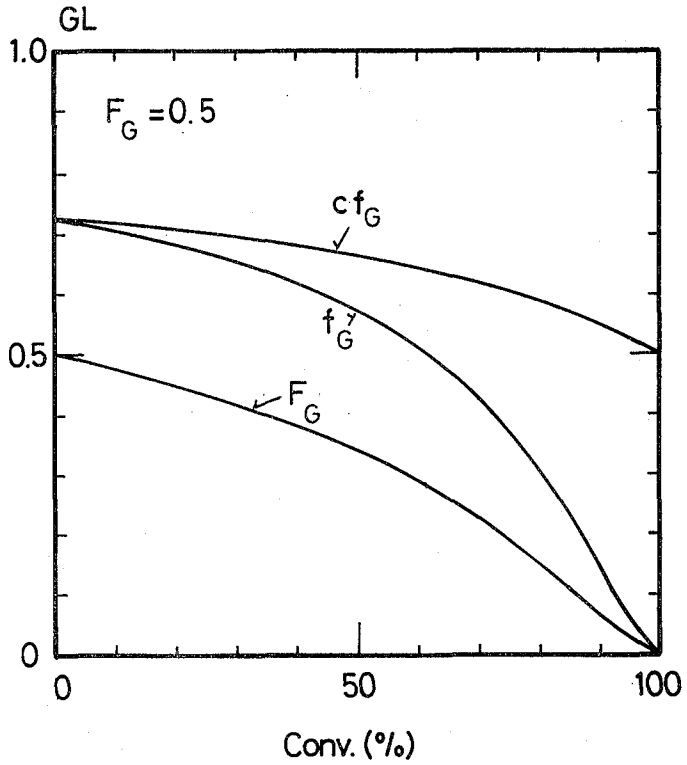

Fig. 3. Variations of the compositions in the feed (F_G), in the instantaneous copolymer (f_G), and in the cumulative copolymer (cf_G) with conversion for copoly(γ-benzyl-L-glutamate/L-leucine).

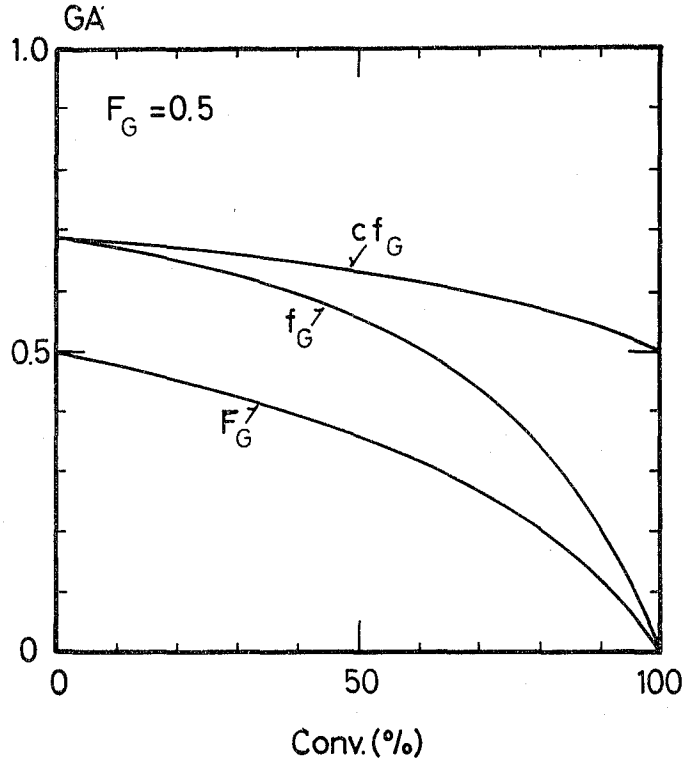

Fig. 4. Variations of the compositions in the feed (F_G), in the instantaneous copolymer (f_G), and in the cumulative copolymer (cf_G) with conversion for copoly(γ-benzyl-L-glutamate/L-alanine).

L-, *A*-, or *P*-monomer in this case, with increasing the degree of conversion.

The similar behavior of these copolymerization parameters, F_G, f_G, and cf_G, as functions of conversion was reported for copoly(γ-benzyl-L-glutamate/L-valine) (GV) system polymerized in 1:1 (v/v) mixture of dioxane and methylene dichloride,[7] in dioxane,[10] and in 1:1 (v/v) mixture of benzene and methylene dichloride,[10] initiated by TEA.

The relative reactivity of a comonomer should depend on a considerable number of factors such as the specificity of the side chain of the available monomer, stereochemical considerations, and solvent effects. Essig, *et al.*[8] have investigated the monomer reactivity ratios for GL-copolymerization initiated by TEA in various solvents, and reported: r_1 (G)=2.7 and r_2(L)=0.3 in dioxane, while, r_1(G)=1.5 and r_2(L)=0.7 in 1:1 (v/v) mixture of benzene and methylene dichloride. While, Nylund and Miller[9] investigated a *n*-hexylamine initiated copolymerization of G and L in DMF. They calculated the values of the monomer reactivity ratios, and reported; r_1(G)=1.57 and r_2(L)=0.61.

Besides of these influence on the propensity of a particular monomer to incorporate into the growing chain, stereochemical phenomena as well as chemical properties of the side chain are of significant importance in influencing monomer re-

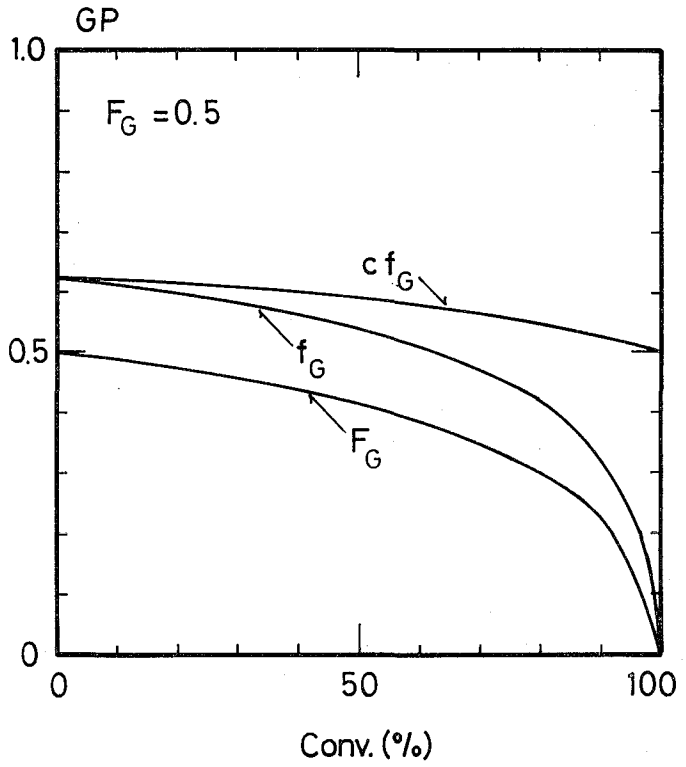

Fig. 5. Variations of the compositions in the feed (F_G), in the instantaneous copolymer (f_G), and in the cumulative copolymer (cf_G) with conversion for copoly(γ-benzyl-L-glutamate/L-phenylalanine).

activity. Therefore, in the copolymerization of two α-helix forming NCAs, the reactivity of one monomer will depend to some degree on its ability to interact with its comonomer and to be incorporated into the growing helical chain. The role of the solvent should be very important on reactivity ratios, which are highly dependent on the ability of the solvent to interact with the activated monomer-NCA (the carbamate ion), stabilize the conformation of the growing chain, and permit stereochemically the incorporation of monomer into the growing chain. Blout, *et al.*[11] concluded from their study of the polymerization of G-NCA in dioxane solution that if the carbamate ion formed from attack of the initiator on the NCA has a high electron density, it dissociates to a greater degree than the carbamate ion with less ionic character and promotes polymerization. Further, the degree of the interaction between solvent and the activated monomer-NCA will be influenced by the stereochemical and physical nature of the side chain.

The more reactive NCA will be the monomer which, upon formation of the ion-pair, interacts most favorably with solvent localizing a negative charge. On the other hand, the less reactive linear carbamate ion will have a smaller electron density, and will incorporate into the growing chain at a slower rate. It seems reasonable from the above-mentioned reason that *G*-NCA has a trend to incorporate

into the growing chain faster than another comonomer-NCA; *i.e.*, *L*-, *A*-, or *P*-NCA.

Next, among these three comonomer-NCA, the order of the rate of the incorporation is shown as follows: *P*-NCA>*A*-NCA>*L*-NCA. Among them *P*-NCA is the only one comonomer that has a phenyl group in the side chain, which will be expected to have stronger affinity to solvent medium.

In addition to the solvent's ability to interact with the activated NCA, the reaction medium brings in stereochemical restrictions on the addition of NCA to the growing polypeptide chain. Methyl group side chain of *A*-NCA seems to be easier to incorporate into the end of the growing chain than *L*-NCA which has an isobutyl side chain group.

It should be very important to investigate sequential distributions of comonomers in copolymer chains in order to make clear the effect on copolymer properties. The experimental results on the molecular conformations and the sequential distributions of comonomers in copolypeptide chains will be reported in our succeeding papers.

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