Polymer Journal, Vol. 10, No. 1, pp 87—91 (1978)

Monomer Reactivity Ratios in Copolymerization of γ-Benzyl L-Glutamate and L-Valine N-Carboxyanhydrides

Kazuo ISHIWARI, Toshio HAYASHI, and Akio NAKAJIMA*

Department of Polymer Chemistry, Kyoto University, Sakyo-ku, Kyoto 606, Japan.

(Received June 12, 1977)

ABSTRACT: Monomer reactivity ratios in the *N*-carboxyanhydride (NCA) copolymerization of γ-benzyl L-glutamate with L-valine initiated by triethylamine (TEA) in a 1 : 1-(v/v) mixture of dioxane and dichloromethane at 25°C were determined. The methods used were a differential linearization graphical method, and a nonlinear least-square method which takes into consideration the change of monomer concentration with increasing conversion. Since the reactivity ratios are very sensitive to copolymer composition, four analytical methods have been employed to determine the copolymer composition, *i.e.*, the amino acid analysis, elemental analysis, and ultraviolet and infrared spectroscopies. The numerical values of the monomer reactivity ratios obtained were r_1(γ-benzyl L-glutamate)=2.96 and r_2(L-valine)=0.17.

KEY WORDS Polypeptide / Copolymer / γ-Benzyl L-Glutamate / L-Valine / Monomer Reactivity Ratio / NCA Copolymerization /

Many studies of the properties of copolypeptides, in particular, comformational studies in solution and in solid state, have been reported by a number of investigators. It is well-known that their properties can be profoundly influenced by the sequence distribution of each comonomer in copolymer chains. However, quantitative studies of the sequence distribution of the comonomers in random (or satistical) copolypeptides are few in number. Therefore, it is useful to determine the monomer reactivity ratios in the NCA copolymerization in order to find out the sequence distribution in copolypeptide chains.

To estimate monomer reactivity ratios, many methods have been proposed. In particular the Fineman—Ross method[1] has been used in many copolymerization systems. However, as pointed out by Tidwell and Mortimer,[2] in this method the experimental data are unequally weighted, and accordingly the calculated r_1 and r_2 values depend on arbitrary factors, such as which monomer is selected as the first component. Recently, Kelen and Tüdös proposed a differential linearization graphical method to overcome the disadvantages described above.[3] As pointed out by Kennedy, *et al.*,[4] the Kelen—Tüdös method not only provides accurate r_1 and r_2 values which are not obtained in many cases by previous methods, but also holds important clues as to the availability of the conventional copolymer composition equation. To determine r_1 and r_2 values by the Kelen—Tüdös method, the polymerization should be stopped generally at a low conversion, where the composition of the monomer is not significantly different from its initial value. However, to prepare copolymers of sufficiently high molecular weight with higher efficiency it is desirable to perform copolymerization up to a somewhat higher conversion. Moreover, there are some experimental difficulties associated with the composition analysis of copolymers formed at low conversion. In this study copolymerization was stopped at a conversion level of about 26%. Therefore the change in composition of the monomer should be taken into consideration to obtain accurate r_1 and r_2 values from higher

* To whom correspondence should be addressed.

conversion data. So, in addition to the Kelen—Tüdös method, a least-square method, taking into consideration the change in composition of the monomer, was used. The change in comonomer composition with comversion can be calculated by the Skeist equation[5] (Skeist method).

In this paper we are concerned with the copolymerization of γ-benzyl L-glutamate and L-valine NCA's, in which the former component is an α-helix former and the latter is a non-α-helix former.[6] It is very important to become able to elucidate the influence of the L-valine residues incorporated into γ-benzyl L-glutamate sequences on the conformation of copolypeptide chains.

EXPERIMENTAL

Materials

The monomers, γ-benzyl L-glutamate *N*-carboxy anhydride (G-NCA) and L-valine *N*-carboxy anhydride (V-NCA), were prepared according to the method proposed by Blout and Karlson,[7] and purified by repeated recrystallizations from an ethyl acetate solution with the addition of petroleum ether. The G-NCA and V-NCA, in desired molar ratios, were dissolved in a 1 : 1-(v/v) mixture of dioxane and dichloromethane. The total concentration of both NCA's was kept at 3 wt%. The polymerization was initiated with triethylamine at an NCA-to-initiator molar ratio of 50. The polymerization was stopped at about 25 to 27-mol% conversion. The course of the polymerization was monitored by titrating 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 methanol including 0.1-*N* HCl. Then the precipitation products were washed in pure methanol and dried under reduced pressure at 50°C. The composition of these copolypeptides was determined by means of four analytical methods, amino acid analysis, elemental analysis, and ultraviolet and infrared spectroscopies. The results of all the copolymerizations are listed in Table I. The compositions determined by means of these four methods show fairly good agreement with each other, as is shown in Table I. Therefore we adopted the average of these values as the composition of the copolypeptides.

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 compositions of the initial monomer mixture, together with the limiting viscosity number $[\eta]$, are summarized in Table I. Infrared (IR) spectra of solid films of the samples cast from chloroform (CF) and from CF—trifluoroacetic acid (TFA) (80/20, v/v) mixture were obtained 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 by Kelen—Tüdös Method

Table I. Copolymerization of γ-benzyl L-glutamate(G) with L-valine(V) by NCA method

Sample no.	Initial monomer ratio, G mol%	Polymer composition, G mol%					Conversion, %	$[\eta]$ (DCA, 25°C), dl/g
		AAA[a]	EA[b]	UV[c]	IR[d]	Average		
1	100						45	1.25
2	90	95	96	96	95	96	27	1.02
3	75	90		87		89	25	1.26
4	50	75	75		75	75	26	1.55
5	35	63	62	62	62	62	25	1.89
6	20	44	45	44	46	45	25	2.04
7	10	26	27		28	27	26	2.22
8	0						38	0.55

[a] Amino anid analysis. [b] Elemental analysis. [c] Ultraviolet spectroscopy. [d] Infrared spectroscopy.

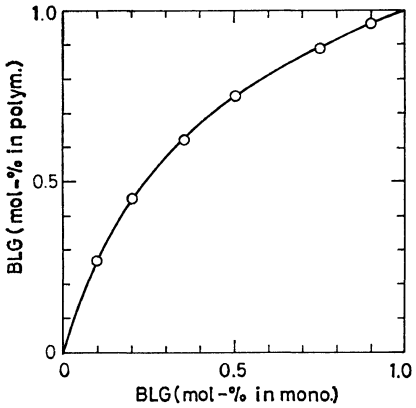

Figure 1. Copolymer composition curve for copoly(γ-benzyl L-glutamate/L-valine) at about 26-% conversion. Data are taken from Table I.

Figure 1 illustrates the copolymer composition curve for the compolymerization of G-NCA with V-NCA at a conversion level of about 26%, taken from the data in Table I. To begin with, the Kelen—Tüdös method was used to test its applicability to this copolymerization system.

Kelen and Tüdös derived the following equation

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

in which $\eta=Y/(\alpha+X)$, $\xi=X/(\alpha+X)$, $X=F^2/f$, $Y=F(f-1)/f$, $F=M_1/M_2$ and $f=m_1/m_2$, where M_1 and M_2 are the molar concentrations of monomers 1 and 2 in the monomer feed, and m_1 and m_2 are the same concentrations in the final polymer. α is an appropriately chosen constant to obtain a uniform spread of the data. Equation 1 is derived based on the conventional copolymer composition equation.

$$f=F\frac{1+r_1F}{r_2+F} \qquad (2)$$

Kennedy, *et al.*,[4] classified copolymerization systems into three classes (symbols I, II, and III) by examining the linearity in the η *vs.* ξ plot, the Kelen—Tüdös plot.

Symbol I indicates that the Kelen—Tüdös plot shows a good linearity and the calculated values are satisfactory for quantitative studies. In this case the simplified assumptions implicit in eq 2 are valid. Model I is called the two-parameter model. Symbols II and III indicate

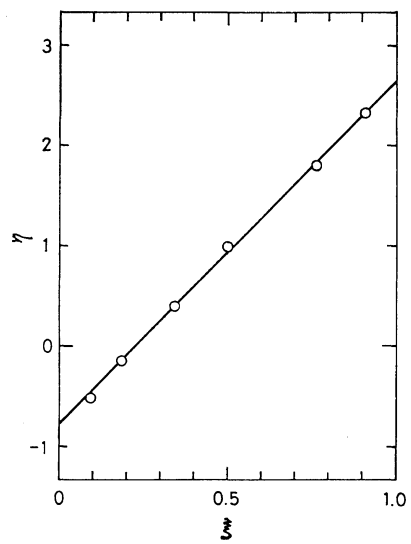

Figure 2. Kelen-Tüdös plot for copoly(γ-benzyl L-glutamate/L-valine).

that the η *vs.* ξ plot exhibits a curvature or gives completely unacceptable scattered data. In such cases, the two-parameter model is unsuitable for the description of the copolymer system, and r values derived from eq 2 are erroneous and misleading.

Figure 2 illustrates the Kelen—Tüdös plot for the present system. The plot shows a good linearity, so this system belongs to class I, and the two-parameter model seems to be suitable for the description of the system, though the polymerization mechanism of NCA initiated by tertiary amine is not still clear. The numerical values obtained were $r_1(G)=2.7$ and $r_2=0.26$.

Monomer Reactivity Ratios from Conversion—Composition Data

Next, to obtain r_1 and r_2 values from conversion—composition data, a nonlinear least-square method, *i.e.*, the Skeist method, was employed. The Skeist method, in brief, is applied as follows.

The change of the mole fraction of the first component in the monomer mixture as a function of conversion F is calculated by the Skeist equation[5]

$$\frac{dF}{dP}=\frac{F-f}{1-P} \qquad (3)$$

where P is the molar conversion, and f is the

mole fraction of the first component in the polymer formed during the differential time interval and is given by the following equation.[8]

$$f=\frac{F(r_1F+1-F)}{F(r_1+1-F)+(1-F)\{F+r_2(1-F)\}} \quad (4)$$

Using F calculated from eq 3 and 4, the cumulative mole fraction C of the first component in the polymer is calculated from

$$C=\frac{F_0-(1-P)F}{P} \quad (5)$$

where F_0 is the initial mole fraction of the first component.

The best possible values of r_1 and r_2 can be obtained from where the quantity $G(r_1, r_2)$ takes its minimum value

$$G(r_1, r_2)=\sum_{i=1}^{N}(C_i-C_{mi})^2 \quad (6)$$

where C_i is the calculated value of C for the ith experimental run, C_{mi} is the measured value of C_i, and N is the number of experimental runs. To obtain r_1 and r_2 values which realized the minimum value of $G(r_1, r_2)$, the Newton iteration method is employed. A necessary condition for the minimum is

$$g_k(r_1, r_2)=\sum_{i=1}^{N}(C_i-C_{mi})\frac{\partial C_i}{\partial r_k}=0 \quad (k=1, 2) \quad (7)$$

Corrections for the jth estimates (r_1^j and r_2^j) of r_1 and r_2, Δr_1^j and Δr_2^j, can be calculated by the following equation.

$$g_k(r_1^j+\Delta r_1^j, r_2^j+\Delta r_2^j)=0 \quad (k=1, 2) \quad (8)$$

Equation 8 can be solved for Δr_1^j and Δr_2^j by a Taylor expansion in which higher-order terms than the second order of Δr_1^j and Δr_2^j are neglected. Then, the $(j+1)$th estimates of r_1 and r_2 are given as $r_1^j+\Delta r_1^j$ and $r_2^j+\Delta r_2^j$, respectively. If the $(j+1)$th value of eq 6 is smaller than the jth one, and Δr_1^j and Δr_2^j are both less than 0.001, then r_1^{j+1} and r_1^{j+1} can be regarded as the most possible values. Contrary, if both Δr_1^j and Δr_2^j are not less than 0.001, the same procedure is repeated. If the $(j+1)$th value of eq 6 is not smaller than the jth one, $\Delta r_1^j/2$ and $\Delta r_2^j/2$ are the corrections of r_1^j and r_2^j. But this situation did not occur in this study.

The differential equations involved in this procedure were solved numerically by the Runge—Kutta method. Using the Skeist method, $r_1(G)=2.96$ and $r_2(V)=0.17$ were obtained.

Figure 3 illustrates the change of monomer concentration of the G component as a function of conversion calculated for $r_1=2.96$ and $r_2=0.17$ by eq 1 and 2. Points in Figure 3 indicate those calculated from the experimental values in Table I according to the following mass balance equation

$$F_G=\frac{F_{G,0}-Cf_G\times P}{1-P} \quad (9)$$

where Cf_G is the cumulative mole fraction of G residue in copolymer chains. In Table II are given experimental values of F_G from eq 9 and the calculated values of F_G for r's values obtained by the Kelen—Tüdös method and the Skeist method. The fact that the calculated values of F_G obtained with the Skeist method agree with the experimental values shows that the Skeist method allows a reliable determination of reactivity ratios of copolymerization of NCA's. The fact that values by the Skeist method shows better agreement with the experimental values than those by the Kelen—Tüdös method indicates that even at 26-% conversion the change of monomer concentration should be taken into consideration to determine accurate monomer reactivity ratios. The change is significant particularly for those points where the more reactive monomer (G monomer in this system) is

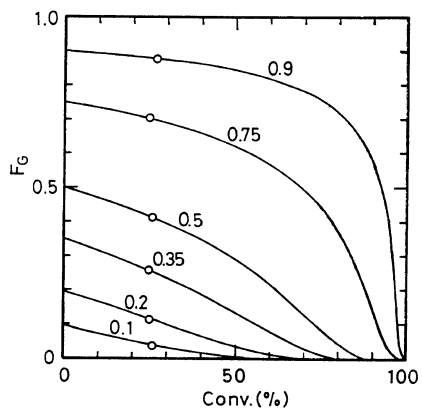

Figure 3. Variations of γ-benzyl L-glutamate NCA monomer composition against conversion. Points in figure indicate experimental values.

Table II. Mole fraction of γ-benzyl L-glutamate in monomer mixture

Feed concentration, $F_{G,0}$	Conversion, %	F_G (from eq 9)	F_G (K-T value)	F_G (Skeist value)
0.10	26	0.0403	0.0549	0.0407
0.20	25	0.116	0.133	0.116
0.35	25	0.260	0.271	0.258
0.50	26	0.412	0.423	0.413
0.75	25	0.703	0.706	0.702
0.90	27	0.878	0.878	0.878

present in relatively small amounts, as shown in Figure 3, and the disagreement between the experimental values and the values by the Kelen—Tüdös method in Table II is larger for those points where the G monomer is present in relatively small amounts. And these facts might indicate that the r_2(V) calculated by the Kelen—Tüdös method would have a somewhat higher value than that by the Skeist method.

The reactivity of NCA is influenced by a number of factors, such as the stereochemical structure and polarity of the monomer, the kinds of initiator, and the solvent effects. G NCA trends to incorporate into the growing chain faster than V NCA in this study, and also faster than leucine, alanine, and phenylalanine NCA's[9]. For the NCA polymerization initiated by tertiary bases, two mechanisms have been proposed: one is the mechanism[10] involving a carbamate ion formed by the addition of a tertiary base to the 5-carbonyl group of NCA, and the other is the mechanism[11] involving an activated monomer formed by the removal of the proton from the endocyclic NH group. Blout, *et al.*,[10] showed that the formed carbamate ion which has high electron density dissociates to a greater degree than one with less ionic character and also polymerizes faster. It seems reasonable from the explanation described above that G NCA polymerizes faster than other NCA's. Bamford and Block[11] showed that which of the mechanisms described above is more predominant depends on whether the tertiary base acts as a Lewis base or as a Brönsted base. So it seems that some ionic species are involved in NCA polymerization. Therefore a faster polymerization is expected when the polarity of the solvent is higher. Hayashi, *et al.*,[12] obtained r_1(G)=2.7 and r_2(V)=0.62 for the polymerization initiated by triethylamine, in which the solvent is a 1 : 1-(v/v) mixture of benzene and dichloromethane *i.e.*, lower r_1 and higher r_2 values. Benzene has a higher polarity than dioxane, and thus polymerization is generally faster in benzene than in dioxane.[13] Thus, in the solvent with high polarity, faster polymerization may disturb the discrimination of the more reactive monomer; thus, lower r_1(G) and higher r_2(V) values were obtained with benzene. Further, it might be accepted that the affinity of the phenyl group of G NCA toward benzene weakens the movement of G NCA.

REFERENCES

1. M. Fineman and S. D. Ross, *J. Polym. Sci.*, **5**, 259 (1950).
2. P. W. Tidwell and G. A. Mortimer, *J. Macromol. Sci., Revs. Macromol. Chem.*, **C4**, 281 (1970).
3. T. Kelen and F. Tüdös, *J. Macromol. Sci., Chem.*, **A9**, 1 (1975).
4. J. P. Kennedy, T. Kelen, and F. Tüdös, *J. Polym. Sci., Polym. Chem. Ed.*, **13**, 2277 (1975).
5. I. Skeist, *J. Am. Chem. Soc.*, **68**, 1781 (1946).
6. G. D. Fasman, "Poly-α-Amino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N.Y., 1967, p 499.
7. E. R. Blout and R. H. Kalson, *J. Am. Chem. Soc.*, **78**, 941 (1956).
8. F. T. Wall, *ibid.*, **66**, 2050 (1944).
9. K. Ishiwari, T. Hayashi, and A. Nakajima, *Bull. Inst. Chem. Res., Kyoto Univ.*, in press.
10. M. Idelson and F. R. Blout, *J. Am. Chem. Soc.*, **80**, 2387 (1958).
11. C. H. Bamford and H. Block, *J. Chem. Soc.*, 4989 (1961).
12. T. Hayashi, A. Nakajia, W. Sederel, S. Deshmane, and J. M. Anderson, *Polymer Preprint, Japan*, **25**, 1189 (1976).
13. M. Szwarc, "Carbanions Living Polymers and Electron Transfer Processes," Interscience Publishers, New York, N.Y., 1968, p 575.