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Polymerization of DL-β-Phenylalanine N-Carboxyanhydride by Poly(N-*n*-Propylglycine) Diethylamide*

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ABSTRACT: DL-β-Phenylalanine N-carboxyanhydride(NCA) was polymerized by poly(N-*n*-propylglycine) diethylamide (DEA) at a much faster rate than by low molecular weight amines having a similar base strength. This phenomenon (the chain effect) had been observed also with other polypeptides such as polysarcosine DEA and poly(N-ethylglycine) DEA. The chain effect was found to diminish in the order: polysarcosine DEA > poly(N-ethylglycine) DEA > poly(N-*n*-propylglycine) DEA. In order to compare these polymer catalysts, the equilibrium constant for the NCA adsorption on to the polymer chain by hydrogen bonding was determined. It was found to be almost the same for the three polymer catalysts. The intrinsic reactivity of the terminal base group of the polymer catalyst was also determined. It was found to decrease as the N-alkyl substituent became bulkier (that is, methyl > ethyl > *n*-propyl). As a consequence, the difference in the chain effect was ascribed mostly to the latter effect. However there still remains some difference in the reactivity of polymer catalysts, which can be explained in terms of the flexibility of the polymer chain.

KEY WORDS DL-β-Phenylalanine NCA / Poly(N-*n*-propylglycine) DEA / NCA Polymerization / Polymer Catalyst / Chain Effect / Flexibility / Block Copolypeptide / Hydrogen Bond

It has been shown that DL-β-phenylalanine N-carboxylanhydride (NCA) is polymerized by polysarcosine dialkylamide at a much faster rate than by low molecular weight amines having a similar base strength. This phenomenon has been called the chain effect by Ballard and Bamford.[1] A comprehensive kinetic study revealed that a hydrogen bond is formed between the amide carbonyl group of the polymer catalyst and the NH group of NCA resulting in an increase of NCA concentration in the vicinity of the terminal base group of the polymer catalyst, and that the reaction rate is increased. The formation of a hydrogen bond was confirmed later by Bamford and Price[2] using infrared spectroscopy.

The mechanism proposed by Bamford suggests that the chain effect is operative in the polymerization of any N-unsubstituted amino acid NCA by any polymer of an N-substituted amino acid. In the latter the amide carbonyl group is available for intermolecular hydrogen bonding with NCA. In fact some N-unsubstituted amino acid NCA's have been shown to be polymerized by the chain effect mechanism.[1,3] Poly(N-ethylglycine) diethylamide (DEA) has been reported to be effective as a catalyst for the chain-effect polymerization.[4] However, Ballard[5] has found that no chain effect is operative in polymerization catalyzed by polyproline dimethylamide.

These facts suggest that the chain effect is influenced strongly by the nature of the polymer chain that is determined by the nature of N-substituent. In the present investigation, the behavior of poly(N-*n*-propylglycine) DEA as catalyst for the chain-effect polymerization was studied. Particular attention was focused on the effect of the increasing bulk of N-substituent on the nature of the polymer catalyst such as the hydrogen bond formation with NCA, the inherent reactivity of terminal base group, and the polymer chain flexibility. The results were discussed in comparison with those obtained with polysarcosine DEA and poly(N-ethylglycine) DEA.

EXPERIMENTAL

Materials

* This is the fourth in the series of "Polymerization of Amino Acid Derivatives by Polymer Catalysts". For the third paper in the series, see ref. 4.

N-n-Propylglycine NCA

Monobromoacetic acid was mixed with an excess of *n*-propylamine to synthesize N-*n*-propylglycine. The resulting amino acid was, without isolation, made to react with ethyl chloroformate to synthesize N-carboethoxy-N-*n*-propylglycine.[6] The latter was converted to N-*n*-propylglycine NCA by the action of thionyl chloride.[7] The crude NCA was reprecipitated from ethyl acetate–petroleum ether (bp 30–70°C) repeatedly. The purified NCA thus formed was a white down-like crystal (mp ~20°C).

N-n-Propylglycine DEA

Chloroacetyl DEA[8] was added to an excess of *n*-propylamine to synthesize N-*n*-propylglycine DEA.[6] The amide was distilled *in vacuo* twice and titrated with hydrochloric acid. The titration result agreed closely with the theoretical value, with an error of ±1%.

Poly(N-n-Propylglycine) DEA

The corresponding amino acid NCA and amino acid DEA were mixed to react in nitrobenzene at room temperature. From the amount of carbon dioxide evolved, the reaction was found to have proceeded quantitatively. The resulting polymer solution was homogeneous. From acid titration the terminal base group was found to be intact when the reaction was complete. Thus the number-average degree of polymerization of the polymer, n, is given by Eq. 1.[9]

$$n = [\text{NCA}]/[\text{DEA}] + 1 \qquad (1)$$

Polysarcosine DEA and poly(N-ethylglycine) DEA were synthesized as reported previously.[4] When the NCA adsorptivity on various polypeptides was measured, the terminal base group of the polymers was blocked by adding N-phenylglycine NCA and made inactive for DL-β-phenylalanine NCA. DL-β-Phenylalanine NCA was synthesized and purified as reported previously.[4] Solvents were purified in the usual manner.[4]

Apparatus and Technique

All polymerizations were carried out in nitrobenzene solution at 25°C. The polymerization rate was followed by measuring the pressure of carbon dioxide evolved using a constant-volume apparatus equipped with a manometer. In this investigation, the correction for the amount of carbon dioxide absorbed in nitrobenzene was made according to Henry's law. To determine the absorption coefficient, DL-β-phenylalanine NCA was polymerized under a variety of conditions and when no further increase in pressure was observed, indicating 100% conversion, the manometer reading was taken. The validity of this treatment was confirmed by the fact that the absorption coefficient determined under a variety of conditions was constant. A small variation in the final conversion does not affect subsequent discussions. The volume of carbon dioxide was calculated and compared with the theoretical amount of carbon dioxide that was taken to be equal to moles of NCA initially present. The absorption coefficient (1.82×10^{-3} mol/(l cmHg)) thus determined was constant for all NCA and catalyst concentrations studied. This proves that Henry's law holds for the present system, and is in agreement with the experimental results of Ballard and Bamford.[10] However, the absorption coefficient in the present experiment was 38% higher than the value in the literature[11] for pure nitrobenzene.

The equilibrium constant for the adsorption of DL-β-phenylalanine NCA on to the polymer catalyst was determined by the method of Bamford and Price[2] using the intensity of infrared absorption of the free NH group of NCA. Measurements were made with a Japan Optics DS-402G grating spectrophotometer using a sodium chloride cell, the thickness being 0.5 mm.

RESULTS AND DISCUSSION

Equilibrium Constant for the Adsoption of DL-β-*Phenylalanine NCA on to the Polymer Catalyst*

To investigate chain-effect polymerization kinetically, the equilibrium constant (K) for the adsorption of DL-β-phenylalanine NCA on to various polymer catalysts must be known. Measurements of this type have so far been made only with polysarcosine dimethylamide.[2]

By analogy with Bamford and Price, K was determined by the intensity of the absorption peak due to the NH group of NCA which is free from hydrogen bonding. The terminal base group of the polymer catalyst coexisting with the NCA was made inactive by N-phenylglycine residue. The NCA concentration was varied within the range of 1.5×10^{-2} to 3×10^{-2} mol/l, and the concentration of the adsoption site

(carbonyl group of polymer catalyst) was altered in the range of 7×10^{-2} to 2×10^{-2} mol/*l*. The mean value and the standard deviation of K were calculated from nine different experiments. The measurements were carried out at room temperature and the temperature in the cell was not specifically controlled, though it would not have differed greatly from the 29°C at which Bamford and Price's measurements were made. The solvent used for the determination of K was methylene chloride instead of the nitrobenzene used for the polymerization. This was because absorptions due to nitrobenzene interfere with the NH absorption band and methylene chloride is more suitable for a precise determination of K. The results are shown in Table I together with value reported by Bamford and Price.[2] With polymers having $n \simeq 30$, only slight difference in K_{MC} is observed among the three polymers. K_{MC} represents K determined in methylene chloride solution. With polysarcosine DEA the effect of n on K_{MC} was checked and found to be of minor importance.

Table I. Equilibrium constant (K) for hydrogen bonding between DL-β-phenylalanine NCA and various poly (N-alkylamino acid) DEA's in methylene chloride solution

Polymer	n	K (*l*/mol)
Polysarcosine DMA[a]		3.5±0.3[2]
Polysarcosine DEA	31	3.3±0.4
〃	21	4.2±0.2
〃	11	3.8±0.2
Poly(N-ethylglycine) DEA[b]	29	3.3±0.5
Poly(N-*n*-propylglycine) DEA	31	3.4±0.3

[a] DMA: Dimethylamide.
[b] Containing a terminal N-phenylglycine unit.

To explain the chain effect kinetically, Ballard and Bamford[1] assumed that K is independent of the position of the site on which NCA is adsorbed. This would mean K is independent of n. On the other hand, the alterative assumption was that the sites near the terminal base group are more liable to adsorb NCA molecules. From Table I it can be seen that with polysarcosine DEA the variation of K_{MC} from n is far less marked than the variation of the polymerization rate from n (see Figure 2). This means that the first assumption is more reasonable, and it was adopted to explain the experimental results in the present investigation. It was also assumed that K in nitrobenzene (K_{NB}), which has not yet been actually determined, would be constant for the three polymer catalysts by analogy with K_{MC}. $K_{NB} = 5$ *l*/mol that has been reported by Bamford and Price[2] for polysarcosine was used for the three polymer catalysts.

Polymerization of DL-β-*Phenylalanine NCA by Poly(N-n-Propylglycine) DEA*

DL-β-Phenylalanine NCA was polymerized by poly(N-*n*-propylglycine) DEA having various n's. Figure 1 represents the time-conversion curves for the polymerization where the concentration of the terminal base group was kept constant. It is clearly shown that the initial rate of polymerization increases remarkably as n of the initiator increases. Hence the chain effect is operative with poly(N-*n*-propylglycine) DEA as well as with polysacrcosine DEA or poly(N-ethylglycine) DEA. The initial rate of polymerization (R_{pi}) was determined from the maximum slope of the initial section of the time-conversion curve. Figure 2 shows the plot of R_{pi} against n. Figure 2 shows that the initiator having $n = 11.5$ gives the fastest rate. The following kinetics which has been proposed by Ballard and Bamford,[1] was employed to explain the experimental results.

$$R_{pi} = \bar{k}_n[E] \tag{2}$$

$$\bar{k}_n = \frac{1}{n}\sum_{i}^{n} k_i \tag{3}$$

In the above, $[E]$ represents the concentration of NCA adsorbed on the polymer catalyst, and is

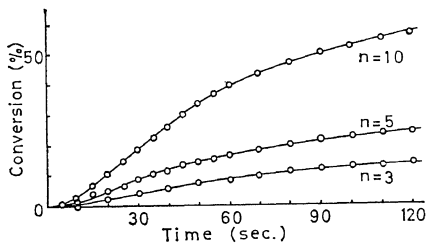

Figure 1. Polymerization of DL-β-phenylalanine NCA initiated by poly(N-*n*-propylglycine) DEA having various degrees of polymerization (n). $[M]_0$, 1.0×10^{-1} mol/*l*, $[X]$, 5.0×10^{-3} mol/*l*, Nitrobenzene solution, 25°C.

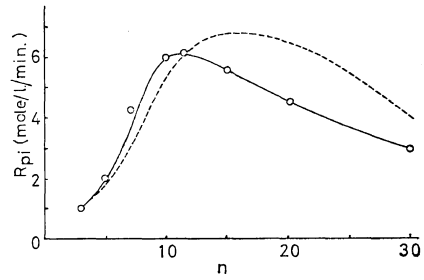

Figure 2. Initial rate of polymerization (R_{pi}) *vs.* degree of polymerization of catalyst (n).
——○——: Observed.
— — — —: Calculated.

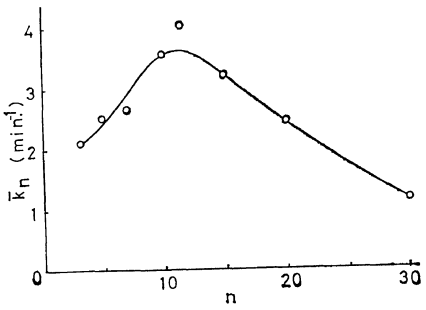

Figure 3. Mean rate constant (k_n) *vs.* degree of polymerization of catalyst (n).

determined at the initial stage of polymerization by the initial concentrations of NCA ($[M]_0$) and the adsorption site (carbonyl group) ($[S]_0 = n[X]$) and the equilibrium constant for adsorption (K). k_i represents the rate constant for the reaction between the terminal base group and the NCA adsorbed on the i-th carbonyl group, counting from the terminal base group along the polymer chain. $\bar{k}_n$ is the mean value of k_i for $i = 1 - n$. The assumption that k_i is dependent on but K is independent of the position of adsorption was discussed in the preceding paragraph.

To determine $\bar{k}_n$, the polymerization was carried out at 25°C with variable n's, a constant $[M]_0$ (1.0×10^{-1}mol/l) and a constant $[S]_0$ (2.5×10^{-2} mol/l). Assuming $K = 5$ l/mol, $[E]$ was calculated to be 7.9×10^{-3} mol/l under present conditions. The subsequent discussions are not affected by the choice of experimental conditions. Since $[E]$ is known and R_{pi} is measurable, $\bar{k}_n$ is determined according to Eq. 2. Figure 3 represents the plot of $\bar{k}_n$ against n.

Using $\bar{k}_n$ thus determined, R_{pi} under the conditions of Figure 2 was calculated according to Eq. 2. The dotted line in Figure 2 represents the calculated relationship between R_{pi} and n. The calculated R_{pi} is in fairly close agreement with the experimental R_{pi}, which supports the validity of $\bar{k}_n$ values. The calculated line tends to deviate from the experimental one at large n's. This cannot be ascribed entirely to experimental error, since a similar phenomenon has been observed also with polysarcosine DEA[2] and poly(N-ethylglycine) DEA.[4] The viscosity of the polymerization system would have a bearing on this. The decrease of the polymerization rate with increasing viscosity of the system has been observed by Bamford.[1,2]

The $\bar{k}_n$ values in Figure 3 give the k_i value from a further calculation based on Eq. 3. Figure 4 represents the plot of k_i against i for poly(N-*n*-propylglycine) DEA as well as polysarcosine DEA or poly(N-ethylglycine) DEA for comparison. k_i values for the last two polymers have been corrected for carbon dioxide dissolved in nitrobenzene (see the experimental section), so they are slightly different from the values reported previously.[4] The maximum k_i value appeared at $i = 10$, which implies that the carbonyl group at the 10th position from the terminal base group is the best position for the reaction.

The terminal base group of poly(N-*n*-propylglycine) DEA is a secondary amine. Ballard and Bamford[1] have found that the polymerization of DL-β-phenylalanine NCA by polysarcosine DEA is initiated by a primary-amine mechanism. But

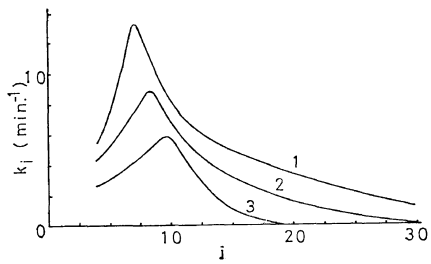

Figure 4. Initial rate constant (k_i) *vs.* reaction site on polymer catalyst (i).
1: Polysarcosine DEA.
2: Poly(N-ethylglycine) DEA.
3: Poly(N-*n*-propylglycine) DEA.

Bamford and Block[12] have shown that polymerization by a secondary amine carrying bulky substituents is initiated partly by a primary-amine mechanism and partly by a tertiary-amine mechanism. This was substantiated later by Goodman and Hutchison,[13] and by Peggion *et al.*[14] using radioactive amines as an initiator. So the initiation mechanism of the present polymerization must be investigated.

Three types of polypeptides were synthesized and their specific viscosities were compared. Poly(N-*n*-propylglycine) DEA, which was used as an initiator in the chain-effect polymerization illustrated in Figure 1 and has an expected DP of 20, was isolated from the nitrobenzene solution (Polymer A). DL-β-Phenylalanine NCA was polymerized by N-*n*-propylglycine DEA under the same conditions as in Figure 1, and the resulting polymer having an expected DP of 20 was isolated from the nitrobenzene solution (Polymer B). The polymer resulting from the chain-effect polymerization initiated by Polymer A under the conditions described in Figure 1 was also isolated from the nitrobenzene solution (Polymer C). The reduced viscosity (0.5 g polymer in 100 m*l* dichloroacetic acid; 25°C) of Polymer C ($\eta_{sp}/c = 0.48$) was higher than that of the mixture of Polymers A and B ($\eta_{sp}/c = 0.25$). Hence, Polymer C was thought to be a block copolymer of N-*n*-propylglycine and DL-β-phenylalanine, which is a result of a primary amine type initiation.

Comparison of Three Poly(N-Alkylglycine) DEA's in Chain-Effect Polymerization—Correction for the Reactivity of Terminal Base Group

In Figure 4 three poly(N-alkylglycine) DEA's are compared for reactivity in chain-effect polymerization. The facts discovered for the first time in the present investigation are as follows.

(i) k_i values decrease in general as the N-substituent in the polymer catalyst becomes bulkier.

(ii) The position i corresponding to the maximum k_i value becomes further removed from the terminal base group as the N-substituent in the polymer catalyst becomes bulkier.

The authors consider that the nature of the N-substituent in the polymer catalyst affects chain-effect polymerization in the following three ways.

(i) Hydrogen bonding between the NCA and carbonyl groups in the polymer is affected.

(ii) The inherent reactivity of the terminal base group (–NHR) of the polymer catalyst is affected.

(iii) The intramolecular collision frequency between the adsorbed NCA and the terminal base group (the flexibility of the polymer chain) is affected.

In this discussion, only an intramolecular reaction is assumed to occur. This consideration has some support from experimental evidence available.[1,5] Of the three points above, item (i) was discussed in the preceding section and taken into account in interpreting the experimental results. To investigate item (ii) the reactions between DL-β-phenylalanine NCA and various N-alkylglycine DEA's were studied, the latter being equivalent to the terminal unit of the polymer catalyst. This type of reaction is almost free of the chain effect and therefore provides information about item (ii). So that only the reaction between the amino acid NCA and amino acid DEA, and not the propagation reaction could be studied, the reaction was carried out at [NCA]/[DEA] = 1/2. The second-order rate constant k for the reaction was determined according to the equation

$$k = -\frac{1}{[M]_0\,[X]_0}\left(\frac{d[M]}{dt}\right)_{t=0}$$

where $[M]_0$ and $[X]_0$ represent the initial concentrations of NCA and DEA, respectively, and $-(d[M]/dt)_{t=0}$ represents the rate of NCA consumption as measured by the rate of CO_2 evolution at time 0. In this experiment, the reaction product was not isolated and identified. k values are indicated in Table II together with the values in the literature. It is observed that the reactivity of the base tends to decrease as the N-substituent becomes bulkier (methyl > ethyl > *n*-propyl). This is explained in terms of the steric effect of the N-substituent because the basicity has been found to be little affected by the N-substituent.[9] It was assumed that the relative reactivity of the terminal base group in the polymer catalyst is equal to the relative reactivity of the corresponding N-alkylglycine DEA, and k_i values in Figure 4 were corrected for item (ii) using k values in Table II, polysarcosine DEA being taken as

Table II. Second-order rate constant (k) for the reaction between DL-β-phenylalanine NCA and various N-alkylglycine dialkylamides in nitrobenzene solution at 25°C

Amides	$_pK_b$ of amides[a]	k (l/mol·min)[b]
Sarcosine DMA[9]	5.21	18.55
Sarcosine DEA		21.2
N-ethylglycine EDA	5.2[15,c]	17.5
N-*n*-propylglycine DEA		11.5
N-isopropylglycine DMA[9]	5.32	10.80
N-cyclohexylglycine DMA[9]	5.10	10.51

[a] In water at 20°C.

[b] $k = -\frac{1}{[M]_0[X]_0}\left(\frac{d[M]}{dt}\right)_{t=0}$.

[c] Value at 26°C.

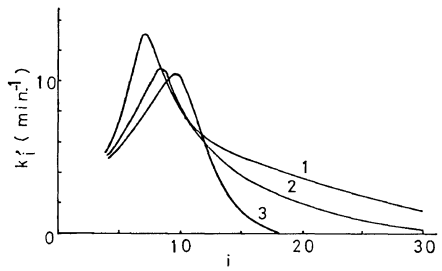

Figure 5. Corrected initial rate constant (k_i') *vs.* reaction site on polymer catalyst (i).
1: Polysarcosine DEA.
2: Poly(N-ethylglycine) DEA.
3: Poly(N-*n*-propylglycine) DEA.

standard. The correction was made with poly(N-ethylglycine) DEA, for instance, by multiplying k_i by 21.2/17.5 (k of sarcosine DEA/k of N-ethylglycine DEA). The corrected k_i values were denoted by k_i'. Figure 5 shows the plot of k_i' against i.

When corrected for the reactivity of the terminal base group, three poly(N-alkylglycine) DEA's were found to have almost identical reactivity in chain-effect polymerization. However, an inspection of Figure 5 shows that polysarcosine DEA has a slightly larger k_i' value and is more reactive than poly(N-ethylglycine) DEA and poly(N-*n*-propylglycine) DEA, although the difference in k_i' values is subtle. It is also shown that the maximum k_i value appears at the position of larger i (more remote from the terminal base group) as the N-substituent becomes bulkier. This phenomenon could be explained in terms of item (iii). As the N-substituent becomes bulkier, the flexibility of the polymer chain would decrease. Hence the collision frequency between the adsorbed NCA and the terminal base group (k_i') would be decreased. Furthermore, the terminal base group may collide more easily with NCA molecules adsorbed on a distant carbonyl group than those in its vicinity. However, this conclusion is only tentative because the difference of k_i' values is not so significant. Experiments where far less flexible polymer catalysts are involved are being carried out to make this point clearer.

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