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The Polymerization of α-Amino Acid *N*-Carboxy Anhydride (4-Alkyl-oxazolidine-dione). IV. A Novel Synthesis of a Block Copolypeptide

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It has been found that, when the polymerization of NCA is initiated with *n*-butylamine in acetonitrile, the amino end group in the polymer produced which is insoluble in the solvent is active (Y. Iwakura, K. Uno and M. Oya, *J. Polym. Sci., Part A-1*, **8**, 2165 (1968)). The amino end group in the polymer isolated without drying could initiate the polymerization of NCA in acetonitrile. By using this characteristic of the polymer, block copolypeptides consisting of various kinds of α-amino acid and of various chain lengths were synthesized. The solubility of the block copolypeptides in a solvent was different from that of the random copolypeptides obtained by the usual copolymerization of a mixture of NCA in acetonitrile. Sometimes the infrared spectra of the block copolypeptides were different from those of the random copolypeptides.

A block copolypeptide has been synthesized by polymerizing α-amino acid *N*-carboxy anhydride, with a soluble, low-molecular weight polypeptide, *i.e.*, polysarcosine dimethylamide, poly-γ-benzyl-L-glutamate, or poly-DL-lysine, as the initiator.[1-3] Because an insoluble polypeptide in an inert solvent for NCA's, *i.e.*, polyglycine and poly-L-alanine, could not initiate the polymerization of NCA, it has generally been considered that a block copolypeptide consisting of insoluble polypeptides would be difficult to synthesize. Therefore, the kind of block copolypeptides would be limited when this method was used.

It has been considered that the polymerization of NCA's initiated with primary amine in dioxane and tetrahydrofuran proceeds by both route by nucleophilic attack and by proton abstraction from the nitrogen of the NCA's.[4] When the polymerization is initiated with the amino end group of polypeptide by proton abstraction from 3N on NCA, a block copolypeptide containing the initiator polymer would not be produced. Further, it is very difficult to synthesize block copolypeptides consisting of three kinds of α-amino acids.

When the polymerization of NCA was carried out in acetonitrile, with *n*-butylamine as an initiator, we found that the polymer formed existed in the precipitation state that the initiator was consumed completely in the earlier stage of the polymeriza-

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[1] C. H. Bamford, *Proc. Roy. Soc., Ser. A*, **223**, 495 (1957).

[2] P. Doty, *J. Amer. Chem. Soc.*, **79**, 3961 (1957).

[3] R. T. Ingwall and H. A. Scheraga, *Biopolymers*, **6**, 331 (1968).

[4] M. Goodman and J. Hutchinson., *J. Amer. Chem. Soc.*, **88**, 3627 (1966).

tion, that further propagation was performed by the attack of the amino end group of the precipitated polymer on the C-5 carbonyl carbon of NCA in acetonitrile, with almost no termination, and that the degree of polymerization increased with an increase in the reaction period in the precipitation state.[1] These case, it was found that the amino end group of the polymer was active in the precipitation state in acetonitrile. Such a consideration indicated the possibility of preparing various kinds of block copolypeptides which are insoluble in an inert solvent for NCA's.

In this paper the preparation of block copolypeptides containing an insoluble polypeptide in an inert solvent for NCA's and more than two kinds of α-amino acid will be described.

Results

The polypeptide obtained by the polymerization of the first α-amino acid NCA with *n*-butylamine in acetonitrile was isolated by filtration and washed well with absolute acetonitrile in order to remove any residual NCA. The isolated polypeptide was without drying put into a second NCA solution in acetonitrile. The polymerization was initiated by the attack of the amino end group of the first polymer on the C-5 carbonyl of the second NCA; a block copolypeptide consisting of two kinds of α-amino acid was thus produced. This block copolypeptide was isolated again by filtration, washed well with absolute acetonitrile, and put into a third NCA solution in acetonitrile. Block copolypeptides consisting of three kinds of α-amino acid were thus produced. The polypeptides obtained at the first or second step became inactive upon drying. The polypeptides obtained were extracted in solvents consisting of homopolypeptides in order to remove any coexisting homopolymer. As the polymerization was carried out in a heterogeneous system, there was no need to use the first polypeptide (initiator polymer) which is soluble in the polymerization solvent, acetonitrile. Any α-amino acid NCA could be used for this synthesis.

The average sequence length of each component in the block copolypeptides could be controlled by changing the polymerization period, the temperature and the monomer concentration for each step.

By this method, various kinds of block copolypeptides were synthesized. The block copolypeptides thus obtained are shown in Tables 1 and 2. The solubility of the block copolypeptides obtained was different in several solvents compared with those of the homopolypeptides consisting of the α-amino acids in the block copolypeptides. For example, the block copolypeptide consisting of L-leucine and DL-alanine (B-1) was soluble in formic acid in spite of the insolubility of poly-L-leucine in this solvent. However, the solubility of the block copolypeptide was essentially dependent on the sequence length of each component. Copolypeptide (B-2), which was constructed of a chain of L-leucine rather longer than that of B-1, became insoluble in formic acid and was still insoluble in hot benzene. The solubility of the block copolypeptide was different from that of the corresponding random copolypeptide obtained by the polymerization of the mixture of two or three kinds of NCA's. For example, the B-2 block copolypeptide in Table 1 was insoluble in dichloroacetic acid, while the corresponding random copolypeptide, R-1, was soluble in dichloroacetic acid and highly swelled in ether. Moreover, the solubility of the B-4 and B-5 block copolypeptides is different from that of the corresponding random copolypeptides, R-2 and R-3

TABLE 1. BLOCK AND RANDOM COPOLYPEPTIDES CONSISTED OF TWO COMPONENTS

No.	N%	$\eta_{sp/c}$	Component (wt% ratio)	Solvent of polymer	Non solvent of polymer
Block					
B-1	17.88	0.68	L-Leu 15 DL-Ala 85	Formic acid	Hot benzene
B-2	16.50	0.96	L-Leu 35 DL-Ala 65	Trifluoroacetic acid	Formic acid, dichloro acetic acid, hot benzene
B-3	11.80	0.55♯	γ-Me-L-Glu 73 DL-Ala 27	Dichloroacetic acid	Formic acid, hot chloroform
B-4	13.77	0.56♯	γ-Me-L-Glu 54 DL-Ala 46	Dichloroacetic acid	Formic acid, hot chloroform
B-5	20.14	0.68	L-Ala 68 Gly 32	Trifluoroacetic acid	Dichloroacetic acid
Random					
R-1	16.50	0.67	L-Leu 35 DL-Ala 65	Dichloroacetic acid Ether (Highly swelled)	Benzene
R-2	14.00	0.25	γ-Me-L-Glu 55 DL-Ala 45	Formic acid	
R-3	20.41	0.44	L-Ala 55 Gly 45	Dichloroacetic acid	

♯: $\eta_{sp/c}$ in DCA.

TABLE 2. BLOCK COPOLYPEPTIDES CONSISTED OF THREE COMPONENTS

No.	N%	$\eta_{sp/c}$	Component	Solvent	Nonsolvent
B-6	18.23	0.34	γ-Bz-L-Glu DL-Ala Gly	Trifluoroacetic acid	Formic acid, dichloroethane, tetrahydrofuran
B-7	16.95	0.24	γ-Bz-L-Glu L-Leu Gly	Trifluoroacetic acid	Formic acid, hot dichloroethane
B-8	11.24	0.99	L-Ala γ-Bz-L-Glu L-Leu	Trifluoroacetic acid	Dichloroethane, hot benzene tetrahydrofuran

N% (found) of homopolymers: Polyglycine 22.45%, Poly-DL-alanine 18.99%, Poly-L-leucine 10.99%, Poly-L-alanine 18.81%, Poly-γ-methyl-L-glutamate 9.7%.

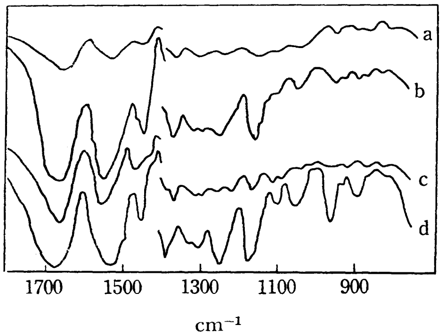

Fig. 1. Infrared spectra of block copolymer and random copolymer consisted of DL-alanine and L-leucine.

a. Block copolypeptide B-2 shown in Table 1.
b. Random copolypeptide R-1 shown in Table 1.
c. Poly-L-leucine obtained in acetonitrile.
d. Poly-DL-alanine obtained in acetonitrile.

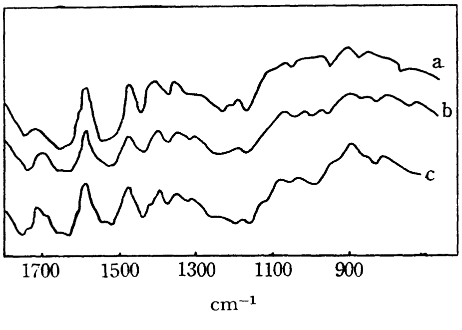

Fig. 2. Infrared spectra of block copolymer and random copolymer consisted of DL-alanine and γ-methyl L-glutamate.

a. Random copolypeptide R-2 in Table 1.
b. Block copolypeptide B-3 in Table 1.
c. Poly-γ-methyl-L-glutamate.

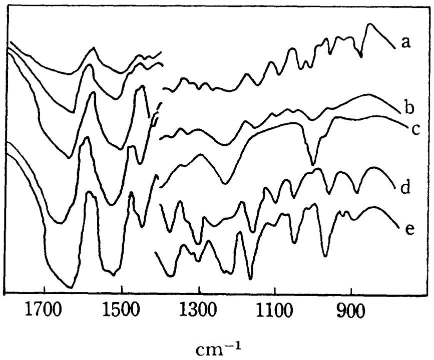

Fig. 3. Infrared spectra of block copolymer and random copolymer consisted of L-alanine and glycine.

a. Block copolypeptide B-5 in Table 1.
b. Random copolypeptide R-3 in Table 1.
c. Polyglycine.
d. α-Forms poly-L-alanine.
e. β-Forms poly-L-alanine.

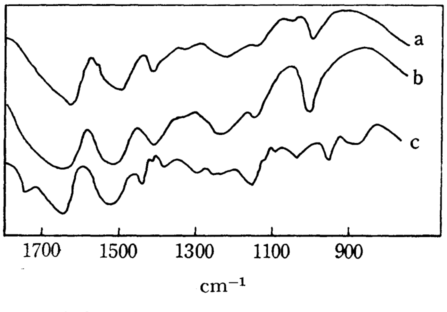

Fig. 4. Infrared spectra of block copolymer consisted of three kinds of α-amino acid.

a. Block copolypeptide B-6 in Table 2.
b. Block copolypeptide B-7 in Table 2.
c. Block copolypeptide B-8 in Table 2.

respectively. Such behavior supports the theory that the copolypeptide obtained is of the block type. The infrared spectra of the block copolypeptides described in Tables 1 and 2 are shown in Fig. 1—4. Sometimes, the spectra of the block copolypeptides were clearly different from those of the random copolypeptide with the same components. For example, the spectra of the block copolypeptide consisting of L-alanine (68 mol%) and glycine (32 mol%) showed the spectra of both poly-L-alanine and polyglycine (an absorption at

1305 cm^{-1} which is characteristic of the α-helix of poly-L-alanine), but it was different from that of the random copolypeptide with the same component no absorption appeared at 1305 cm^{-1} (Fig. 3).

Experimental

The NCA's used in this study were prepared in a high degree of purity by methods reported previously.5)

Synthesis of Block Copolypeptides. (L-Leu)$_m$-(DL-Ala)$_n$: First, L-leucine NCA (0.5 g) was polymerized in acetonitrile (10 m*l*), using 1.0 mol% of *n*-butylamine (to NCA) as the catalyst, for 1.7 hr at 30°C. The polymer precipitated thus was isolated by filtration and washed with absolute acetonitrile. The polymer isolated without drying was added to 10 m*l* of a DL-alanine NCA solution in acetonitrile (0.05 g/m*l*), after which the mixture was kept standing for 120 hr at 30°C. The polymer thus formed was washed with hot benzene in order to remove the coexisting homopolymer. The B-1 block copolymer (0.384 g) was thus obtained. When the polymerization was stopped at the first step, the specific viscosity ($\eta_{sp/c}$) of the poly-L-leucine obtained was 0.20 (in TFA), and its yield was 0.048 g.

The first and second steps of the polymerization described above lengthed 18 and 120 hr respectively, and the polymer isolated was washed with formic acid and hot benzene. A block copolymer (B-2) (0.376 g) was thus obtained. When the polymerization was stopped at the first step, the $\eta_{sp/c}$ value of the poly-L-leucine obtained was 0.68, and its yield was 0.20 g.

(γ-Me-L-Glu)$_m$-(DL-Ala)$_n$: First, γ-methyl-L-glutamate NCA (0.5 g) was polymerized in acetonitrile (10 m*l*), with 1.0 mol% of *n*-butylamine as catalyst, for 57 hr at 30°C. The polymer thus precipitated was isolated by filtration and washed well with absolute acetonitrile. The polymer isolated without drying was added to 20 m*l* of a DL-alanine NCA solution in acetonitrile (0.025 g/m*l*), and the mixture was left to stand for 207 hr at 30°C. The polymer thus obtained was washed with hot chloroform and formic acid to remove the homopolymers. The B-3 copolymer (0.294 g) was thus obtained. When the polymerization was stopped at the first step, the $\eta_{sp/c}$ value of the poly-γ-methyl-L-glutamate obtained was 0.46 g, and its yield was 0.205 g.

Under the polymerization conditions described above, the concentration of the γ-methyl-L-glutamate NCA was reduced to 0.45 g/10 m*l*, the other conditions were the same as those described above. The B-4 polymer (0.129 g) was thus produced. When the polymerization was stopped at the first step, $\eta_{sp/c}$ value of the poly-γ-methyl-L-glutamate obtained was 0.44, and its yield was 0.189 g.

(L-Ala)$_m$-(Gly)$_n$: L-Alanine NCA (0.65 g) was polymerized in acetonitrile (13 m*l*), with 0.5 mol% of *n*-butylamine as an initiator, for 1.7 hr at 30°C. The polymer isolated without drying was added to 10 m*l* of a glycine NCA solution (0.044 g/m*l*) after which the mixture was kept standing for 12 hr at 30°C. The B-5 polymer (0.207 g) was thus obtained. In this case, the $\eta_{sp/c}$ value of the poly-L-alanine obtained at the first step was 0.45 (in TFA).

(γ-Bz-L-Glu)$_k$-(DL-Ala)$_m$-(Gly)$_n$: γ-Benzyl-L-glutamate NCA (0.5 g) was polymerized in acetonitrile (10 m*l*), with 1.0 mol% of *n*-butylamine as an initiator, for 4 hr at 20°C. The polymer thus precipitated was isolated by filtration and washed well with absolute acetonitrile. The polymer isolated without drying was added to 10 m*l* of a DL-alanine NCA solution in acetonitrile (0.05 g/m*l*), after which the mixture was kept standing for 16 hr at 20°C. The copolymer was isolated by filtration and washed sufficiently with absolute acetonitrile, and then the copolymer was again added to 10 m*l* of a glycine NCA solution in acetonitrile (0.05 g/m*l*) and the resulting mixture was kept for 23 hr at 20°C. The polymer thus isolated was washed with hot dichloroethane and formic acid to remove the homopolymer. The B-6 block copolymer (0.15 g) was thus produced. When the polymerization was stopped at the first or second step, the specific viscosities of the polymers thus obtained were 0.107 and 0.243, and their yields were 0.045 g and 0.088 g respectively.

(γ-Bz-L-Glu)$_k$-(L-Leu)$_m$-(Gly)$_n$: The first step of the polymerization was carried out under the same conditions as were used for B-6, while the second and third steps of the polymerization were carried out by using 10 m*l* of an L-leucine NCA solution in acetonitrile (0.05 g/m*l*) for 18 hr at 20°C, and by using 10 m*l* of a glycine NCA solution (0.05 g/m*l*) for 23 hr at 20°C respectively. The B-7 copolypeptide (0.118 g) was thus obtained. The polymer obtained in the second step of the polymerization had a specific viscosity of 0.118.

(L-Ala)$_k$-(γ-Bz-L-Glu)$_m$-(L-Leu)$_n$: The first, second, and third steps of the polymerization were carried out by using 10 m*l* of an L-alanine NCA solution in acetonitrile (0.05 g/m*l*) for 3 hr at 20°C, 10 m*l* of a γ-benzyl-L-glutamate NCA solution (0.05 g/m*l*) for 10 hr at 20°C, and 10 m*l* of an L-luecine solution (0.05 g/m*l*) for 10 hr at 20°C, respectively. The copolypeptide was washed with hot dichloroethane and hot benzene. The B-8 block copolypeptide (0.185 g) was thus obtained. The specific viscosities of the polymer obtained at first and second steps were 0.69 (in TFA) and 0.83 (in TFA) respectively.

Analysis of Copolypeptides. The compositions of the copolypeptides were determined by the analysis of the nitrogen in the polymer: the results are presented in Tables 1 and 2.

Further, paper-chromatographic analysis for the hydrolyzate of these copolymers with hydrochloric acid gave two or three spots for the corresponding α-amino acids in the copolymers.

The viscosity of the polymer was measured at a concentration of 0.25 g/100 m*l* in trifluoroacetic acid (no mark or TFA) or dichloroacetic acid (DCA), using an Ostwald viscometer and at 25°C.

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5) M. Oya, K. Uno and Y. Iwakura, *Kogyo Kagaku Zasshi*, **69**, 741 (1966).