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Synthesis and Characterization of Poly(*N*-cyanoethylglycine)

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ABSTRACT: *N*-Cyanoethylglycine was synthesized from glycine and acrylonitrile, and converted into its *N*-carboxyanhydride (NCA) by Leuch's method. The NCA was polymerized in a $HCONMe_2$ solution using an amine as the initiator to give, for the first time, a white, powderic, amorphous polymer having $\bar{P}_n = 15—16$. The rate of polymerization was much smaller than that of *N*-*n*-butylglycine NCA under the same conditions, and the low basicity of the amino group at the growing end, which is a result of an electron-withdrawing effect of cyanoethyl group, was found to be responsible for the slow polymerization. Poly(*N*-cyanoethylglycine) is soluble in aprotic solvents such as $HCONMe_2$, Me_2SO, and Me_2CO, and its main-chain peptide bonds comprise *ca.* 70% of *cis* form and *ca.* 30% of *trans* form in Me_2SO. Poly(*N*-cyanoethylglycine) is therefore flexible and compact in organic solvents. A part of the side chains of poly(*N*-cyanoethylglycine) was reduced into 3-aminopropyl groups by the hydrogenation.

KEY WORDS *N*-Cyanoethylglycine NCA / Polymerizability / Poly(*N*-cyanoethylglycine) / Characterization / Hydrogenation / Poly(*N*-3-aminopropylglycine) /

Polymers carrying pendant reactive groups such as the cyanoethyl group are useful as functional materials.[1] Poly(α-amino acid) carrying pendant cyanoethyl groups, however, has not been reported. Among many possible reactions of the cyanoethyl group, the hydrogenation leading to the 3-aminopropyl group seems to be interesting because the latter adds to α-amino acid *N*-carboxyanhydride (NCA) and thus initiates the polymerization of NCA. Therefore, poly(*N*-cyanoethylglycine) is the precursor of a polymeric initiator for the graft copolymerization of α-amino acid NCA to produce a branched or a multi-chain poly(α-amino acid).

On account of the sensitivity of α-amino acid NCA toward water, alcohols, amines, and acids, the NCA polymerization is usually conducted in aprotic solvents. In contrast to common poly(α-amino acid)s which are soluble only in hydroxylic solvents, poly(*N*-alkylamino acid)s are soluble in aprotic organic solvents. This property of poly(*N*-alkylamino acid)s enables their use as polymeric initiators to synthesize branched poly(α-amino acid)s by the graft copolymerization. In poly(*N*-alkylamino acid)s, the main-chain amide bonds tend to assume either *trans* or *cis* conformation.[2,3] This property of poly(*N*-alkylamino acid)s allows different conformational states to be possible and renders flexibility.[4] Furthermore, the participation of *cis* amide bond makes the polymer structure somewhat more compact.[5]

If the hydrogenated poly(*N*-cyanoethylglycine), that is, poly(*N*-3-aminopropylglycine), is an initiator for the polymerizations of α-amino acid NCA's carrying different functional substituents, the resulting branched poly(α-amino acid) should be differently functionalized along a flexible and compact chain. This situation seems to be very favorable for binding and transporting various kinds of small molecules effectively. Some functional polymers along this line, such as serum albumin[6] and hydrophobic branched

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polyethyleneimine,[7] have been noted to bind and transport small molecules effectively.

As the starting polymer to develop branched poly(α-amino acid)s which bind and transport small molecules effectively, we synthesized and characterized poly(*N*-cyanoethylglycine).

EXPERIMENTAL

Reagents

N-Cyanoethylglycine was synthesized from glycine and acrylonitrile in alkaline solution.[8] *N*-Carbobenzyloxy—*N*-cyanoethylglycine was treated with thionyl chloride at 60°C for 30 min to obtain *N*-cyanoethylglycine NCA.[9] The NCA was purified by recrystallization from AcOEt/*n*-C_6H_{14}; yield 63.4%, mp 108.5°C (lit.[9] mp 105°C).

Anal. Calcd for $C_6H_6N_2O_3$: C, 46.76; H, 3.92; N, 18.18. Found: C, 47.05; H, 3.76; N, 18.36.

A direct synthesis of *N*-cyanoethylglycine NCA from *N*-cyanoethylglycine and trichloromethyl chloroformate[10] was unsuccessful.

N-Cyanoethylglycine NCA was added into a large excess of Et_2NH, and the mixture was distilled in vacuum to obtain *N*-cyanoethylglycine diethylamide.

N-*n*-Butylglycine NCA and *N*-*n*-butylglycine diethylamide were synthesized as reported previously.[11]

Poly(*N*-cyanoethylglycine) was synthesized as follows: *N*-cyanoethylglycine NCA (5×10^{-1} mol/*l*) and *N*-cyanoethylglycine diethylamide (2.5×10^{-2} mol/*l*) were dissolved in $HCONMe_2$. The mixed solution was sealed into a glass ampoule under vacuum and allowed to react at room temperature for 30 days. When the infrared absorptions at 1850 and 1780 cm^{-1} which are characteristic of NCA disappeared, the reaction solution was poured into ether to precipitate the reaction product. The product poly(*N*-cyanoethylglycine) was precipitated three times from acetone solution with either.

Procedures

The rate of nucleophilic addition reaction of amines to *N*-cyanoethylglycine NCA was determined as following. In a constant volume apparatus kept at 25°C, the $HCONMe_2$ solutions of *N*-cyanoethylglycine NCA (0.1 mol/*l*) and appropriate amines (0.1 mol/*l*) were mixed. The amount of carbon dioxide liberated in the reaction was measured,[12] and the conversion of NCA was calculated. From the time—conversion curve thus obtained, the first-order rate constant was determined.

Infrared spectra (KBr disk) of poly(*N*-cyanoethylglycine) was investigated using Shimazu IR 27G spectrometer.

100-MHz nuclear magnetic resonance spectra (Me_2SO-d_6 solution) of poly(*N*-cyanoethylglycine) was investigated at 31.5°C using Varian HA-100 spectrometer.

The molecular weight of poly(*N*-cyanoethylglycine) was determined from the vapor-pressure depression of the aqueous solution using Hitachi-117-type apparatus.

The amino groups in polymers and initiators were determined by the back-titration of *N*/100-HCl solution with *N*/100-NaOH solution using Hiranuma RAT-101-type potentiometer.

X-ray analysis of poly(*N*-cyanoethylglycine) was carried out with powderic samples pretreated at 78°C or 100°C.

RESULTS AND DISCUSSION

Polymerization of N-Cyanoethylglycine NCA

Using *N*-cyanoethylglycine diethylamide as an initiator *N*-cyanoethylglycine NCA was polymerized in $HCONMe_2$ at room temperature, the NCA/initiator molar ratio being 20. To complete the polymerization, it took about three months when the NCA concentration was 0.1 mol/*l*, and about one month when the NCA concentration was 0.5 mol/*l*. This is much slower than the polymerizations of the usual α-amino acid NCA's.[13] Hanby, *et al.*,[14] and Ballard and Bamford[15] have investigated the reactivities of *N*-substituted α-amino acid NCA and found that they were usually less reactive than unsubstituted α-amino acid NCA's. The low reactivity of *N*-substituted α-amino acid NCA has been interpreted in terms of steric hindrance by the *N*-substituent in the propagation reaction. *N*-Cyanoethylglycine diethylamide and *N*-*n*-butylglycine diethylamide were considered as the models for the growing ends of the polymerizations of *N*-cyanoethylglycine NCA and *N*-*n*-butylglycine NCA, respectively, and their pK_a values and the second-order rate constant for the nucleophilic addition reaction

Table I. pK_a values of amine R–NH–CH_2–CO–NEt_2 and their nucleophilic addition reaction[a] to *N*-cyanoethylglycine NCA

R	pK_a[b]	k, $mol^{-1}\,l\,min^{-1}$
$-CH_2CH_2CN$	6.44	6.5×10^{-3}
$-CH_2CH_2CH_2CH_3$	8.95	6.1×10^{-1}

[a] Solvent, $HCONMe_2$; temp, 25°C; $[NCA]_0=0.1$ mol/*l*; $[amine]_0=0.1$ mol/*l*.
[b] Determined by titration.

Table II. Properties[a] of poly(*N*-cyanoethylglycine)

mp, °C	MW	Terminal amine, meq/g	DP[d]	Elemental analysis		
				C%	H%	N%
118—124	1700[b]	0.559[c]	15.2	53.40	5.79	24.87
	(2465)	(0.406)	(21)	(54.40)	(5.76)	(25.59)

[a] Theoretical or calculated values are shown in parentheses.
[b] Determined by vapor-pressure depression.
[c] Determined by potentiometric titration.
[d] Calculated on the basis of terminal amine content.

towards *N*-cyanoethylglycine NCA were determined and are compared in Table I. It is conceivable that the low reactivity of *N*-cyanoethylglycine NCA is due not only to the steric hindrance of *N*-substituent but also to the low basicity of terminal amino group. Despite that the two sorts of amines carry an *N*-substituent of similar size, the nucleophilic reactivity of *N*-cyanoethylglycine diethylamide is only about 1/100 as small as that of *N*-butylglycine diethylamide. Furthermore, the former is less basic by *ca.* 2.5 pK_a unit than the latter. These findings indicate that the terminal amino group in the polymerization of *N*-cyanoethylglycine NCA is deactivated by the strongly electron-withdrawing cyanoethyl group, resulting in a slow polymerization.

Properties of Poly(N-cyanoethylglycine)

The reaction solution was homogeneous throughout the polymerization. The resulting polymer was white and powderic. The amine concentration was unchanged before and after the polymerization. Under the condition that the NCA/initiator molar ratio was 20, the molecular weight of the resulting polymer, which was collected from the polymerization solution at the complete conversion of NCA, was determined to be 1700 by the vapor-pressure depression method. On the other hand, the content of amino group in the polymer was 0.559 meq/g, which corresponded to the molecular weight 1790. Both values are in close agreement with each other. The average degree of polymerization is 15.2, which is a little smaller than 21, the expected values from the NCA/initiator molar ratio.[16] These observations indicate that the polymerization of *N*-cyanoethylglycine NCA proceeds *via* normal-amine-type mechanism,[17] and goes to the completion without termination reactions. The properties of poly(*N*-cyanoethylglycine) are summarized in Table II.

Poly(*N*-cyanoethylglycine) is soluble in $HCONMe_2$, Me_2SO, and water, slightly soluble in Me_2CO (in 30—50 times the amount of solvent), and insoluble in MeOH, dioxane, and ether. The good solubility of polymer in organic solvents, which is undoubtedly related to the *N*-substitution, is favorable for the further reactions of the polymer. However, the solubility of poly(*N*-cyanoethylglycine) is inferior to poly(*N*-*n*-butylglycine) (DP=30) which is also soluble in MeOH and $CHCl_3$.

Structure of Poly(N-cyanoethylglycine)

The IR spectra of *N*-cyanoethylglycine NCA and its polymer are shown in Figure 1. The 1850- and 1780-cm^{-1} absorptions due to the five-membered cyclic acid-anhydride group of NCA do not appear in the spectrum of polymer. In the polymer, a strong absorption due to the peptide carbonyl group is observed at 1660 cm^{-1}. The sharp absorption at 2250 cm^{-1} is characteristic of nitrile group.

The 100-MHz NMR spectrum of poly(*N*-cyanoethylglycine) is shown in Figure 2. Two resonance signals at 4.61 and 4.20 ppm are ascribable to the main-chain $C^{\alpha}H_2$ protons. The upfield signal is assigned to the $C^{\alpha}H_2$ protons adjacent to *trans*-peptide bond, according to the NMR investigations on a series of poly(*N*-alkylglycine)s by Sisido, *et al.*[18,19] The resonance signal at 3.56 ppm is assignable to the $C^{\beta}H_2$ protons and that at 2.67 ppm to the $C^{\gamma}H_2$ protons. For this assignment, the NMR spectra of poly(*N*-*n*-butylglycine) and poly(*N*-*n*-propyl-

glycine) were used. The area ratio of the three kinds of signals are 1 : 1 : 1. The ratio of the peak areas of the high and the low $C^{\alpha}H_2$ signals indicates that the main-chain peptide bonds of poly(*N*-cyanoethylglycine) consists of about 70% of *cis* form and about 30% of *trans* form. According to Sisido, *et al.*,[19] the *trans*/*cis* ratio of peptide bond in *N*-acetyl, *N*-alkyl-L-alanine dimethylamide varies with the *N*-alkyl group: the *cis* content in CH_2Cl_2 at 35°C is 18% for *N*-

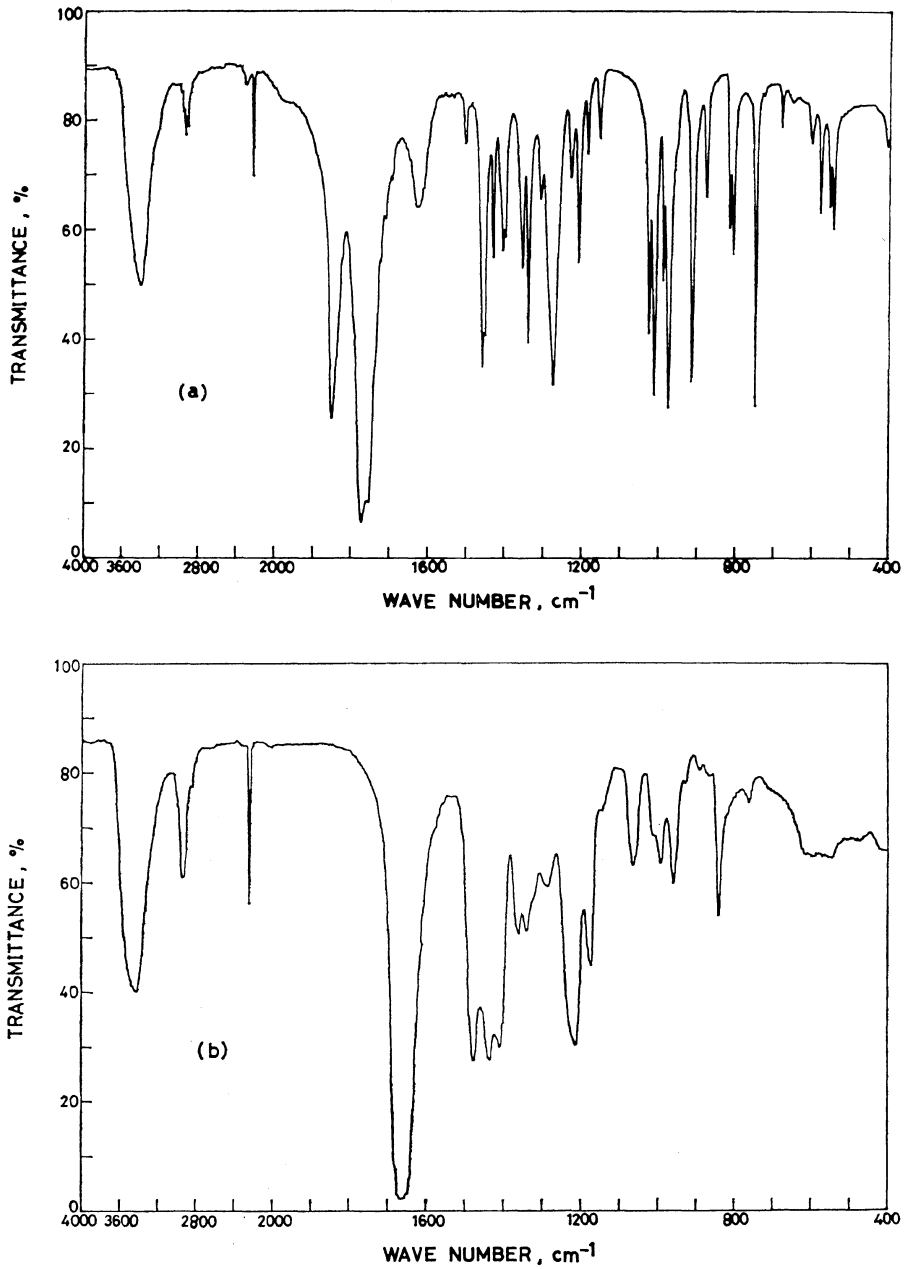


Figure 1. Infrared spectra of *N*-cyanoethylglycine NCA (a) and poly(*N*-cyanoethylglycine) (b); KBr disk.

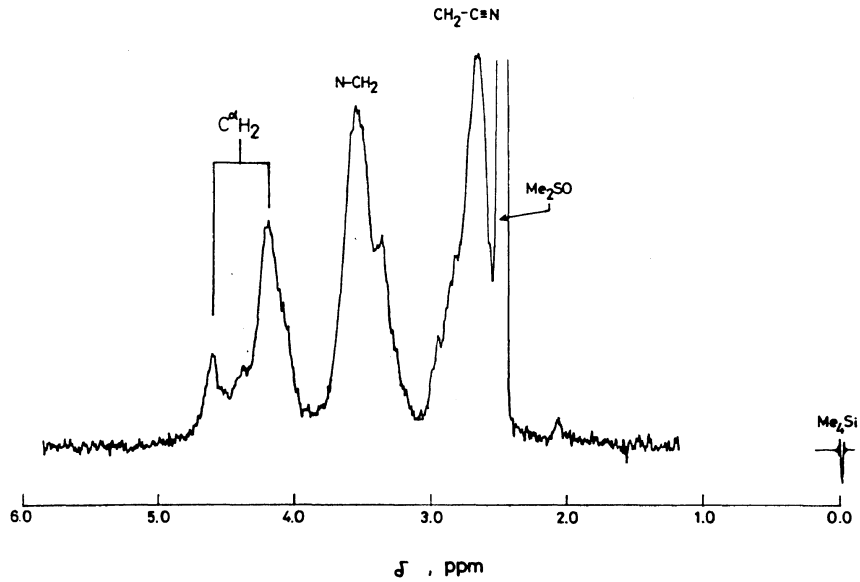
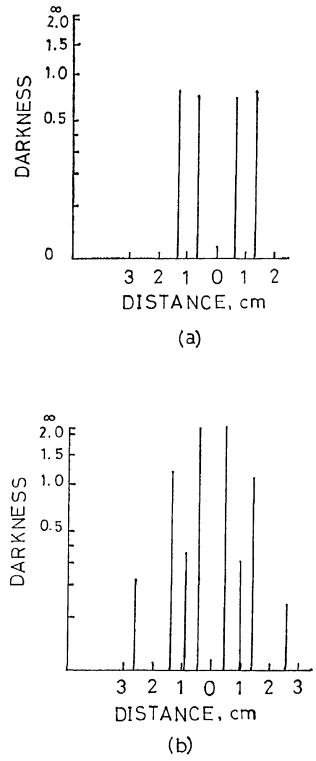


Figure 2. 100-MHz NMR spectrum of poly(*N*-cyanoethylglycine) in Me_2SO-d_6 at 21.5°C, Me_4Si being an internal standard.



methyl, 56% for *N*-ethyl, 74% for *N*-*n*-propyl, and 71% for *N*-*n*-butyl. In general, the *cis* content increases as the solvent is changed from CH_2Cl_2 to Me_2SO.[18] These experimental results together suggest that the *cis* content in poly(*N*-cyanoethylglycine) would be nearly the same as those in poly(*N*-*n*-butylglycine) and poly(*N*-*n*-propylglycine). Since the *cis* content of poly(*N*-alkylglycine) is closely related to the bulkiness of the *N*-substituent,[18] the estimated *cis* content of poly(*N*-cyanoethylglycine) is reasonable. The occurrence of *cis* and *trans* peptide bonds in poly(*N*-cyanoethylglycine) makes the whole molecule flexible[4] and allows the polymer to assume a compact conformation.[5] These conformational features of poly(*N*-cyanoethylglycine) are promising for the design of a functional polymer to bind and transport small molecules effectively.

The X-ray diffraction pattern of poly(*N*-cyanoethylglycine) was obtained and compared with

Figure 3. Schematic representation of X-ray diffraction patterns: (a), poly(*N*-cyanoethylglycine), DP 15, spacing, 4.9 and 2.7 Å; (b), poly(*N*-*n*-butylglycine), DP 30, spacing, 13.8, 6.7, 4.6, and 2.7 Å. Ordinate is a relative transmittance of light from each diffraction. Abscissa is the distance of each diffraction from the center. The camera distance was 39.96 mm.

that of poly(*N*-*n*-butylglycine) in Figure 3. It is understandable that poly(*N*-cyanoethylglycine) is less crystalline than poly(*N*-*n*-butylglycine) because of highly polar pendant nitrile groups.

Reduction of Poly(N-cyanoethylglycine)

The preliminary experiments were carried out to investigate the catalytic hydrogenation of pendant cyanoethyl groups into 3-aminopropyl groups which will be the initiator for the graft polymerization of various α-amino acid NCA's. Poly(*N*-cyanoethylglycine) was dissolved in H-$CONMe_2$ and hydrogenated with Raney Ni as a catalyst. The conversion of nitrile groups into primary amino groups were found by titration to be 12.7 mol%. This is equivalent to about 1.2 amino groups per chain, and is far less than that expected. During the hydrogeneration with H_2/Ni, side reactions took place to produce a crosslinked polypeptide. The gel of crosslinked polypeptide must have covered the catalyst surface and thus deactivated it. To overcome this difficulty, an alternative way leading to poly(*N*-3-aminopropylglycine) is currently being developed. It involves the preparation of *N*-(3-carbobenzyloxyaminopropyl)glycine by the reaction of bromoacetic acid and *N*-carbobenzyloxy-1,3-propanediamine, the polymerization of the amino acid by NCA method, and the decarbobenzyloxylation of the resulting polymer.

To summarize the experimental results, *N*-cyanoethylglycine was synthesized, and then converted into poly(*N*-cyanoethylglycine) by the NCA method for the first time. The polymer having $\bar{P}_n$ *ca.* 15 was obtained and it was amorphous and soluble in various aprotic organic solvents. The main-chain peptide bonds of the polymer were composed of *cis* as well as *trans* bonds, the former being predominant (*ca.* 70%) in Me_2SO.

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