Investigation of the solid-state polymerization of *N*-carboxy α-amino acid anhydrides with reference to their crystal structures*

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Solid-state polymerizations of *N*-carboxy anhydrides (NCAs) of α-amino acids (L-leucine, L-alanine, γ-benzyl-L-glutamate and glycine) were carried out using butylamine as initiator in hexane at 20–50°C. The results were compared with those for polymerization in acetonitrile solution. L-Leucine NCA was the most reactive among the NCAs examined, and formed high-molecular-weight polypeptide in the solid state. In contrast, L-alanine NCA polymerized faster than any other NCAs examined and formed high-molecular-weight polypeptide in acetonitrile compared to the solid-state polymerization. X-ray analysis, electron microscopy and infra-red spectroscopy revealed that L-leucine NCA polymerized to form α-helical polypeptide predominantly along the *c* axis in the crystal, while the α-helical polymer grows in random directions in the crystal of L-alanine NCA. Although γ-benzyl-L-glutamate NCA polymerized a little slower in the solid state than in acetonitrile, its polymerization reached 100% conversion and formed polypeptide with higher molecular weight than the value expected from the molar ratio of the monomer to the initiator. Glycine NCA polymerized very slowly in the solid state. These differences could be explained by their crystal structures. Favourable features for solid-state polymerization were found in the crystal of L-leucine NCA: five-membered rings of the NCA make a layer sandwiched by two hydrophobic side-chain layers and the NCA molecules polymerize to form the α-helical polypeptide in a layer without any interference from the other layers. On the other hand, such a layer structure was not seen in the crystal structure of L-alanine NCA. In the crystal of γ-benzyl-L-glutamate NCA, the layer structure similar to L-leucine NCA was observed and this seemed to activate the polymerization of the NCA, while the long side chains were considered to make molecular rotation difficult to some extent in the crystal. Glycine NCA seemed to be stable because of the dimer structure in the crystal.

(Keywords: solid-state polymerization; amino acid anhydrides; crystal structure; polypeptide; characterization)

INTRODUCTION

It has been expected that, in solid-state polymerization, the crystal structures of the reacting monomers have some effects on their reactivities or the molecular structures of the resulting polymers. Various vinyl monomers, cyclic monomers such as trioxane, tetroxane and trithiane, diacetylene derivatives, and other monomers have been polymerized in the solid state, and topochemical reactions have been found in several cases[1–7]. On the other hand, polycondensation of some cyclic amides and cyclic diesters was carried out in the solid state[8,9]. It is of interest that polymeric sulphur nitride, $(SN)_x$, which gives anisotropic electrical conductivity, is obtained by topochemical solid-state polymerization of cyclic disulphur dinitride, S_2N_2 (refs 10, 11).

N-Carboxy anhydrides of α-amino acids (α-amino acid NCAs) have been extensively used as monomers for the preparation of synthetic polypeptides. Their polymerization mechanism in solution has been investigated by numerous workers[12–15]. It has been known that several amino acid NCAs are very sensitive to moisture in the air and polymerize in the crystalline state at room temperature. Although a few studies were reported on the solid-state polymerization of some α-amino acid NCAs[16–18], no discussion was made on the relation between their reactivity and crystal structures, because the crystal structures of the NCAs have not been determined for a long time since the first synthesis of glycine NCA by Leuchs in 1906[19]. Several methods were considered to carry out the solid-state polymerization of the amino acid NCAs: to expose the crystals to air containing moisture or to heat them to a temperature below their melting points. However, these gave irreproducible results. Oya *et al.* reported that L-valine NCA and L-leucine NCA could be polymerized in the solid state when their crystals were placed in hexane (non-solvent for the NCAs) containing triethylamine as an initiator[20]. We carried out the polymerization of several α-amino acid NCAs in hexane and observed that the polymerization proceeded in the solid state. However, as was pointed out by Morawetz[1], exact kinetic studies are impossible for solid-state polymerization because crystal imperfections should affect the reaction rate. In order to estimte averaged reaction rates, we crushed NCA

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crystals mildly and used only the crystals that passed thorugh a 100 mesh screen for the solid-state polymerization. As a result, it was suggested that the solid-state polymerizability of the amino acid NCAs depended on the kind of amino acid[21]. In parallel to these investigations, we determined the crystal structures of glycine NCA, L-alanine NCA, L-leucine NCA, γ-benzyl-L-glutamate (BLG) NCA and L-valine NCA by X-ray diffraction[22–26]. In the previous paper, we studied the kinetics of the polymerization of L-leucine NCA and L-alanine NCA in the solid state using crystals below 100 mesh (all the crystals were prepared by rapid crystallization using an excess of a precipitant) and discussed the differences in the polymerizability of both NCAs with reference to their crystal structures[27]. As the polymerization of the NCAs proceeds via the evolution of carbon dioxide, their crystals should be considerably deformed during the solid-state polymerization. Nevertheless, L-leucine NCA was more reactive in the solid state than in acetonitrile solution. In addition, L-leucine NCA polymerized much faster than any other NCAs and formed high-molecular-weight polypeptide in the solid state. These facts suggest that the molecular arrangement in the crystal plays an important role in determining the solid-state polymerizability of the NCAs.

In the present article, a review is presented for the relation between the polymerizability of four kinds of NCAs and their crystal structures.

EXPERIMENTAL

Materials

Amino acid NCAs were synthesized in the manner described previously using commercial trichloromethyl chloroformate and amino acids[21,27]. Hexane, ethyl acetate, acetonitrile (ACN), tetrahydrofuran (THF), butylamine, trifluoroacetic acid (TFA), dichloroacetic acid (DCA) and the other reagents were purified by the usual methods.

Polymerization

Fine NCA crystals were prepared by the addition of 100 volumes of hexane into the NCA saturated solution in ethyl acetate (resulting crystals were passed through a 100 mesh screen). A series of kinetic experiments were carried out using crystals prepared at the same time. Polymerization rates were estimated from the amount of resulting carbon dioxide during the polymerization. Polypeptides for characterization were obtained by removing unreactive NCA in ethyl acetate as described previously[21,27]. The molar ratio of each amino acid NCA to butylamine ([A]/[I]) was 200.

Characterization of polymers

Measurements of intrinsic viscosities of polypeptides, i.r. spectroscopy, electron microscopy and X-ray diffraction studies were carried out in the same way as described previously[21,27]. The average degree of polymerization ($\overline{DP}_n$) of poly(γ-benzyl-L-glutamate) was estimated from the intrinsic viscosity [η] in DCA at 25°C using the following equation[28]:

$$[\eta] = 2.78 \times 10^{-5} \bar{M}_n^{0.87}$$

A Nikon POH polarizing microscope was used for the observation of polymerizing L-leucine NCA crystals.

RESULTS AND DISCUSSION

It was confirmed that the NCAs examined in the present work did not dissolve in hexane. When butylamine was added to the NCA crystals in hexane, polymerization took place at the surface of the crystals and proceeded into the inner part of them. One of the observations of the polymerization is given in *Figure 1a*, which is an optical micrograph of an L-leucine NCA crystal polymerizing in hexane with butylamine. A boundary is seen between the outer polymer-rich phase and the inner monomer-rich phase. In addition, a cleavage along the *a* axis is observed. Bubbles of carbon dioxide were evolved from the crystal in the course of polymerization. *Figure 1b* gives a photograph of the polymerizing L-leucine NCA crystal taken under a polarizing microscope. The inner bright part is monomer phase, which shows birefringence. Similar observations could be made on the other NCA crystals, although the rates of polymerization and crystal deformation were different from each other. Thus, it is apparent that the polymerization of the NCAs in hexane proceeds from the surface to the inner part of the crystals in the solid state.

In order to discuss the solid-state polymerization, it is desirable to carry out the polymerization of the NCAs in solution. However, a favourable solvent for the solution polymerization of all of the NCAs could not be

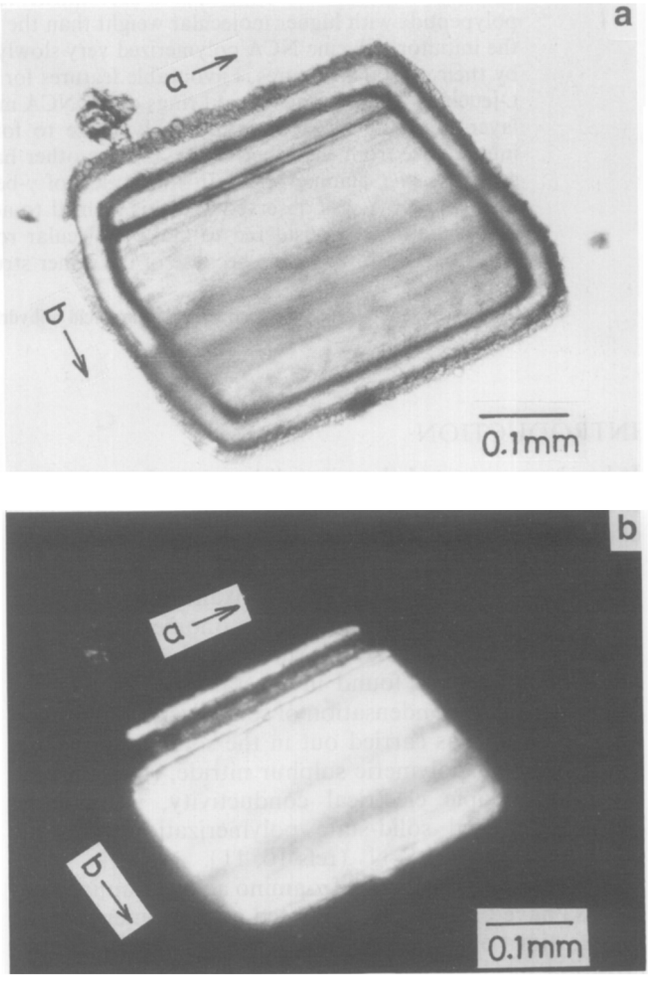


Figure 1 (a) Optical micrograph and (b) polarizing micrograph of a polymerizing L-leucine NCA crystal in hexane with butylamine, viewed along the *c* axis. Arrows in the figure indicate approximate directions of the *a* and *b* axes of the crystal

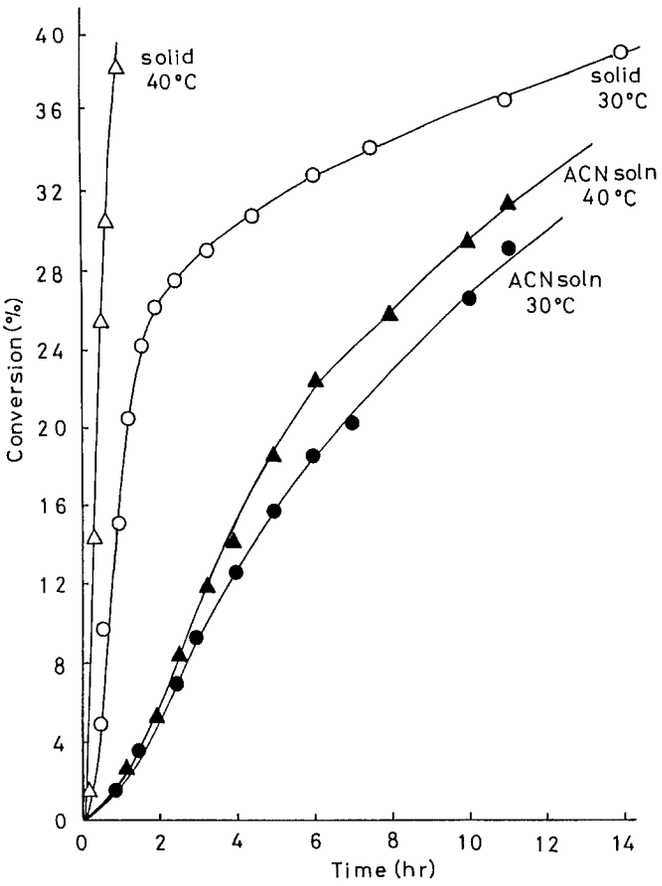


Figure 2 Plots of conversion *versus* polymerization time for the polymerization of L-leucine NCA initiated by butylamine in the solid state and in acetonitrile at 30 and 40°C. In the figure, 'solid' indicates polymerization in the solid state and 'ACN' polymerization in acetonitrile. The molar ratio of NCA to butylamine ([A]/[I]) was 200 and $[I] = 9.50 \times 10^{-3}$ mol l^{-1}

found. Acetonitrile easily dissolves all of the NCAs examined but not polypeptides with low molecular weight. It is known that when the polymerization of NCAs is carried out in acetonitrile, the resulting oligopeptides form crystals and continue heterogeneous polymerization with monomer; the polymerization mechanism has been investigated in detail[29–34]. Therefore, the polymerization of each NCA in acetonitrile has been carried out and, especially, the initial stages of the polymerization are compared with the solid-state polymerization.

Figure 2 gives the time–conversion curves for the polymerization of L-leucine NCA in the solid state (in hexane) and in acetonitrile at 30 and 40°C. This suggests that the polymerization rate in the solid state is much faster than that in acetonitrile. The temperature dependence of the polymerization rate is much higher in the solid state than in acetonitrile. Apparent activation energies of polymerization of L-leucine NCA were 16.9 kcal mol^{-1} (70.7 kJ mol^{-1}) in the solid state and 2.56 kcal mol^{-1} (10.7 kJ mol^{-1}) in acetonitrile, respectively[27]. These results suggest that the mobility of the monomer molecules in the crystal is an important factor for the present solid-state polymerization. The polymerization rates of L-leucine NCA in the solid state above 45°C were too fast to estimate the reaction rate by the present method. It is remarkable that L-leucine NCA was the most reactive among the other NCAs of α-amino acids; glycine, L-alanine, L-valine, γ-benzyl-L-glutamate and β-benzyl-L-aspartate as reported previously[21]. *Figure 3* gives the results for the polymerization of L-alanine NCA. The polymerizability of L-alanine NCA in acetonitrile is much higher than that in the solid state, contrary to L-leucine NCA. However, the apparent activation energies were very similar to the corresponding ones for L-leucine NCA: 16.4 kcal mol^{-1} (68.6 kJ mol^{-1}) in the solid state and 2.60 kcal mol^{-1} (10.9 kJ mol^{-1}) in acetonitrile[26]. L-Alanine NCA was the most reactive in acetonitrile among the six NCAs mentioned above[21]. I.r. spectroscopy showed that poly(L-leucine) and poly(L-alanine) obtained both in the solid state and in acetonitrile formed α-helices[27].

It was pointed out that the number-average degree of polymerization ($\overline{DP}_n$) of polypeptides obtained in the polymerization of L-alanine NCA, DL-alanine NCA and L-leucine NCA initiated by primary amine in acetonitrile agreed well with [W]/[I], which is the molar ratio of the monomer consumed by the polymerization to the initiator used[29–31]; monodisperse polypeptides could be formed in the polymerization system because of the absence of side reactions. Thus, the $\overline{DP}_n$ of the resultant polymer in acetonitrile should be proportional to the conversion at which the polymer is obtained with [A]/[I] (=200) as a proportional constant. In general, the relationship between intrinsic viscosity of polymer solution and number-average molecular weight of the polymer is given as follows:

$$[\eta] = K\bar{M}_n^{\alpha} \qquad (1)$$

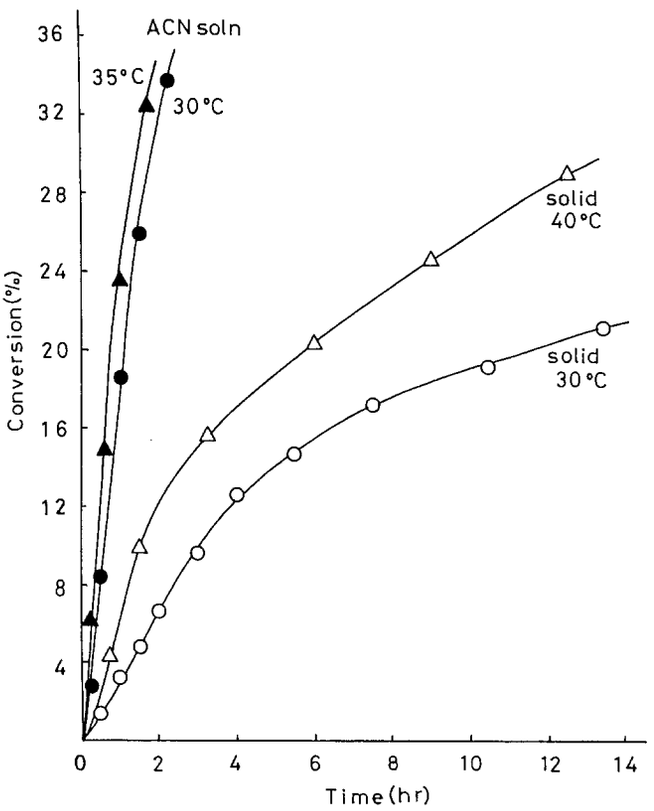


Figure 3 Plots of conversion *versus* polymerization time for the polymerization of L-alanine NCA initiated by butylamine in the solid state at 30 and 40°C and for polymerization in acetonitrile at 30 and 35°C. Notation methods and the other experimental conditions are the same as in *Figure 2*

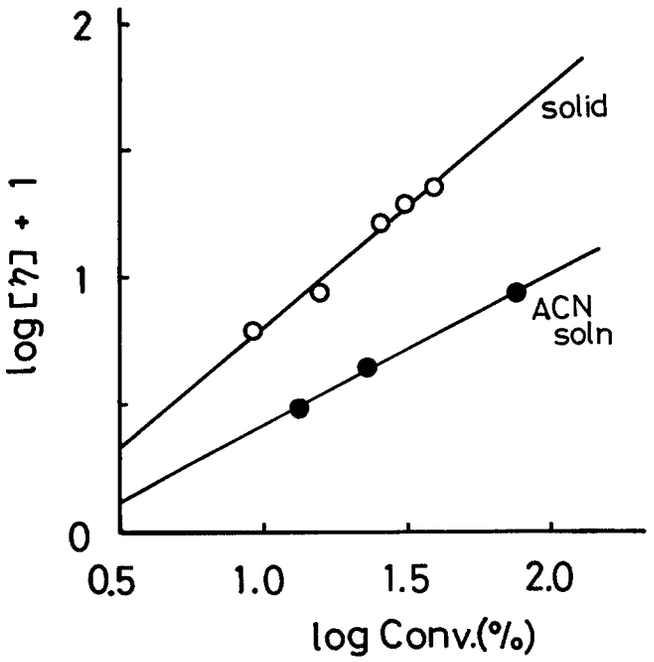


Figure 4 Logarithmic plot of intrinsic viscosity of poly(L-leucine) in TFA at 25°C *versus* conversion (Conv. (%)). In the figure, 'solid' and 'ACN' indicate that the polymers were obtained in the solid state or in acetonitrile, respectively. Polymers were obtained at 30°C

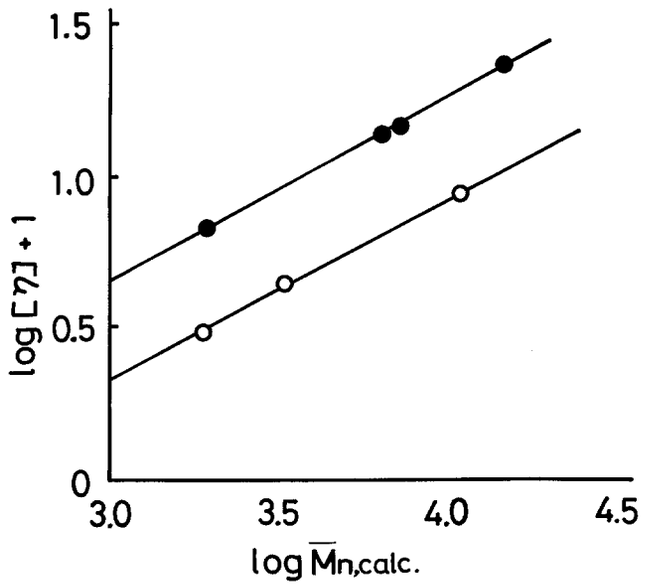


Figure 5 Plots of $\log[\eta]$ *versus* $\log \bar{M}_{n,calc}$ for poly(L-leucine) (○) and poly(L-alanine) (●). Both polymers were obtained via polymerization of their NCA in acetonitrile at 30°C. The viscosity $[\eta]$ ($dl^3\ g^{-1}$) of the former was measured in TFA and of the latter in DCA at 25°C

When $\bar{M}_n$ is proportional to the conversion (Conv. (%)) as mentioned above:

$$\bar{M}_n = ([A]/[I]) \times (\text{Conv. } (\%)/100) \quad (2)$$

the following equations are obtained:

$$[\eta] = K([A]/100[I])^{\alpha}\ (\text{Conv. } (\%))^{\alpha} \quad (3)$$

$$\log[\eta] = \log K + \alpha \log([A]/100[I]) + \alpha \log(\text{Conv. } (\%)) \quad (4)$$

In fact, Komoto *et al.*[35] obtained a linear relationship in the logarithmic scale between $[\eta]$ in TFA at 25°C and the number-average molecular weight ($\bar{M}_{n,calc}$) calculated from the conversion and [A]/[I]. *Figure 4* gives the relationship between the logarithm of $[\eta]$ ($dl^3\ g^{-1}$) of poly(L-leucine) in TFA and the logarithm of conversion (Conv. (%)). Not only the polymer obtained in acetonitrile but also that in the solid state gave linear relationships between $\log[\eta]$ and log(Conv. (%)). The molecular weight ($\bar{M}_{n,calc}$) of poly(L-leucine) obtained in acetonitrile was estimated as the product of the conversion and [A]/[I]. Their logarithmic plots against $\log[\eta]$ gave a linear relationship as shown in *Figure 5*. Thus, the following viscosity *versus* number-average molecular weight relationship was obtained for poly(L-leucine) in TFA solution at 25°C*:

$$[\eta] = 3.24 \times 10^{-3} \bar{M}_n^{0.605} \quad (5)$$

When equation (5) is applied to the polymer formed in the solid state, even $\overline{DP}_n$ of the polymer obtained at 50% conversion is estimated to be 1100 ($\bar{M}_n = 124\,000$). Thus, it is remarkable that the $\overline{DP}_n$ of the poly(L-leucine) obtained in the solid state is much higher in comparison with that of the polymer in acetonitrile at the same conversion, because the $\overline{DP}_n$ of the poly(L-leucine) at 100% conversion obtained in acetonitrile is considered to be about 200.

Figure 6 gives the relationship between $\log[\eta]$ and log(Conv. (%)) for poly(L-alanine). The results are entirely contrary to those of poly(L-leucine); the

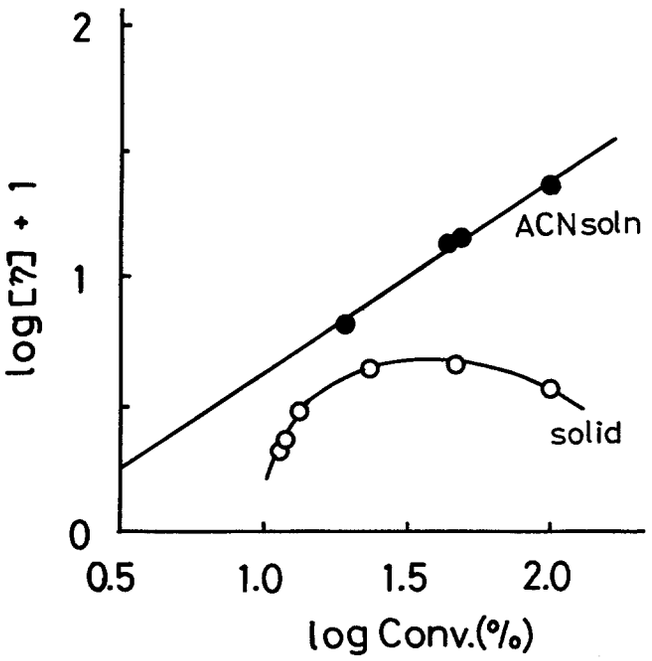


Figure 6 Logarithmic plot of intrinsic viscosity of poly(L-alanine) in TFA at 25°C *versus* conversion (Conv. (%)). In the figure, 'solid' and 'ACN' are the same as in *Figure 4*. Polymers were obtained at 30°C

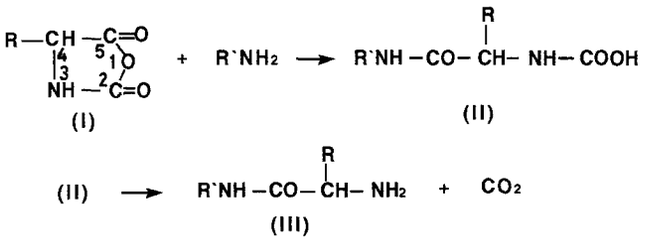
* Equation (5) is different from that presented in the reference in the same way as the present one[32], which gives $\overline{DP}_n = 1100$ ($\bar{M}_n = 124\,000$) for poly(L-leucine) at 100% conversion in the solid state. Since a small amount of initiator or impurities should affect the estimation of molecular weight in this manner, we had better estimate approximate molecular weights from the relationship

molecular weight of poly(L-alanine) obtained in acetonitrile increased linearly with conversion, while that obtained in the solid state gives a maximum at around 30–40% conversion. The plot between $\log(\bar{M}_{n,calc})$ and $\log[\eta]$ for poly(L-alanine) obtained in acetonitrile also gave a good linear relationship as shown in *Figure 5*. The following equation was estimated for poly(L-alanine):

$$[\eta] = 6.53 \times 10^{-3} \bar{M}_n^{0.612} \quad (6)$$

From this equation, the maximum $\overline{DP}_n$ of the poly(L-alanine) obtained in the solid state was estimated to be about 15 ($\bar{M}_n = 1060$). These results are considered as follows: When the polymerization of L-alanine NCA proceeds in the crystals, the crystals are broken irregularly and new initiation should start at the resulting new crystal surface. As a result, the $\bar{M}_n$ should be lowered by low-molecular-weight species at relatively high conversions.

The polymerization of NCA initiated by a primary amine is considered to follow the scheme[12,16,36]:



Primary amines initiate the polymerization of the NCAs by nucleophilic attack by an amino group on the C5 carbonyl of NCA. The derivative (III) can react with another NCA in similar manner to the scheme, leading to polymeric products. The elimination of carbon dioxide seems to prevent the polymerization of NCA in the solid state. Nevertheless, L-leucine NCA gave a high reactivity and high-molecular-weight polymer in the solid state. The above results suggest that the crystal structure of L-leucine NCA should have some favourable features for polymerization, compared to L-alanine NCA or the other NCAs.

X-ray analysis revealed the differences between the reactivities of L-leucine NCA and L-alanine NCA. *Figure 7a* is an *h0l* Weissenberg photograph of an L-leucine NCA single crystal before polymerization. *Figure 7b* is that of L-leucine NCA polymerized partially by moisture in air (the mechanism of the polymerization of the NCA initiated by primary amine is considered to be similar to that initiated by water[12,13]). It is remarkable that a couple of new broad reflections appeared in the vicinity of the c^* axis in *Figure 7b* (these are represented by thick arrows in the figure). The intensity of these reflections increased with the progress of polymerization, while the reflections due to the monomer crystal disappeared with time. The spacing for these reflections is estimated to be 5.11 Å. In general, the α-helical pitch of proteins containing 3.6 amino acid residues is approximately 5.4 Å[37,38]. However, the observable spacing of the reflection on X-ray diagrams has been considered to be different from the pitch length because of the slight inclination of the peptide chain. Pauling, Crick and Bamford *et al.* observed reflections with spacing of 5.0–5.2 Å for the α-helix[39–42]. Thus, the above 5.11 Å is considered to be due to the α-helical pitch of the polymer (this reflection was also observed *0hl* Weissenberg photographs for polymerizing L-leucine NCA crystal[27]). In addition, it was confirmed that α-helical poly(L-

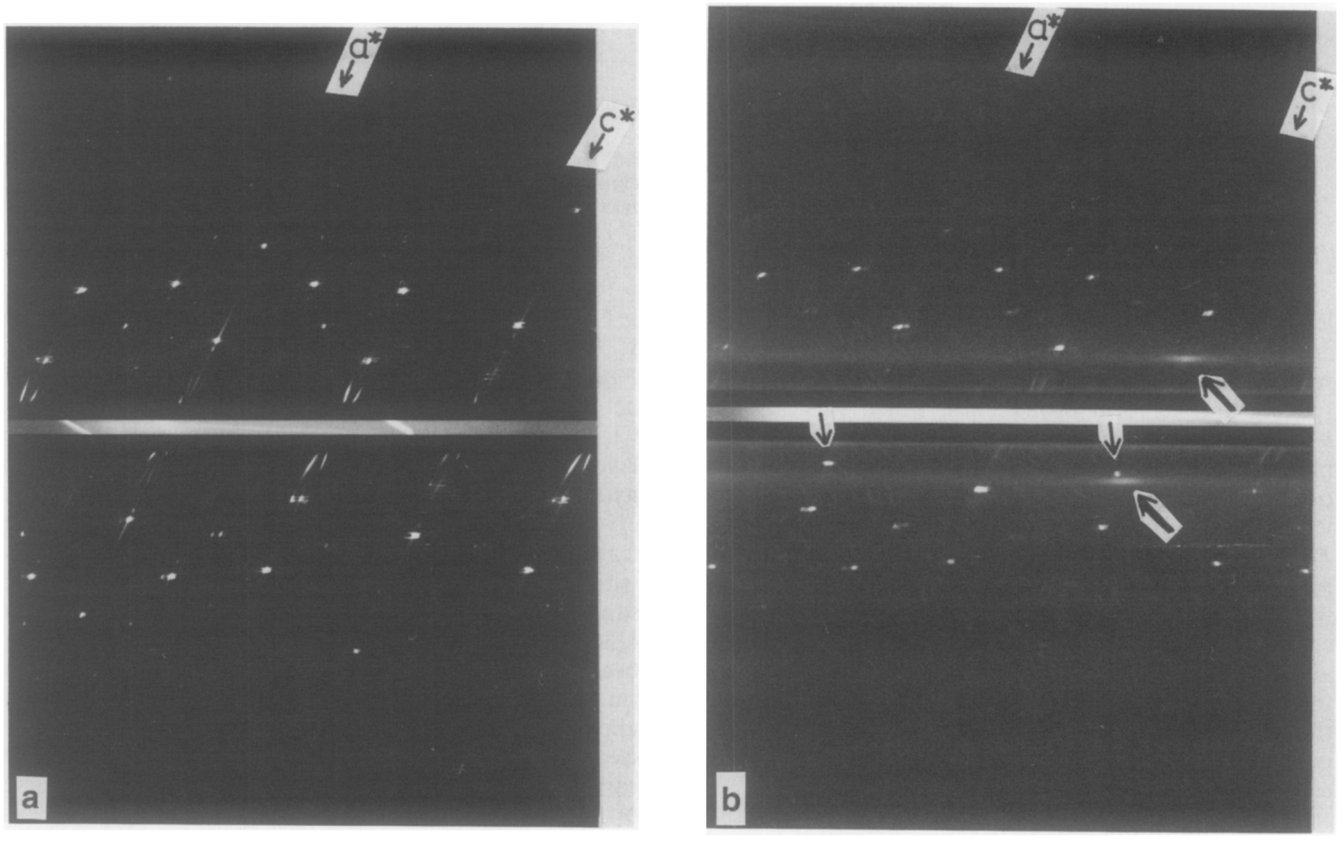


Figure 7 The *h0l* Weissenberg photographs of L-leucine NCA: (a) before polymerization; (b) after 15 h in air at room temperature. Exposure, 12 h

Table 1 Crystal data for five amino acid NCAs at 20°C

NCA	Formula	*MW*	Space group[a]	*a* (Å)	*b* (Å)	*c* (Å)	*U* (Å^{-3})	*Z*	ρ_x (g cm^{-3})
L-leucine	$C_7NO_3H_{11}$	157	$P2_12_12_1$	6.518 (4)	23.983 (14)	5.531 (5)	864.7 (11)	4	1.21
L-alanine	$C_4NO_3H_5$	115	$P2_12_12_1$	7.749 (2)	10.699 (3)	6.063 (2)	502.7 (3)	4	1.52
L-valine	$C_6NO_3H_9$	143	$P2_12_12_1$	5.787 (1)	22.740 (3)	5.395 (1)	709.1 (1)	4	1.33
BLG	$C_{13}NO_5H_{13}$	263	$P2_12_12_1$	7.766 (1)	27.470 (4)	5.948 (1)	1268.9 (4)	4	1.40
Glycine	$C_3NO_3H_3$	101	$C2/c$	9.298 (2)	5.156 (2)	16.900 (4)	771.6 (4)	8	1.74

[a]Crystal system of glycine NCA is monoclinic ($\beta = 107.88$ (2)°) and those of the others are orthorhombic

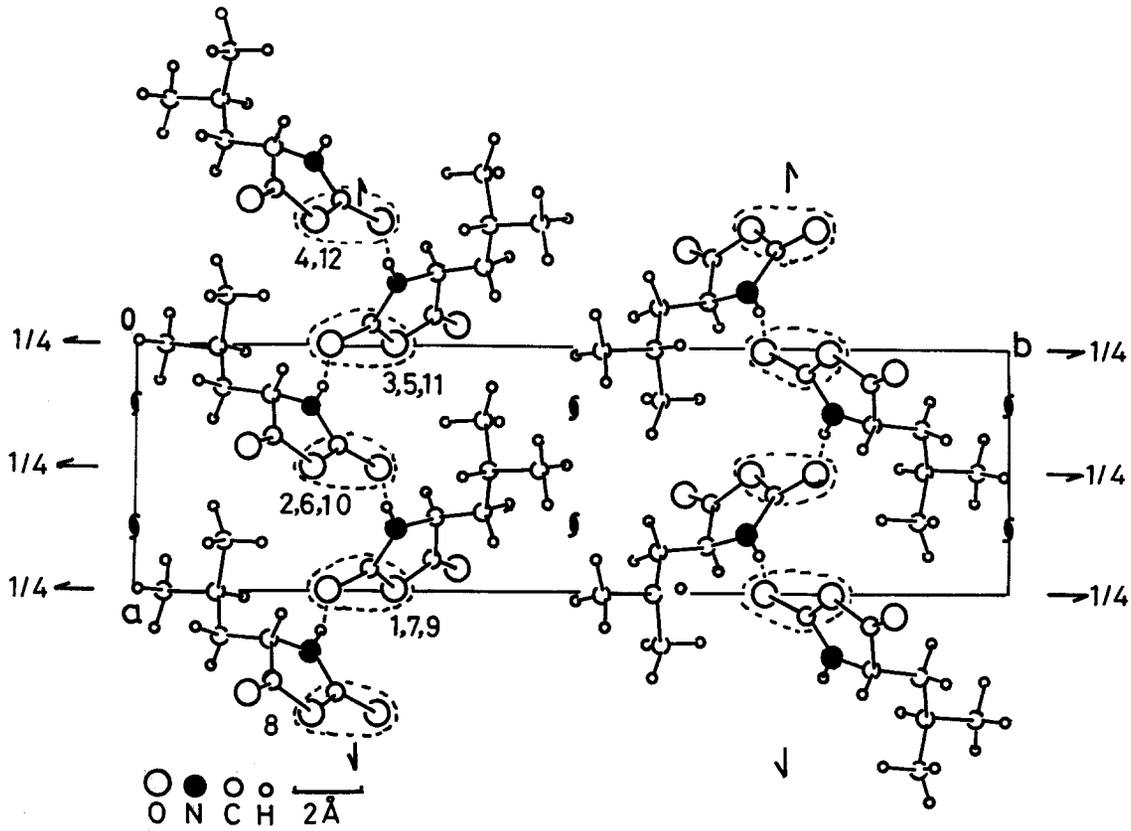


Figure 8 Projection of the crystal structure of L-leucine NCA along the *c* axis. General coordinates: molecule 6 (x, y, z); molecule 5 ($-\frac{1}{2} + x, \frac{1}{2} - y, 1 - z$). Hydrogen bonds are shown by broken lines and the constituent atoms of carbon dioxide evolved in the process are enclosed by broken lines. Numbers from 1 to 12 represent the order of polymerization given in *Figure 10*

leucine) was formed both in the solid state and in acetonitrile by i.r. spectroscopy. In *Figure 7b*, weak reflections seem to spread from the reflections of 5.11 Å spacing and to form broad lines, which suggests that α-helices are formed disordered to some extent in the *ac* plane of the monomer crystal during polymerization. The other new reflections are observed as sharp spots in the vicinity of the c^* and a^* axes as shown by arrows in *Figure 7b*. However, it is not clear whether the extinction rule does not hold any more for polymer formation and whether some particular reflections from the broken crystal appeared incidentally on both axes. Anyway, the above results suggested that L-leucine NCA polymerizes to form α-helical poly(L-leucine) predominantly along the *c* axis in the crystal, although the polymerization proceeds somewhat in various directions parallel to the *ac* plane of the crystal.

On the other hand, X-ray analysis showed that L-alanine NCA did not give any preferred directions for polymerization[27].

Crystal data of four kinds of NCAs are given in *Table 1*. The crystal structure of L-leucine NCA is shown in *Figure 8*. L-Leucine NCA molecules form hydrogen bonds between the imino group and the C5 carbonyl group, which makes a ribbon along the *a* axis. Hydrophilic layers of five-membered rings are sandwiched between hydrophobic side-chain layers in the L-leucine NCA crystal. This structure gives the following favourable features for polymerization: (1) Polymerization proceeds within one sandwich without destroying neighbouring sandwiches. (2) The side chains can move easily to form α-helical poly(L-leucine) in the course of polymerization because of the loose packing. (3) The five-membered rings are not hindered by the side chains from approaching each other. (4) The crystal should be easily cleaved between sandwiches, which makes it easy for carbon dioxide to escape from the crystal in the course of polymerization. Such a cleavage of the crystal was observed in *Figure 1*. In addition, the above consideration is supported by electron microscopy. *Figure 9* gives an

electron micrograph of polymerized L-leucine NCA, in which lamellar crystals of poly(L-leucine) are stacked along the *b* axis of the crystal. It is remarkable that the surface of the polymer crystal including the *ac* plane is rather smooth, while many voids that seem to be formed by evolved carbon dioxide were observed on the other surfaces. This can be realized from *Figure 8*; carbon dioxide goes out of the crystal along the directions perpendicular to the *b* axis. *Figure 10* shows the projection of one layer of the NCA molecules viewed along the *c* axis. The molecules should polymerize easily in this layer to form an α-helix along the *c* axis. It is known that 3.6 amino acid residues are involved in an α-helical pitch of 5.4 Å as mentioned above. It is noteworthy that the length of the *c* axis, 5.527 Å, is close to the α-helical pitch. Considering the coordinates of the molecules and the distances between the imino groups and the C5 atom of the NCA, the most likely molecular linkage for the polymerization is represented in *Figure 10*. In this figure, the numbers and arrows represent the order of polymerization; the arrows do not illustrate the backbone of a resulting α-helix. As seen in the figure, the four molecules numbered 5, 6, 7 and 8 are involved in the distance of the *c* axis. The distance from the N atom of molecule 1 to the C5 atom of molecule 2 and the corresponding distances between molecules 2 and 3, molecules 3 and 4, molecules 9 and 10, molecules 10 and 11 and molecules 11 and 12 are all 4.407 Å. The distance between the N atom of molecule 4 and the C5 atom of molecule 5 is 6.760 Å. The corresponding distances between molecules 5 and 6, molecules 6 and 7 and molecules 7 and 8 are 5.460 Å, and that between molecules 8 and 9 is 5.949 Å. These distances seem to be too great to allow polymerization. However, if it is

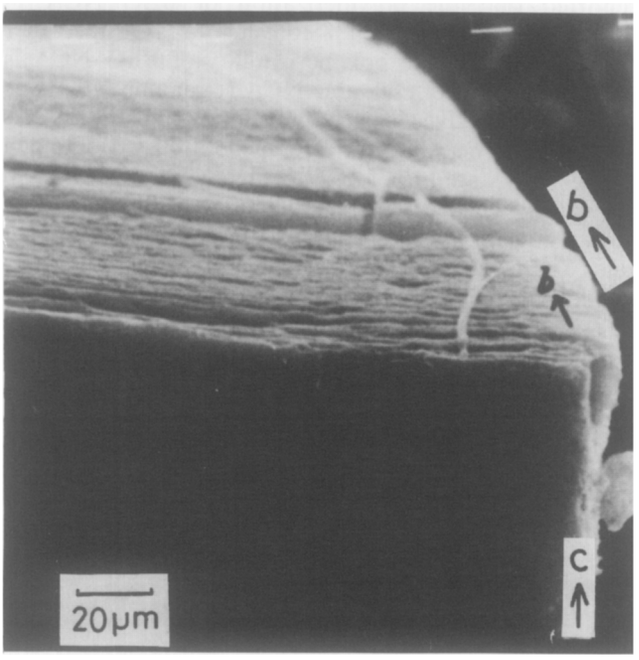


Figure 9 Electron micrograph of polymerized L-leucine NCA

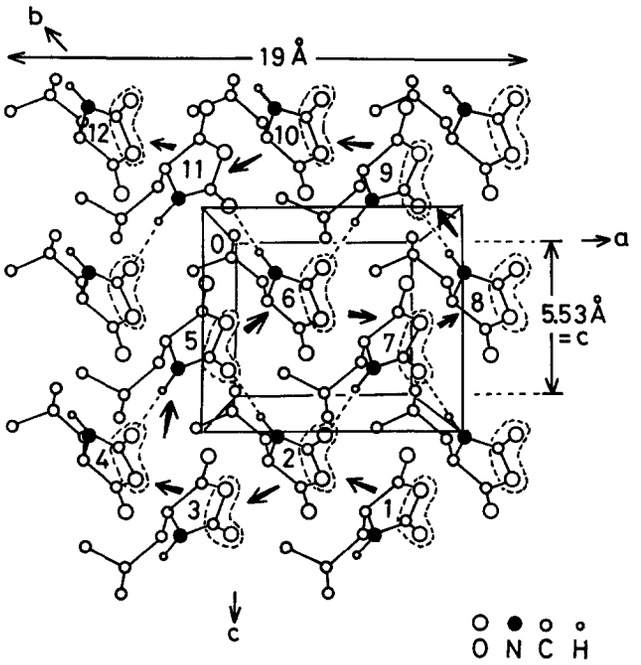


Figure 10 Projection of the crystal structure of L-leucine NCA along the *b* axis. Numbers represent one example of the order in which the molecules polymerize. Arrows do not give the backbone of the α-helix but the way of linking the molecules. Broken lines show carbon dioxide similarly to *Figure 8*

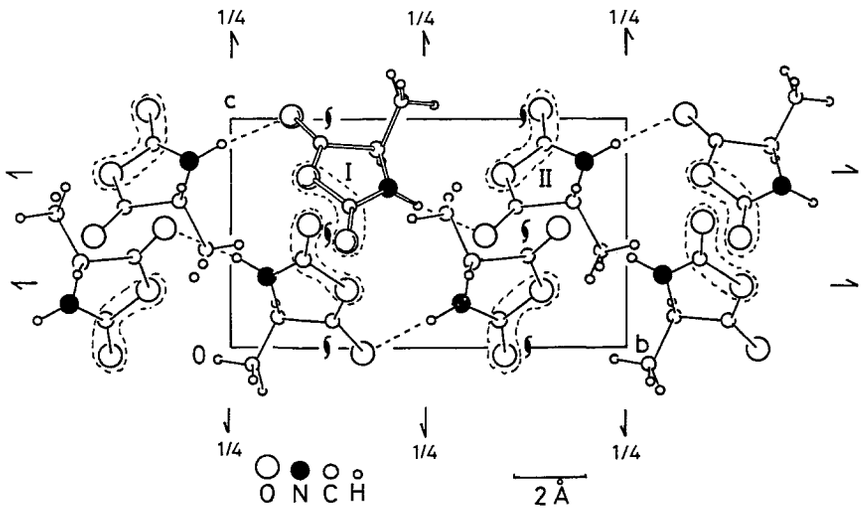


Figure 11 Projection of the crystal structure of L-alanine NCA along the *a* axis. General coordinates: I (x, y, z); II $(1-x, \frac{1}{2}+y, \frac{3}{2}-z)$. Notation methods are the same as in *Figure 8*

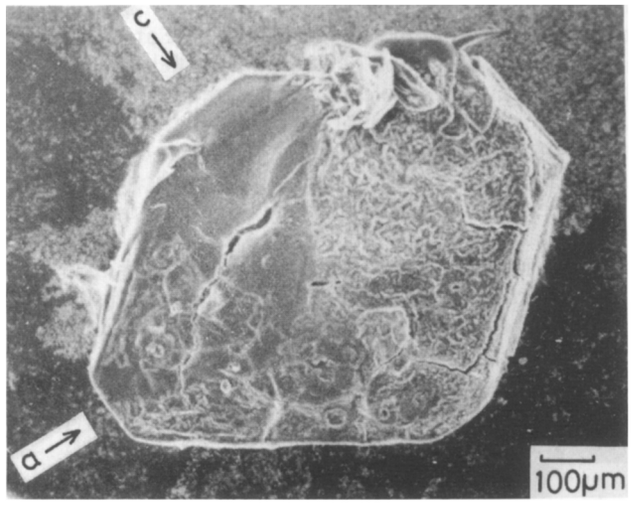


Figure 12 Electron micrograph of polymerized L-alanine NCA viewed along the *b* axis

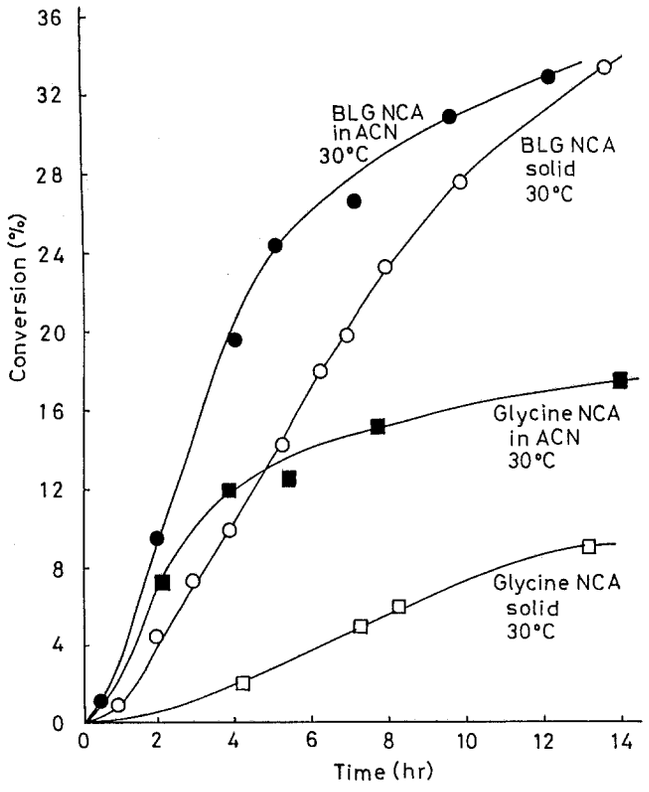


Figure 13 Plots of conversion *versus* polymerization time for the polymerization of BLG NCA and glycine NCA initiated by butylamine in the solid state and in acetonitrile at 30°C. Notation methods and the other experimental conditions are the same as in *Figure 2*

assumed that each monomer rotates around its centre of gravity, polymerization would be highly possible. The reacting monomers given in *Figure 10* occupy a volume element with dimensions $(1/2)b = 11.992$ Å along the *b* axis and $(5/2)a = 16.295$ Å along the *a* axis (see also *Figure 9*). On the other hand, the diameter of an α-helix of poly(L-leucine) was estimated approximately to be 19 Å using a molecular model. Therefore, the reacting monomers seem to form easily α-helical poly(L-leucine) within the occupied volume in the crystal.

On the other hand, the crystal structure of L-alanine NCA does not show favourable features compared to L-leucine NCA. *Figure 11* gives the crystal structure. The hydrogen bonding is similar to that of L-leucine NCA. However, the sandwich structure as seen in the L-leucine NCA crystal is not observed clearly in the L-alanine NCA crystal. In addition, the methyl group of L-alanine NCA seems to hinder the access of the imino groups to the C5 atom, which is necessary for polymerization. *Figure 12* gives an electron micrograph of polymerized L-alanine NCA, in which an irregular deformation in the crystal is observed. This suggests the difficulty of polymerization of L-alanine NCA proceeding in the solid state. This coincides with the results of the polymerization mentioned above.

Figure 13 gives time–conversion curves for the polymerization of BLG NCA both in the solid state and in acetonitrile and those for glycine NCA. The polymerizability of BLG NCA in the solid state seems a little lower than that in acetonitrile as far as the initial stages of polymerization are concerned. Komoto *et al.* reported that the polymerization of BLG NCA in acetonitrile stopped at 54% conversion[43]. On the other hand, the solid-state polymerizability of BLG NCA is higher than that of L-alanine NCA (L-alanine NCA was the most reactive in acetonitrile among the NCAs examined). The values of $\overline{DP}_n$ of poly(BLG) obtained in the solid state are plotted against conversion (%) in *Figure 14*. The $\overline{DP}_n$ increased linearly with increasing conversion until about 20% and increased slowly after that. These values are rather high compared to the value of [A]/[I], 200. The slowing down in the increasing rate of $\overline{DP}_n$ after 20% is considered to be caused by crystal deformation during polymerization. Thus, the BLG NCA crystal should have some features favourable for polymerization compared to the L-alanine NCA crystal. *Figure 15* gives the crystal structure of BLG NCA. In the crystal, hydrogen bonds are formed between the imino groups and the carbonyl groups in the side benzyl ester groups[24]. The hydrophilic layer of five-membered

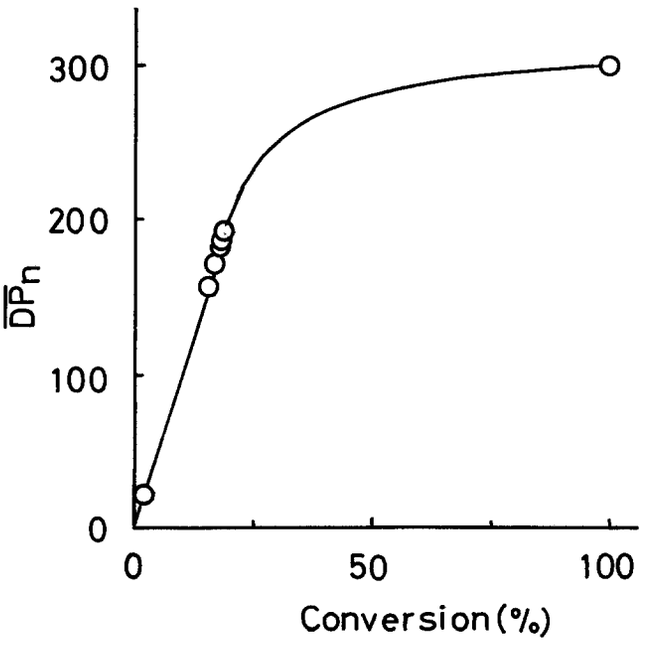


Figure 14 Plot of $\overline{DP}_n$ *versus* conversion (%) for the polymerization of BLG NCA in the solid state at 30°C

rings is sandwiched by the side-chain layers similarly to L-leucine NCA. In addition, it was known that BLG NCA formed polymer with the α-helical conformation in the course of polymerization in the solid state[21]. Because of the layer structure, BLG NCA can polymerize more easily than L-alanine NCA in the solid state. On the other hand, BLG NCA is not so reactive as L-leucine NCA in the solid state. The reason is considered to be as follows: The long side chains and the hydrogen bonds between the imino groups and the side chains prevent somewhat the rotation of the NCA molecules around their centres of gravity necessary for the reaction to form the α-helix, and the rotation of such long side chains may cause considerable crystal deformation during polymerization.

Figure 13 shows that glycine NCA is less reactive in the solid state than in acetonitrile. Its polymerizability in the solid state is the lowest among the NCAs examined[26]. The reason can be clearly explained by the crystal structure given in *Figure 16*. In the crystal, glycine NCA molecules form a dimer structure with hydrogen bonds between the imino group and the C2 carbonyl group. Glycine NCA molecules must break such hydrogen bonds and move in the crystal for polymerization, which should be very difficult. In fact, glycine NCA molecules are packed closely in the crystal (see the density in *Table 1*) and are very stable in the air at room temperature.

It is concluded that when an amino acid NCA has a

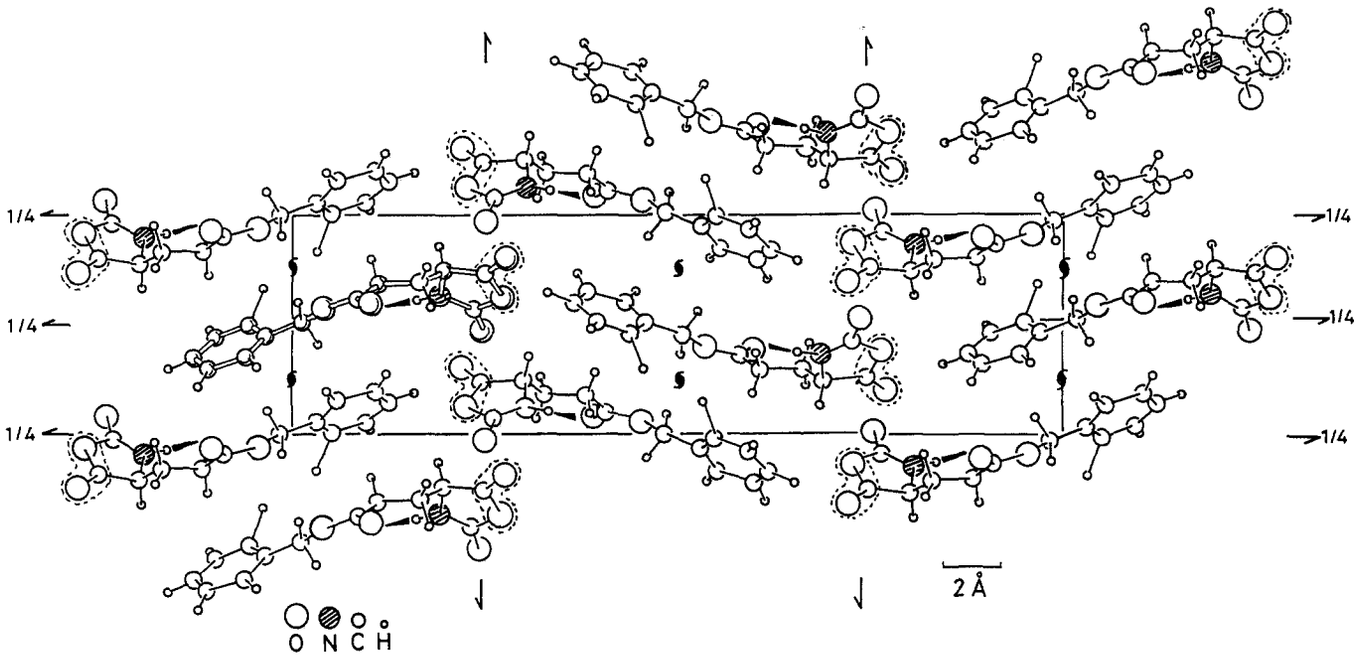


Figure 15 Projection of the crystal structure of BLG NCA along the *c* axis. Hydrogen bonds are formed between the imino group of a molecule (x, y, z) and the carbonyl group in the side chain of a molecule $(x, y, -1 + z)$ and indicated by black triangles. General coordinates: I (x, y, z); II $(-\frac{1}{2} + x, \frac{1}{2} - y, 1 - z)$. The other notation methods are the same as in *Figure 8*

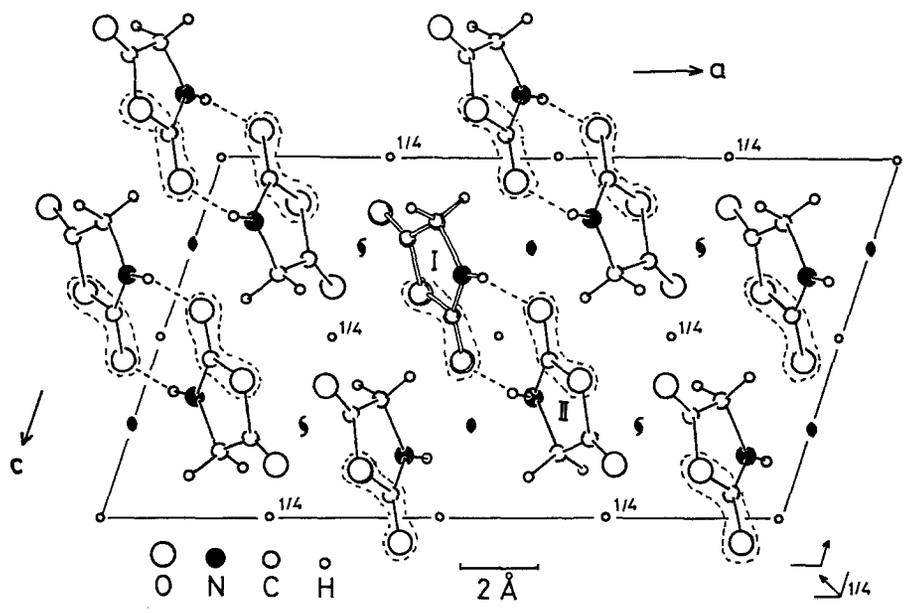


Figure 16 Projection of the crystal structure of glycine NCA along the *b* axis. General coordinates: I (x, y, z); II $(1 - x, 1 - y, 1 - z)$. Notation methods are the same as in *Figure 8*

layer structure in the crystal and the molecules that are to react with each other occupy a space a little smaller than the resulting polymer, polymerization in the solid state is enhanced compared to that in solution. The ease of rotation of the molecules around their centre of gravity in the crystal is another requirement for high reactivity.

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