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# POLYMERIZATION OF α-AMINO ACID N-CARBOXYANHYDRIDE IN THE PRESENCE OF PREFORMED POLY(α-AMINO ACID) — FROM CHAIN EFFECT TO STEREOSELECTIVE POLYMERIZATION —

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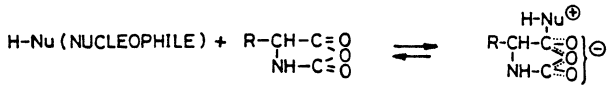
Abstract — The nucleophilic-addition-type polymerization of phenylalanine (Phe) N-carboxyanhydride(NCA) initiated by poly(N-alkylglycine) was much faster than those initiated by corresponding low-molecular-weight amines, and the polymerization rate increased with increasing degree of polymerization of poly(N-alkylglycine) initiator. This was explained in terms of the adsorption of Phe NCA to poly(N-alkylglycine) initiator by hydrogen bonding and the subsequent rapid intramolecular reaction between the bound Phe NCA and the terminal secondary amine along a flexible polymer chain. Changing the extent of hydrogen bonding the copolymerization of two kinds of α-amino acid NCAs were controlled, and changing the flexibility of polymer initiators, the intramolecular reaction was controlled. In the activated-NCA-type polymerization, an activated α-amino acid NCA adds to an α-amino acid NCA unit bound to the C-terminal of a growing chain, which was found to be stereoselective for the first time. The reaction was preferred for the species having the same configuration of the asymmetric carbon atom. Using N-[acylglycyl-oligo(α-amino acid)]α-amino acid NCA as a model for a growing chain, the participations in the enantiomer selection of terminal unit, a penultimate unit, and a growing-chain conformation were experimentally shown. Changing the conformation of the model peptide, the enantiomer selection in the reaction was controlled. Both types of the influence of preformed polymer on the polymerization of α-amino acid NCA are interesting in relation to the mechanism of the efficiency and the enantiomer specificity of enzyme reactions.

## MECHANISM OF POLYMERIZATION OF α-AMINO ACID NCA

α-Amino Acid NCA is easily polymerized by bases such as aliphatic primary or tertiary amines, but the mechanism of polymerization is strongly dependent on the nature of initiator used. With reference to the polymerization mechanism, some ambiguities still remain to be solved. However, it has been made clear by the investigations of Bamford's school that the polymerization initiated by primary amines and by tertiary amines proceeds via the nucleophilic-addition-type mechanism [Eq. 1—3] (Ref. 1) and via the activated-NCA-type mechanism [Eq. 4—6] (Ref. 2), respectively, These mechanisms have been substantiated by a number of investigations subsequently.

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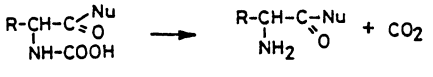

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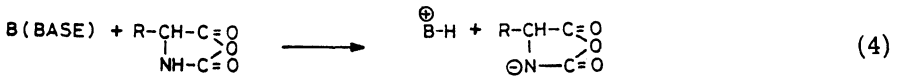
I will describe below the chain-effect polymerization of Phe NCA according to the nucleophilic-addition-type mechanism and the stereoselective polymerization of α-amino acid NCA according to the activated-NCA-type mechanism. In both of them the polymerization of α-amino acid NCA takes place under a strong regulation by preformed poly(α-amino acid) chains.



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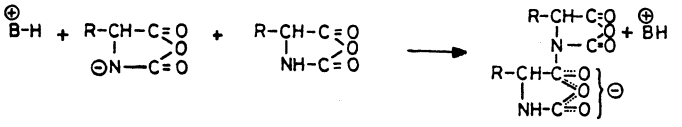

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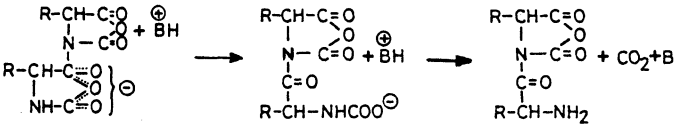

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CHAIN-EFFECT POLYMERIZATION OF α-AMINO ACID NCA

Background

In 1956 Ballard and Bamford (Ref.3) attempted the synthesis of block copolypeptides which consist of water-soluble polysarcosine and water-insoluble polyphenylalanine. They investigated the polymerization of DL-Phe NCA in nitrobenzene($PhNO_2$) initiated by polysarcosine having different degrees of polymerization, and they found an increasing rate of polymerization with increasing degree of polymerization of polysarcosine initiator as shown in Fig. 1. In these reactions a terminal secondary amino group of polysarcosine initiates the polymerization according to the nucleophilic-addition-type mechanism. The increase of n of the initiator leads to increasing numbers of electron-withdrawing peptide group and should therefore decrease the basicity of the terminal amine, hence the polymerization rate. This expectation was, however, in a sharp contrast to the experimental results. In the above experiments the

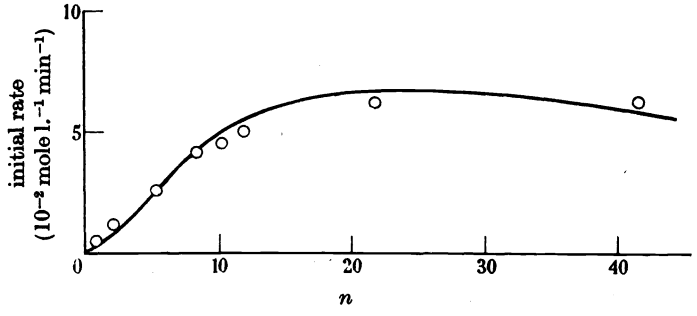



Fig. 1. Polymerization of DL-Phe NCA initiated by polysarcosine dimethylamides having different degrees of polymerization (n). $PhNO_2$ solution ; temp. 15°C; $[NCA]_o$= 0.1M, [Amine] = 5.4 mM. [Reproduced by permission of the copyright owner from Proc. R. Soc. London,Ser. A 236, 384 — 396 (1956).]

concentrations of Phe NCA and the terminal amine were kept constant. Therefore, the only variable with increasing n of polysarcosine initiator was the concentration of peptide bond of the initiator chain. Ballard and Bamford therefore interpreted the experimental results in terms that Phe NCA is bound to a peptide carbonyl group of polysarcosine initiator by hydrogen bonding to result in an increased local concentration of the NCA around the terminal amino group, and they named this sort of polymerization as the chain-effect polymerization. Their explanation is schematically shown in Fig. 2, which involves the intramolecular cooperation of a binding site (carbonyl group) and a catalytic site (terminal amine). Their explanation has been supported by the experimental fact that the acceleration is observed only when the N-unsubstituted amino acid NCA, which can be a hydrogen-bond donor, is polymerized with poly(N-substituted amino acid) as an initiator which can be a hydrogen-bond acceptor. In dimethylformamide($HCONMe_2$) no acceleration has been observed. Since this solvent is strongly accepting for hydrogen bond, the hydrogen bonding between the NCA and the polymer initiator must have been destroyed, leading to a polymerization without acceleration. When

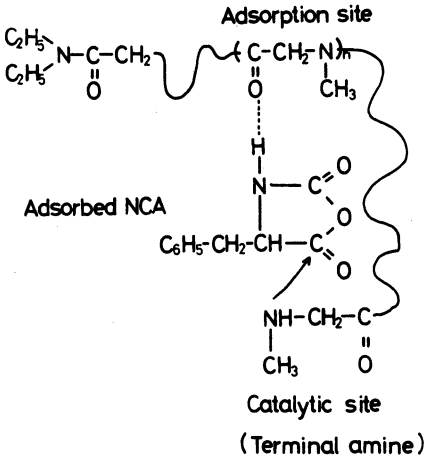



Fig. 2. Mechanism of accelerated polymerization of DL-Phe NCA by polysarcosine diethylamide. [Reproduced by permission of the copyright owner from Biopolymers 12, 1505 — 1513 (1973).]

Phe NCA was polymerized with sarcosine dimethylamide in the presence of polysarcosine acetylated at the terminal-N which is incapable of initiating the polymerization, only a minor extent of acceleration was observed. This indicates that the cooperation between a binding site and a catalytic site of polysarcosine initiator is due undoubtedly to the intramolecular one, and supports the mechanism shown in Fig. 2.

The significance of the above experimental results is twofold. Firstly, we could utilize the chain effect for the sequential regulation of copolymerization in which N-substituted and N-unsubstituted α-amino acid NCAs are involved. If the conditions are suitably chosen, the latter NCA may be polymerized faster than the former according to the chain effect. Secondly, the reaction of the type shown in Fig. 2 could reproduce the features of enzyme catalysis. The catalysis by enzyme which is an intramolecular multiple catalyst is represented schematically in Fig. 3. Step(1) represents a binding of substrate to an enzyme induced by a secon-

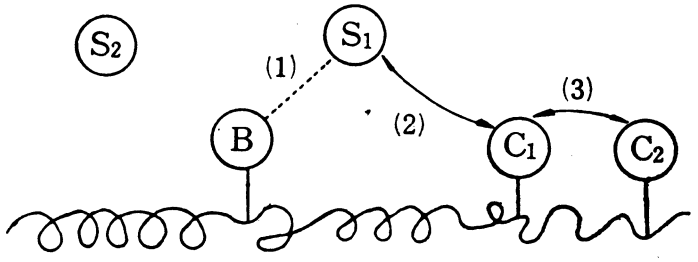



Fig. 3. Schematic representation of intramolecular multiple (enzyme) catalysis. [Reproduced by permission of the copyright owner from Kobunshi Shokubai, p. 80, Kodansha Scientific, Tokyo (1976).]

dary valance force which involves hydrophobic interaction, charge-transfer interaction, electrostatic interaction, hydrogen bonding, and so on. Step(2) represents an intramolecular attack by a catalytic group toward a bound substrate. Step(3) represents an intramolecular cooperation of two or more functional groups to increase their reactivities. In step(2) and step(3), two or more functional groups exhibit the maximum degree of cooperation when they are brought into an optimum arrangement that is determined by the flexibility of polymer chain and the orientation of functional groups. In view of the similarity between Fig. 2 and Fig. 3, the chain-effect polymerization is useful to investigate the dynamic chain flexibility in the intramolecular reaction.

Induction of secondary structure in copolymerization

γ-Ethyl-L-glutamate[L-Glu(OEt)] NCA and sarcosine(Sar) NCA are nearly equally reactive in the nucleophilic-addition-type polymerization. Therefore, in their copolymerization initiated by a nucleophile a random copolymer is expected to be produced. However, when their copolymerization is conducted with polysarcosine as an initiator, L-Glu(OEt) NCA must be bound to the initiator chain and polymerized preferentially to Sar NCA because the former possesses an

NH hydrogen and the latter lacks it. This leads to the expectation that a copolymer having a block-copolymer-like composition will be formed in the polymerization initiated by polysarcosine. Under this expectation a copolymer $(S_{20})G_{20}S_{20}$ was prepared which is copolymer having the overall chain length 60 produced by copolymerizing an equimolar mixture of Sar NCA and L-Glu(OEt) NCA in nitrobenzene using a polysarcosine having $\bar{n}$=20 as an initiator (Ref. 4). If several units of L-Glu(OEt) continue in the copolymer, it leads to a formation of some secondary structure which should be confirmed by the optical rotatory property of copolymer. The optical rotatory dispersion of $(S_{20})G_{20}S_{20}$ was measured and compared with that of a copolymer $(S_{20},G_{20})S_{20}$ which was produced by polymerizing an equimolar mixture of Sar NCA and L-Glu(OEt) NCA in nitrobenzene using sarcosine dimethylamide as an initiator, and adding Sar NCA after the completion of the first polymerization. Therefore, $(S_{20},G_{20})$ represents a random copolymer of equimolar Sar and L-Glu(OEt) units having overall chain length 40, and $S_{20}$ represents a block segment consisting of 20 Sar units which is equivalent to polysarcosine initiator incorporated into $(S_{20})G_{20}S_{20}$.

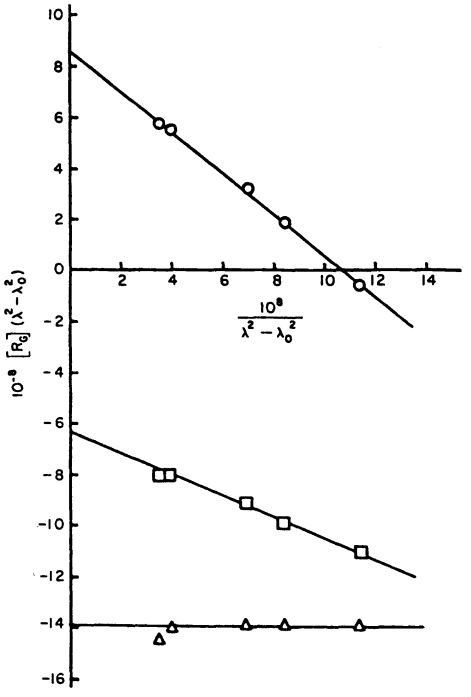



Fig. 4. Moffitt—Yang plots for poly[Sar-co-L-Glu(OEt)] in chloroform solution at 20°C:(○) poly[L-Glu(OEt)]; (□) $(S_{20})G_{20}S_{20}$; (△) $(S_{20},G_{20})S_{20}$. Copolymer designations are explained in the text. $[R_G]$ is the partial molar residue rotation of the L-Glu(OEt) residue corrected to unit refractive index. $\lambda_0$ has been taken as 210 nm. [Reprinted by permission of the copyright owner from Biopolymers 4, 1067—1072 (1966).]

The Moffitt—Yang plot of the optical rotation is shown in Fig. 4. For the copolymer $(S_{20}, G_{20})S_{20}$, the plot is horizontal and no evidence for the copolymer taking a particular secondary structure was obtained. On the other hand, for the copolymer $(S_{20})G_{20}S_{20}$, the plot shows a definite slope indicating the presence of a secondary structure. Undoubtedly this secondary structure in the copolymer $(S_{20})G_{20}S_{20}$ is related to a sequence of L-Glu(OEt) units. If one assumes that the secondary structure in the copolymer is the same type as that in poly[L-Glu(OEt)], nearly 30% of the L-Glu(OEt) units are involved in the secondary structure of the copolymer. The presence of secondary structure in the copolymer has been confirmed as well by other means such as the solubility difference.

The induction of secondary structure in the copolymerization of L-Glu(OEt) NCA and Sar NCA initiated by polysarcosine has been achieved under different conditions. It is very inte-

resting to show the possibility to control the copolymerization of amino acid NCAs through the physico-chemical interaction with preformed polymers.

Chain-effect polymerization of α-amino acid NCA by different poly(N-alkylamino acids)

Experimental data shown in Fig. 1 and the reaction mechanism illustrated in Fig. 2 represent the importance of the initiator being a polymer carrying a catalytic group and substrate-binding groups. At the same time they manifest clearly the effect of chain length between a catalytic group and a substrate-binding group upon their intramolecular cooperation. It is reasonably assumed that NCA molecules are bound to carbonyl groups of the initiator chain with an equilibrium constant which is independent of the position of the carbonyl group in the chain. However, the intramolecular reaction of the terminal catalytic group should take place with the highest rate constant $k_i$ on the NCA molecule bound to the $i$th carbonyl group counting from the terminal along the chain, to which the terminal amine approaches with the greatest ease. Ballard and Bamford analyzed the experimental results shown in Fig. 1 and determind the ($k_i$,$i$) relationship, which is shown in Fig. 5 (Ref. 3). A sharp maximum of $k_i$

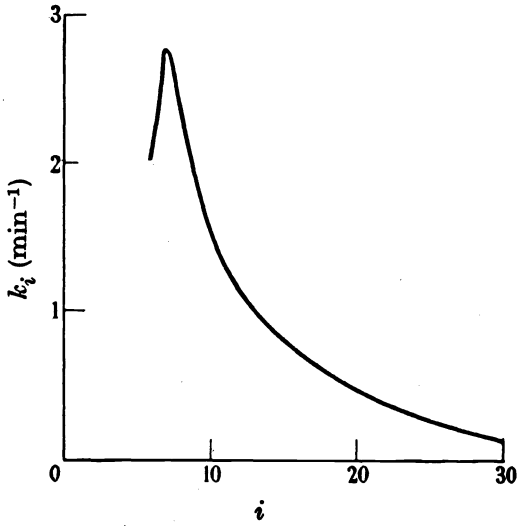



Fig. 5. The intramolecular rate constant $k_i$ in the polymerization of DL-Phe NCA initiated by polysarcosine dimethylamide as a function of the position $i$ of the sarcosine residue in the initiator chain. Nitrobenzene solution; temperature, 15°C. [Reprinted by permission of the copyright owner from Proc. R. Soc. London, Ser. A 236, 384—396 (1956).]

is observed at $i$=7. It is concluded that the terminal amino group of polysarcosine chain encounters most frequently with the NCA molecule bound to the seventh carbonyl group counting from the terminal.

The reaction mechanism illustrated in Fig. 2 can be regarded as a cyclization reaction of polysarcosine chain. The maximum $k_i$ value at $i$=7 means that a polysarcosine chain is cyclized most easily to give 20 - 25-membered ring (except for small-membered ring corresponding to smaller $i$ values than 5). The cyclization of a chain is governed by the flexibility of the chain. The latter is determined by the internal rotation around the main chain, which is affected seriously by the substituents. If we determine the ($k_i$,$i$) relationship for the poly(N-alkylamino acids), the effect of alkyl substituents on the chain flexibility and consequently on the intramolecular cooperation will be evaluated.

The polymerizations of Phe NCA in nitrobenzene initiated by poly(N-ethylglycine)(Ref. 5) and poly(N-n-propylglycine)(Ref. 6) were investigated. In both cases the acceleration by polypeptide initiator was observed as in the case of polysarcosine initiator, and the ($k_i$,$i$) relationship was determined for both cases according to the same procedure. After corrections were made for the different intrinsic reactivities of the terminal amino groups and the different abilities of carbonyl groups to bind NCA were taken into consideration, ($k_i'$,$i$) relationship was determined and is shown in Fig. 6, where $k_i'$ represents the corrected value of $k_i$.

When poly(N-ethylglycine) and poly(N-n-propylglycine) were used as initiator for the polyme-

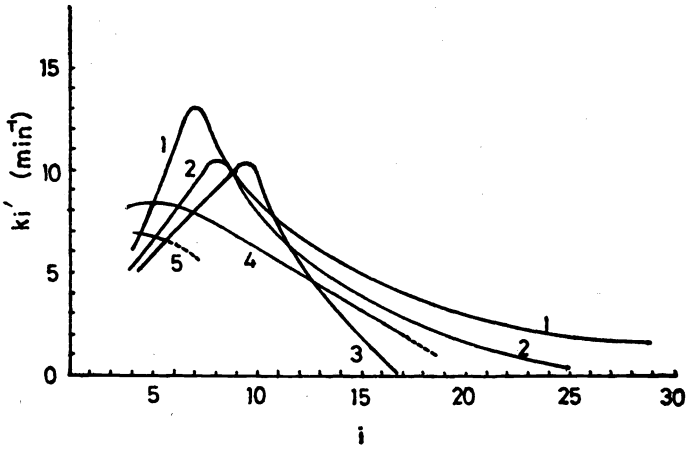



Fig. 6. Relationship between $k_i'$ and $i$ with various poly(N-alkylglycines):1, polysarcosine;2, poly(N-ethylglycine);3, poly(N-n-propylglycine);4, Poly(N-n-butylglycine);5, Poly(N-isobutylglycine). [Reprinted by permission of the copyright owner from Kobunshi Kagaku 30, 61—66 (1973).]

rization of Phe NCA, the ninth and 11th carbonyl groups counting from the terminal along the chain were found to be the most suitable binding site for the intramolecular collision, respectively. Apparently, as the N-alkyl group of the poly(N-alkylglycine) initiator increases in size, the overall reactivity of the polypeptide initiator which is comparable with the area underneath the corresponding ($k_i'$, $i$) curves in Fig. 6 decreases considerably, and the optimum binding site for the intramolecular collision definitely moves away from the terminal catalytic group. These observations clearly demonstrate the significance of the flexibility of polymer chain. A bulky substituent hinders the internal rotations around the main chain and thus hinders the intramolecular reaction which needs to assume a small cycle of the main chain. Instead, it makes an intramolecular reaction proceeding via a larger cyclic intermediate relatively easy.

The polymerization of Phe NCA by poly(N-n-butylglycine) and poly(N-isobutylglycine) as initiator was carried out in nitrobenzene (Ref. 7). These polypeptide initiators having bulkier N-alkyl substituent were more efficient initiators than the corresponding low-molecular-weight amines, but the difference between them was not very large. The bulky N-substituents must have made the polypeptide chain less flexible than others.

It is interesting to note the polymerization of Phe NCA in nitrobenzene using poly(N-benzylglycine) and poly(N-methyl-DL-alanine) as initiator, the initiator activity was only comparable to that of low-molecular-weight amine (Ref. 8). Since poly(N-methyl-DL-alanine) was found to bind Phe NCA in chloroform, the absence of the polymer effect in the polymerization should be due to the rigidity of the polymer chain imposed by the bulky substituent in poly(N-benzylglycine) and by the two substituents in a unit of N-methyl-DL-alanine. Ballard (Ref. 9) carried out the polymerization of Phe NCA in nitrobenzene using poly(L-proline) as initiator which assumes in inert solvents a secondary structure, and observed that the initiator efficiency was comparable to that of low-molecular-weight amine.

The initiation of Phe NCA polymerization with copolypeptides in which some phenylalanyl units have been replaced for sarcosyl units of polysarcosine, which was the most efficient initiator among those investigated above, was then investigated (Ref. 10). For the polymerization of Phe NCA in nitrobenzene at 25°C, in general, the replacement of phenylalanyl units for sarcosyl units in the polypeptide initiator lowered the initiator efficiency. The deteriorating effect of phenylalanyl units was multiplied when they came close to the terminal amino group. A peptide bond involving phenylalanyl unit takes a trans conformation only, whereas that involving sarcosyl unit can take either cis or trans conformation. Furthermore, peptide bonds involving phenylalanyl NH form intramolecular and intermolecular hydrogen bond. These properties of phenylalanyl peptide bond certainly reduce the thermodynamic flexibility of a polypeptide initiator and have bearing on the reduced dynamic flexibility as the polymerization initiator.

Results of the chain-effect polymerization

In enzyme reactions a catalytic group and a bound substrate undergo an intramolecular reaction, but in this case the spatial arrangement of the two functional groups is strictly determined by the higher-order structure. On one hand, this is the most important feature of enzyme reactions, but on the other hand, it is too specific for each reaction catalyzed by the corresponding enzyme. Therefore, in order to have a good grip of the generalized chara-

cter of enzyme reactions, it is urgently needed to investigate the reaction by using enzyme-model compounds. The chain-effect polymerization is a very useful enzyme-model reaction and has shed much light on this problem. However, quantitive investigations about the intramolecular reactions proceeding on a polymer chain carrying two interacting groups at each terminus seemed more effective for that purpose. We have done this line of investigation and relieved generalized features of intrachain reactions, through which several features of enzyme reactions have been comprehended (Ref. 11).

## STEREOSELECTIVE POLYMERIZATION OF α-AMINO ACID N-CARBOXYANHYDRIDE

### Background

In enzyme reactions the substrate specificity as well as the high efficiency is the important feature. In continuation of the investigation on the chain-effect polymerization which has supplied much information on the high efficiency of enzyme reaction, we were interested in an asymmetric chain-effect polymerization using chiral polypeptide as initiator as the model for the enantiomer selection of enzyme reaction. Poly(N-methyl-L-alanine) was used as a chiral poly(N-alkylamino acid) initiator for the polymerization of L-, D-, and DL-Phe NCA, and the asymmetric selectivity was investigated. In this system the asymmetric selectivity is controlled according to three mechanisms as follows. (1) terminal-unit control; enantiomer selection by the chiral active end of the growing chain: (2) penultimate-unit control; enantiomer selection by the chiral penultimate unit in the growing chain: (3) conformation control; enantiomer selection by the chiral conformation of the growing chain. Poly(N-methyl-L-alanine) has been reported to form a secondary structure in inert solvents (Ref. 12, 13 & 14), and in the polymerization of Phe NCA by it all mechanisms (1), (2) and (3) may participate. We have carried out the polymerization in nitrobenzene expecting the asymmetric selectivity $R_L > R_{DL} > R_D$ or $R_D > R_{DL} > R_L$, where R represents the polymerization rate of each enantiomorphic NCA. However, the experimental results were quite unexpected as follows (Ref. 15). (A) the polymerization induced by poly(N-methyl-L-alanine) was much slower than that induced by low-molecular-weight amine; (B) the polymerization rate was $R_L = R_D = 2R_{DL}$, which is not asymmetric selective but stereoselective; (C) the same type of stereoselectivity was observed in the polymerization induced by N-methyl-L-alanine dimethylamide, so that the conformational-control mechanism is not operating. The same sort of polymerization was conducted with initiators having different but similar structures, and the following observation was made (Ref. 16). (D) the same type of stereoselectivity was observed either with poly(N-methyl-DL-alanine) or with sarcosine dimethylamide as initiator, so that the stereoselectivity is not controlled either by the terminal-unit or by the penultimate-unit chirality.

In the chain-effect polymerizations induced by poly(N-alkylglycine) only the nucleophilic-addition mechanism was considered. But a different mechanism seems to operate in the polymerization induced by poly(N-methylalanine) as initiator. Therefore, we started the investigation on the relationship between the stereoselectivity and the reaction mechanism.

### Stereoselective polymerization in the activated-NCA-type mechanism

The above-described experimental results that the polymerization rates of optically pure Phe NCAs were equal and twice as large as that of racemic Phe NCA indicate apparently the perfect stereoselection. A mathematical calculation on the basis of the assumption that a terminal unit reacts exclusively with the same enantiomorphic NCA results in the observed relationship $R_L = R_D = 2R_{DL}$. We used this relationship as a criterion for the occurrence of stereoselection, and investigated what sort of amines induce the stereoselection (Ref. 17). The experimental results are summarized in Table. 1, which shows that a tertiary amine and secondary amines which are sterically crowded and reluctant to undergo the nucleophilic-addition reaction to NCA induce the stereoselective polymerization.

Next, we investigated what sort of α-amino acid NCAs are more apt to undergo the stereoselective polymerization (Ref. 18). The experimental results are summarized in Table 2, which shows that the NCAs of α-amino acids the NH of which is easily accessible to the initiator amine to start the activated-NCA-type polymerization undergo the stereoselective polymerization.

Finally, we investigated the solvent effect on the stereoselectivity in the polymerizations of some α-amino acid NCAs investigated by different secondary amines (Ref. 19). The experimental results are summarized in Table 3, which shows that the stereoselectivity is observed in the solvents which are polar and convenient for the acid-base reaction.

All of the above experimental results clearly indicate that the activated-NCA-type polymerization is stereoselective. The transition-state model for the enantiomer selection according to the terminal-unit control in the activated-NCA-type polymerization was proposed as that in Fig. 7. In the propagation reaction an activated NCA approaches to the terminal cyclic group from the opposite side of the space where the terminal α-C substituent resides. The orientation of two cyclic species is such that the overlapping of the electronic orbital

Table 1. Stereoselectivity in the polymerization of Phe NCA by various amines[a]

| Amine | Selectivity[b] | Amine | Selectivity[b] | Amine | Selectivity[b] |
|---|---|---|---|---|---|
| n-$HexNH_2$ | X | MeOCOCH(-$(CH_2)_3$-)NH | X | (RS)-MeCH(-$(CH_2)_4$-)NH | o |
| $Me_2NCOCH_2NH_2$ | X | DL-$Et_2NCOCH(Me)NHMe$ | o | n-$Bu_2NH$ | o |
| L-$Me_2NCOCH(Bz)NH_2$ | X | L-$Et_2NCOCH(Me)NHMe$ | o | $PhCH_2NHMe$[c] | X |
| $Me_2NCOCH_2NHMe$ | Δ | -$(CH_2)_5$-NH | X | (RS)-PhCH(Me)NHMe | o |
| $Me_2NCO(CH_2)_2NHMe$ | o | (RS)-EtOCOCH(-$(CH_2)_2$-)(-$(CH_2)_2$-)NH | X | $PhCH_2NHEt$ | o |
| $Me_2NCO(CH_2)_3NHMe$ | o | (RS)-MeCH(-$(CH_2)_3$-)(-$CH_2$-)NH | X | n-$Bu_3N$ | o |

a 25°C, Nitrobenzene, [NCA]/[Amine] = 20
b o = stereoselective, Δ = weakly stereoselective, X = nonstereoselective
c [NCA]/[Amine] = 10

Table 2. Stereoselectivity in the polymerization of six kinds of α-amino acid NCAs by different amines at 25°C in nitrobenzene

| α-Amino acid | Stereoselectivity[a] | | | | | |
|---|---|---|---|---|---|---|
| | n-$HexNH_2$ | $PhCH_2NHMe$ | $Et_2NCOCH_2NHMe$ | L-$Et_2NCOCH(Me)NHMe$ | n-$Bu_2NH$ | n-$Bu_3N$ |
| Phe | X | X | Δ | o | o | o |
| Glu(OEt) | X[b] | X | X | o | | |
| Ala | X | X | X | o | | o |
| Leu | | X | X | o | | |
| Val | X | X | X | X | X | o |
| Ile | | | X | X[c] | X | o |

a o = stereoselective, Δ = weakly stereoselective, X = nonstereoselective
b n-octylamine was used.
c DL-N-methylalanine diethylamide

of nitrogen anion and the π-orbital of carbonyl group is maximum and two acid anhydride groups are kept as remote as possible. Under these conditions a transition state for the reaction between an activated L-NCA and L-terminal group is more stable than that between the species having different configurations. The stability difference is due to the different extent of interactions between the α-C substituents and the carbonyl groups.

We do not consider that the reaction depicted in Fig. 7 is perfectly stereoselective. Other stereoregulating mechanism as well as the terminal-unit control may cooperate, leading to the apparently perfect stereoselection in polymerization. Investigations on the stereoselective polymerization using suitable model compounds are necessary.

Stereoselectivity in the model reactions designed for the terminal-unit control and the penultimate-unit control

For the reasons described, the model reactions (8) & (9) were designed and compared with the propagation reaction (7). In the model reactions an activated hydantoin(HDT) was used for an activated NCA. They have very similar structures, and HDT is easily activated by tertiary amines but not polymerizable. Therefore, using an activated HDT the behavior of an activated NCA can be investigated in detail. On the other hand, N-alkyl NCA is not activated by

Table 3 Stereoselectivity in the polymerization of Phe NCA in different solvents at 25°C

| Amine | Stereoselectivity[a] | | |
|---|---|---|---|
| | m-$(MeO)_2Ph$ | $PhNO_2$ | $HCONEt_2$ |
| DL-$Me_2NCOCH(Me)NHMe$ | X | o | o |
| $Me_2NCOCH_2NHMe$ | X | Δ | o |
| $Me_2NCO(CH_2)_2NHMe$ | X | o | o |
| $PhCH_2NHMe$ | X | X | o |
| $Me_2NCOCH_2NH_2$ | | X | X |

a o = stereoselective, Δ = weakly stereoselective, X = nonstereoselective.

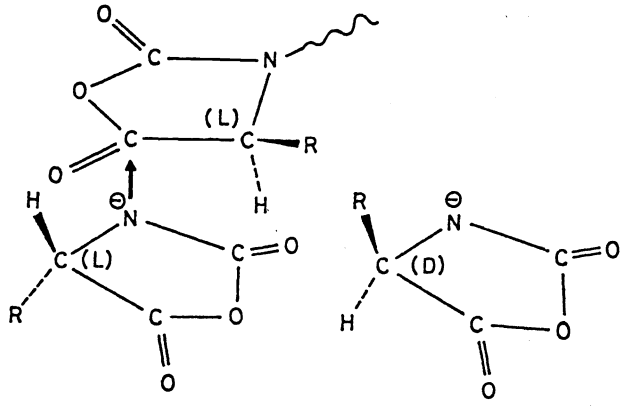
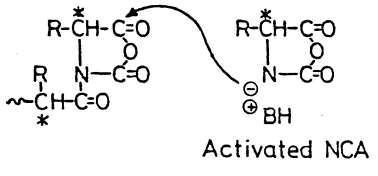
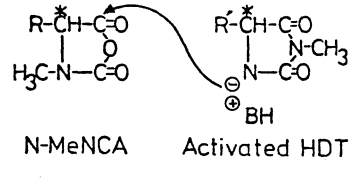


Fig. 7. Transition-state model of enantiomer selection in reaction of two chiral compounds. [Reproduced by permission of the copyright owner from Biopolymers 15, 2407—2420 (1976).]




(7)

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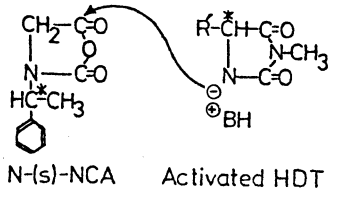



(9)

tertiary amine, and is susceptible to a nucleophilic attack by an activated HDT but is not homopolymerizable if it carries a bulky α-C substituent. Therefore, in the reaction of an activated NCA and an N-alkyl NCA, we can investigate the details of the stereoselectivity in the elementary reactions of NCA polymerization.

At first, the model reaction for the terminal-unit control (Eq. 8) was investigated. Various L-α-amino acid HDTs were activated by tertiary amine and reacted with L- and D-Phe or L- and D-N-methylphenylalanine NCA in nitrobenzene and dimethylformamide (Ref. 20). In any case, activated L-NCA was found to react preferentially with L-NCA. This result implies that the terminal cyclic group of a growing chain in NCA polymerization tends to select the same enantiomorphic activated NCA. However, the enantiomer excess estimated in the model reaction was much inferior to that expected for the perfect stereoselection. It is therefore obvious that the stereoselection in the polymerization is a result of the cooperation of many stereo-controlling mechanisms.

Next, the model reaction (9) was investigated to make clear the contribution of a penultimate unit to the stereoselectivity (Ref. 21). In this reaction, Gly NCA carrying asymmetric substituent on the nitrogen is used as a model for the terminal NCA ring acylated at the nitrogen with a chiral polypeptide chain. In the reaction in nitrobenzene and acetonitrile, a preferential reaction took place between the species having the opposite configuration. But in the reaction in dimethylformamide and dimethylacetamide, the reaction between the species having the same configuration was preferential. The varied stereoselectivity with changing the solvent was ascribed to the varying conformation of the side chain of NCA. Anyway it is certain that the chirality of the N-substituent of NCA affects the stereoselectivity in the addition of activated NCA. This suggests that the penultimate unit of a growing chain participates in the enantiomer selectivity in the NCA polymerization according to the activated-NCA-type mechanism.

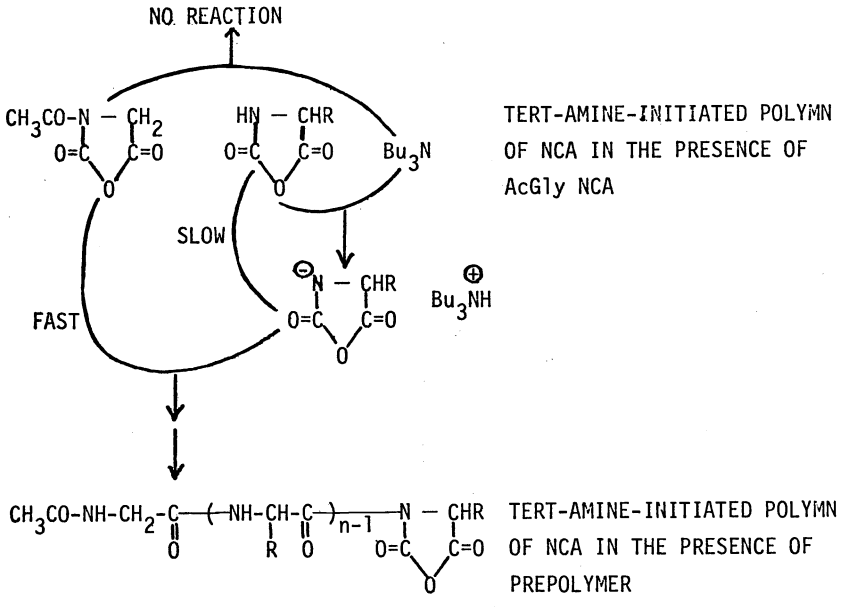
Stereoselectivity in the addition reaction of an activated NCA to N-[AcGly-(α-amino acid)$_{n-1}$]-α-amino acid NCA

The investigations about the stereoselective activated-NCA-type polymerization prompted us to design a good model for a growing chain. The instability of a growing chain in the activated-NCA-type polymerization has been advocated. The most important reason for the instability is the bifunctionality of the growing chain, that is, the latter carries an acylated NCA and a free amine at both ends. To have more stable model compound for the growing chain, we acylated its terminal-N .

N-Acetylglycine (AcGly) NCA has been synthesized by Kricheldorf (Ref. 22). When a usual α-amino acid NCA is activated with tertiary amine in the mixture of usual NCA and AcGly NCA, an NCA anion adds almost exclusively to AcGly NCA, because the latter is more electrophilic than usual NCA. The reaction product is N-acetylglycyl-α-amino acid NCA and this in turn reacts with activated NCA produced subsequently. Repetition of these steps leads to an oligopeptide which carries an N-acylated NCA ring at the terminal-C and is acetylglycylated at the N-terminal-N. This prepolymer is a good model of a growing chain in the activated-NCA-type polymerization and is more stable than the growing chain. The degree of polymerization of the prepolymer is determined by the molar ratio of NCA against AcGly NCA. Since the conformation of the prepolymer is controlled by the degree of polymerization, we can prepare the growing-chain models assuming different conformations by the choice of the experimental conditions. If we polymerize NCA by tertiary amine in the presence of the prepolymer, we can determine the reaction rate for the nucleophilic addition of an activated NCA to the cyclic terminus of the prepolymer. If we use prepolymer having n = 1,2, and those larger than 3, we will deal with the enantiomer selection in the activated-NCA-type polymerization according to the terminal-unit control, the penultimate-unit control, and the growing-chain control, respectively. This process is illustrated in Scheme 1.

L- or D-α-amino acid NCA was polymerized by tri-n-butylamine in the presence of AcGly NCA. The α-amino acids tested were Phe, Glu(OEt), valine (Val), and β-benzyl aspartate [Asp(OBz)]. Solvent for the reaction was nitrobenzene or dimethylacetamide. To the solution of L- or D-prepolymer the corresponding D-NCA was added and activated with tri-n-butylamine. The reaction temperature was always 25°C. The initial reaction rate was determined and the reaction rate of activated D-NCA to D-prepolymer ($R_D$) was compared with that to L-prepolymer ($R_L$) as a measure of the stereoselectivity. The experimental results are summarized in Table 4 (Ref.23).

A number of useful and interesting conclusions were drawn from the experimental results of Table 4. With the prepolymer having n = 1, R(D←D$^-$) was larger than R(L←D$^-$). This experimental result indicates the same enantiomer selectivity and agrees well with the experimental results using activated HDT as a model for activated NCA. With the prepolymer having n = 2, R(L-L ← L$^-$) > R(D-L ← L$^-$) ≒ R(L-D ← L$^-$) > R(D-D ← L$^-$). (note that these experiments were carried out with prepolymers specially prepared, and that the results are not shown in Table 2.) This experimental result indicates the cooperative selection by a penultimate unit for the enantiomer having the same configuration as the terminal unit, which agrees very well with




Scheme 1. Preparation of a prepolymer and the activated-NCA-type polymerization in the presence of prepolymer.

Table 4. $R_D/R_L$ in the addition of D-NCA activated by tri-n-butylamine to N-[AcGly-(D- or L- α-amino acid)$_{n-1}$]-D- or L-α-amino acid NCA

| n | Phe NCA/$PhNO_2$ | Glu(OEt) NCA/$PhNO_2$ | Glu(OEt) NCA/$AcNMe_2$ |
|---|---|---|---|
| 1 | 1.42 | 1.53 | 1.30-2.94 |
| 2 | 2.70 | 1.74 | 1.63 |
| 5 | 3.29-6.58 | 1.97-5.55 | 1.64-2.79 (1.6% R) |
| 10 | 6.50-∞ | 2.14-6.00 | —— (42% R) |
| 15 | 6.00-∞ | 2.00-22.6 | —— (55% R) |
| **n** | **Val NCA/$PhNO_2$** | **Asp(OBz) NCA/$PhNO_2$** | **Asp(OBz) NCA/$AcNMe_2$** |
| 1 | 1.49 | 1.50 | 1.44 |
| 2 | 1.77 | 1.54 | 1.50 |
| 5 | 2.50 | 1.29-2.00 (0%) | 1.30-1.87 |
| 10 | 4.15 | 1.32-3.76 | —— |
| 15 | — | 3.19 (4.8% R) | —— (8.6% R) |

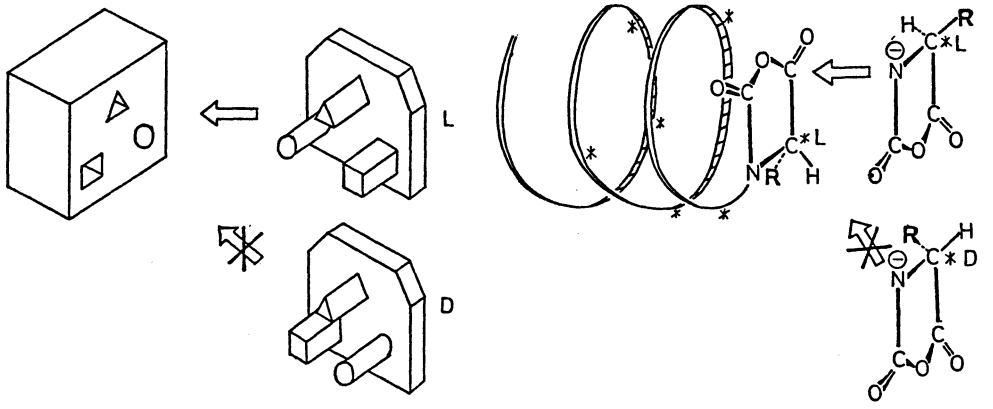
( ), α-HELIX CONTENT ; R, RIGHT-HANDED.

the experimental results using NCA carrying chiral N-alkyl substituents as a model. With increasing n of prepolymer, the tendency for the same enantiomer selectivity increased. With the prepolymer having n=5, β-type extended conformation may appear under suitable conditions. Under this circumstance $R_D/R_L$ was in the order : Phe/nitrobenzene (β-type)≒Glu(OEt)/nitrobenzene (β-type)> Glu(OEt)/dimethylacetamide (random)≒Asp(OBz)/nitrobenzene (random). Obviously, β-type extended chain conformation assists the same enantiomer selectivity. When n reaches 10, α-helix conformation is possibily formed under suitable conditions. With prepolymers having n≥10, $R_D/R_L$ was in the order : Phe(α-helix)≒Glu(OEt) (α-helix)> Val(β-type) >

Asp(OBz)(random) in nitrobenzene. Apparently, an α-helical conformation of a growing chain is more convenient for the same enantiomer selectivity than a β-type extended conformation and a randomly coiled conformation. Thus, the contribution from the conformation of growing chain to the enantiomer selectivity was clearly shown.

Results of the stereoselective polymerization

The achievement of the investigation of stereoselective NCA polymerization is threefold. Firstly, the polymerization of α-amino acid NCA according to the activated-NCA-type mechanism was found to be stereoselective for the first time, and the transition-state model leading to the same enantiomer selectivity was proposed. Secondly , using an activated HDT as a model for an activated NCA, the enantiomer selection according to the terminal-unit control and the penultimate-unit control was elucidated. In consequence, several stereoselective or asymmetrically selective reactions involving activated HDT were discovered. Finally, through the reactions of N-acylated oligopeptide carrying N-acylated NCA ring at the C-terminal, the contribution of the conformation of growing chain to the enantiomer selection was clearly shown. This phenomenon is interesting in reference to the enantiomer selection by enzymes which assume a rigid higher-order structure. The fundamental similarity of the enantiomer selection between NCA polymerizations and enzyme reactions is illustrated in Scheme 2.




Enzyme Reaction NCA Polymerization

Scheme 2. Some fundamental similarities of enantiomer specificity in enzyme reactions and enantiomer-selective NCA polymerizations.

Extention of the investigations on the stereoselective and asymmetrically selective polymerization of α-amino acid NCA will shed more light on the mechanism of the perfect enantiomer selection in enzyme reactions, and the development of the artificial enantiomer-selective catalyst is expected as a goal of these investigations.

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