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# Stereo-differentiation in the Coupling Reaction of Boc-DL-Amino Acid with Poly-L(or D)-Amino Acids Attached to Polystyrene Resin

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Boc-DL-amino acids(Ala, Val, Leu) were coupled with known length of poly-L(or D)-amino acids(Ala, Val) attached to polystyrene resin by dicyclohexylcarbodiimide(DCC). Preferential couplings of Boc-L-Val with poly-L-Ala resin(e.e.$\leq$23%) and of Boc-L-Leu with poly-D-Val resin(e.e.$\leq$54%) were observed.

Stereo-differentiating$^{1)}$ polycondensation of N-carboxyanhydride(NCA) of racemic $\gamma$-benzyl glutamate to form L-rich polymer was reported by using poly-L-$\gamma$-benzyl glutamate as an initiator.$^{2)}$ Polycondensations of NCA monomers of $\alpha$-amino acids having different D/L ratios were also carried out.$^{3-5)}$ In both studies, the formation of an $\alpha$-helix of oligopeptide composed of L-amino acid was considered to be an important factor in the stereo-differentiating polycondensation reaction. And stereo-differentiating coupling reactions of L-amino acid esters with N-acyl DL-amino acids have been studied.$^{6,7)}$ However, stereo-differentiating coupling reactions of poly-L-amino acid with DL-amino acid derivatives in the presence of condensation reagent have not yet been studied.

In order to study the stereo-differentiation of poly-L(or D)-amino acid having known length of residue, we carried out the coupling reactions of racemic Boc-amino acid(N-t-butyloxycarbonyl amino acid) with various length of oligo- or poly-L-(or D)-amino acids attached to 1% divinylbenzene-polystyrene resins using DCC (dicyclohexylcarbodiimide) in DCM(dichloromethane) or DMF(N, N-dimethylformamide).

N-Protected poly-amino acid resins were prepared by the stepwise elogenation method.$^{8)}$ The N-terminal Boc groups of these poly-L(or D)-amino acid resins were deprotected with 50% trifluoroacetic acid(Tfa)/ DCM (v/v) and neutralized with 10% triethylamine in methanol(v/v). The deprotected poly-L-amino acid resins(20 mg, containing 0.010 mmol free amino groups) were coupled with racemic Boc-amino acid (0.025 mmol) in 0.8 ml DCM at 20 °C for 24 h using DCC(0.025 mmol) as shown in Scheme 1. Poly-D-amino acid resins were prepared by the same manner as poly-L-amino acid resins. All coupling yields were almost 100% according to the quantitative nihydrin test.$^{9)}$ After the coupling reaction, the resin was washed with methanol and DCM and hydrolyzed in 50% propionic acid/12 M-HCl(v/v) at 110 °C for 24 h.$^{10)}$ The hydrolyzed resin was filtered off and the filtrate was evaporated to dryness. The residual amino acids were derivatized with 3 M-HCl/isopropyl alcohol(Pr$^{i}$OH) and then trifluoroacetic anhydride(Tfaa)/ DCM to give N-trifluoroacetyl(Tfa) amino acid isopropyl ester. Enantiomeric excess(e.e.) of Boc-L( or D)-amino acid in Boc-DL-amino acid coupled to poly-amino acid resin was determined by gas chromatographic resolution of the derivatized enantiomers of amino acid using Chirasil-Val.[11)]

$$(\text{L-AA}^1)_n\text{-OCH}_2\text{-resin} \quad (\text{ 1\% divinylbenzene-polystyrne })$$

$$\xrightarrow[\text{DCC/DCM or DMF at 20 °C for 24 h}]{\text{Boc-DL-AA}^2} \begin{array}{l}\text{Boc-L-AA}^2\text{-(L-AA}^1)_n\text{-OCH}_2\text{-resin} \\ \text{Boc-D-AA}^2\text{-(L-AA}^1)_n\text{-OCH}_2\text{-resin}\end{array}$$

$$\xrightarrow[\text{At 110 °C for 24 h}]{\text{12M-HCl/CH}_3\text{CH}_2\text{COOH(1:1)}} \text{L-AA}^2,\ \text{D-AA}^2,\ \text{L-AA}^1$$

$$\xrightarrow[\text{2) Tfaa/DCM}]{\text{1) Pr}^i\text{OH/HCl}} \begin{array}{l}\text{Tfa-L-AA}^2\text{-OPr}^i,\ \text{Tfa-D-AA}^2\text{-OPr}^i \\ \text{Tfa-L-AA}^1\text{-OPr}^i\end{array}$$

Scheme 1. $AA^1$: Ala, Val; $AA^2$: Ala, Val, Leu; Boc: t-Butyloxycarbonyl; DCC: Dicyclohexylcarbodiimide; DCM: Dichloromethane; DMF: N,N-Dimethylformamide; $Pr^i$: Isopropyl; Tfa: Trifluoroacetyl; Tfaa: Trifluoroacetic anhydride.

The values of e.e. of Boc-L-Val in Boc-DL-Val coupled to poly-L-Ala resin became higher with the increase of chain length of the poly-L-Ala in two solvents as shown in Fig. 1. The value of e.e. reached 23% when the poly-L-Ala having 6 residues on the resin was used in DCM and became constant after that.

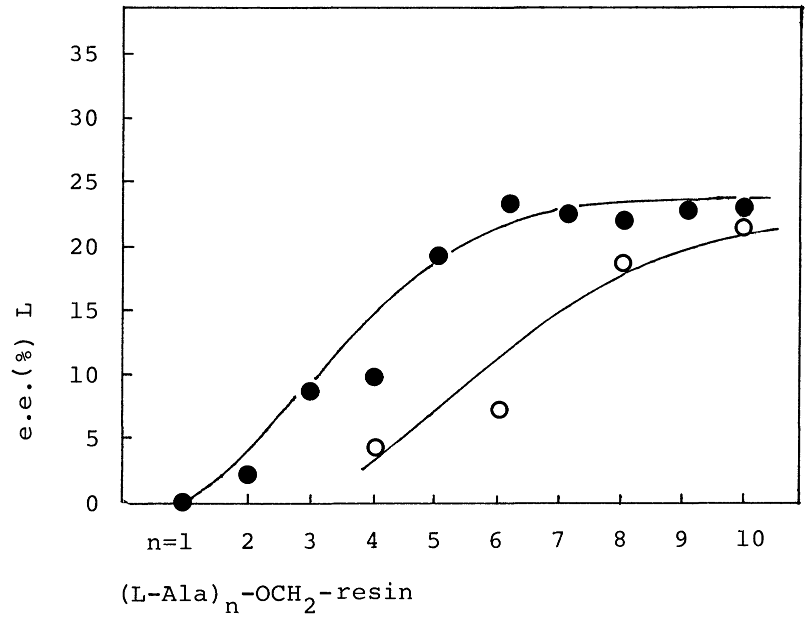

Fig. 1. Enantiomeric excess of Boc-L-Val in Boc-DL-Val coupled with poly-L-Ala resins [$(\text{L-Ala})_n\text{-OCH}_2\text{-resin}$]. (●): in DCM; (○): in DMF.

The results indicate that at least 6 amino acid residues of poly-L-Ala are necessary to form a stable chiral or chiral-recognition media on the resin. Lundberg and Doty have shown[12] that at least 7-13 residues of poly-L-$\gamma$-benzyl glutamate in dioxane are necessary to form a stable $\alpha$-helical structure in the solvent, and it is well known that dioxane, DCM, and DMF are good $\alpha$-helix promoting solvents. In DCM, poly-L-Ala having more than 6 residues on the resin would take a stable $\alpha$-helical structure, which would relate to the stereo-differentiation in the coupling reactions.

Figure 2 shows e.e. of Boc-Ala and Boc-Leu which coupled with poly-L-Val or poly-D-Val attached to polystyrene resin. When the chain length#(n) of poly-L-Val was 1 to 4, Boc-L-Ala and Boc-L-Leu preferentially condensed with this peptide resin. On the contrary, when the chain length#(n) of poly-L-Val resin was 5 to 14, Boc-D-Ala and Boc-D-Leu preferentially coupled with the peptide resin. Boc-D-Leu in Boc-DL-Leu coupled with poly-L-Val in high e.e.(54%), when the chain length#(n) was 8. In the coupling reactions of poly-D-Val attached to polystyrene resin, similar but reversed phenomenon was observed(see Fig.2). These results obtained above suggest that the residue length-depending stereo-differentiation reaction may be caused by the conformational change of poly-L(or D)-Val attached to polystyrene resin.

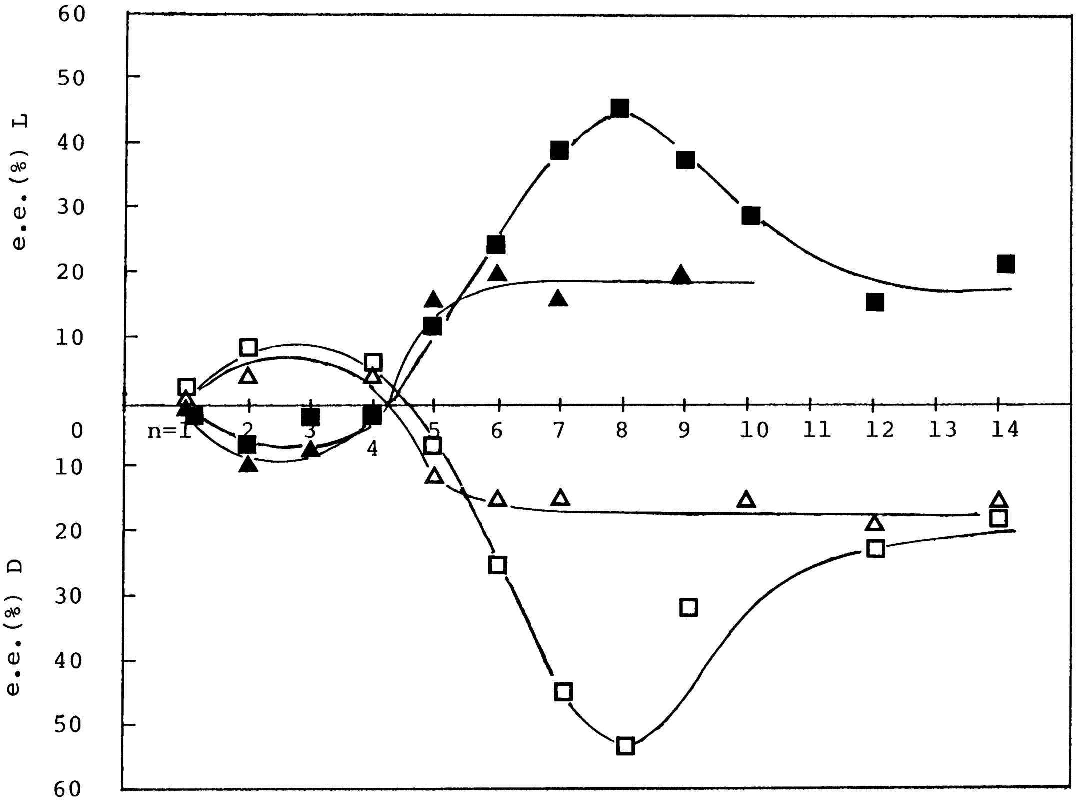

Fig. 2. Enantiomeric excess of Boc-Ala( △ , ▲ ) or Boc-Leu( □ , ■ ) coupled with poly-L-(or D)-Val resins [$(L\text{-}Val)_n\text{-}OCH_2$-resin or $(D\text{-}Val)_n\text{-}OCH_2$-resin]. ( △ , □ ): Coupling with $(L\text{-}Val)_n\text{-}OCH_2$-resin; ( ▲ , ■ ): Coupling with $(D\text{-}Val)_n\text{-}OCH_2$-resin.

We could conclude that poly-L-Ala on the resin recognized the configuration of Boc-Val and preferentially coupled with Boc-L-Val. This stereo-differentiation was not only caused by the chilarity of L-Ala itself but also by the secondary structure(perhaps α-helix) of the poly-L-Ala.[13,14] On the other hand, poly-L-Val on the resin could recognize the configuration of Boc-D-Ala(or Boc-D-Leu) and preferentially condense with these enantiomers rather than Boc-L-Ala(or Boc-L-Leu) when the chain length#(n) was longer than four. The longer poly-L-Val chains($n \geqq 5$) might interact each other to take different conformation like β-structure.[14] Fasman and Itoh have shown that the conformations of longer($n \geqq 6$) poly-L-Val differ from those of shorter($n \leqq 4$) poly-L-Val.[14] The stereo-differentiation in our study might be caused by the secondary structure of the poly-L-Val. This study indicates that one handed oligo- or polyamino acid on the resin could provide a chiral field in which chiral molecules are concentrated or stereo-differentiating reactions proceed. The details on the stereochemistry of the reaction are unknown at this time and are currently under investigation.

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