Sequential Polypeptides. The Steric Hindrance of L-Valine Residues in the Polycondensation of Peptide Active Esters

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Five tripeptide *N*-hydroxysuccinimide esters with *N*-terminal L-valine, L-phenylalanine, and L-alanine were polymerized in *N,N*-dimethylformamide with a variety of concentration of the monomers. The yields and molecular weights of the resulting sequential polypeptides were then compared in order to demonstrate the presence of the steric hindrance of the L-valine residue in polycondensation. The results of the polycondensation and the conformational study of the resulting polypeptides suggest that the side chain of the L-valine residue at the *N*-terminal position of the monomers hinders the polycondensation of the monomers.

L-Valine is a very interesting amino acid in polypeptide chemistry from synthetic as well as conformational aspects. Poly(L-valine) takes predominantly the β-structure and does not form the α-helix, although the L-valine residues can be incorporated in the stable α-helical conformation in naturally occurring proteins[1] and synthetic sequential polypeptides.[2,3] These facts can be explained by the steric hindrance of the side chain of the amino acid.

In a previous study,[4] we reported on the synthesis and conformation of two sequential polypeptides, $(\text{L-Ala–L-Val–Gly})_n$ and $(\text{L-Val–L-Ala–Gly})_n$. In that study, we found that the HBr·H–L-Ala–L-Val–Gly–ONSu monomer polymerized to give a sequential polypeptide, $(\text{L-Ala–L-Val–Gly})_n$, with high molecular weights and in higher yields than the HBr·H–L-Val–L-Ala–Gly–ONSu monomer.[5] This fact was tentatively explained by the presence of the steric hindrance of the side chain of L-valine at the *N*-terminal position of the latter monomer. However, a question if the lowering of the yield and the molecular weight of $(\text{L-Val–L-Ala–Gly})_n$ results only from the steric hindrance of the L-valine residue remains unresolved, because there are many factors responsible for the yield and the molecular weight of the sequential polypeptides. Among them, the conformations of the growing polypeptide chains may greatly influence the results of polycondensation. The previous study compared the results of the polycondensation of different polypeptides which may take different conformations in the polymerization system.

We chose in this study monomers with the same composition, but with different sequences of amino acids, which give the same sequential polypeptide—for example, HCl·H–L-Val–L-Phe–L-Phe–ONSu and HCl·H–L-Phe–L-Val–L-Phe–ONSu, which give the same polymer $(\text{L-Val–L-Phe–L-Phe})_n$. In the polycondensation of these monomers, the conformational effect is removed and the sterical effect of the L-valine residue may be clearly demonstrated. We polymerized five monomers: the above two monomers, HCl·H–L-Val–L-Ala–L-Ala–ONSu, HCl·H–L-Ala–L-Val–L-Ala–ONSu, and HCl·H–L-Val–L-Val–Gly–ONSu. The results are discussed, together with a conformational study in the solid state.

Results and Discussion

Synthesis of Monomers. For such a study as this, in which the mechanism of the polycondensation of peptide active esters is deduced from the yields and the molecular weights of the resulting polypeptides, exact and reproducible values of the yield must be obtained in polycondensation. Thus, a large amount of the peptide active esters as a monomer for polycondensation must be prepared for repeated polycondensations. The peptide active esters are comparatively difficult to synthesize because many treatments are needed for their synthesis. An easy synthetic method giving a high yield must be adopted to prepare a large amount of the monomers.[6] In this study, the monomers were prepared by means of the *o*-nitrophenylthio *N*-carboxy α-amino acid anhydride (Nps-NCA) method,[7] which can rapidly produce in a high yield a peptide derivative protected by the Nps group.[8] This method is especially useful for the synthsis of a large amount of peptides. The Nps-NCA method gives no by-products such as the *N,N'*-dicyclohexylurea in the dicyclohexylcarbodiimide method, and the resulting product is easily purified by simple recrystallization.

An amino acid ethyl ester was treated with an Nps-NCA to give the Nps-dipeptide ethyl ester in a yield above 85%. The subsequent removal of the Nps group of the dipeptide derivative by treatment with hydrochloric acid in dioxane gave, almost quantitatively, the dipeptide ethyl ester hydrochloride, which was then treated with another Nps-NCA to give the Nps-tripeptide ethyl ester in a yield above 82%. The Nps-tripeptide ester was saponified to lead to Nps-tripeptide free acid, which was active-esterified by treating it with *N*-hydroxysuccinimide and dicyclohexylcarbodiimide.[9] The resulting Nps-tripeptide ONSu ester was purified by recrystallization from tetrahydrofuran.

A series of treatments for the synthesis leading to Nps-tripeptide ONSu esters was started from the 0.2 mol scale of the amino acid ester. Though many reactants were used, every condensation with Nps-NCA produced in a yield above 82%, and all the products were easily purified by recrystallization. The results of the syntheses are shown in Table 1.

Polycondensation of the Monomers. All the polymerizations of the tripeptide ONSu esters were done in *N,N*-dimethylformamide for one day at room temperature.[4,6] Every polymerization system was treated with different procedures for the isolation of the resulting polypeptide. We supposed that the yield of polycondensation may vary with each isolation pro-

TABLE 1. RESULTS OF SYNTHESES OF Nps-TRIPEPTIDE ONSu ESTERS

N-Protected monomer	Yield[a] %	Mp °C	$[\alpha]_D$ (c 1.0, $HCONMe_2$)	Found, % / Calcd, % C	H	N
Nps-L-Phe-L-Val-L-Phe-ONSu	64.8	185—190 (dec)	+22.0	59.97 59.89	5.45 5.33	10.47 10.59
Nps-L-Val-L-Phe-L-Phe-ONSu	71.4	185—190 (dec)	−12.7	59.84 59.89	5.40 5.33	10.61 10.59
Nps-L-Ala-L-Val-L-Ala-ONSu	67.5	158—160 (dec)	−34.5	49.39 49.50	5.44 5.34	13.81 13.75
Nps-L-Val-L-Ala-L-Ala-ONSu	61.4	170—175 (dec)	−76.4	49.51 49.50	5.39 5.34	13.84 13.75
Nps-L-Val-L-Val-Gly-ONSu	58.2	185—190 (dec)	−23.7	50.53 50.47	5.64 5.58	13.29 13.38

a) The yields are the values from the starting amino acid ethyl ester hydrochloride.

TABLE 2. RESULTS OF POLYCONDENSATION OF HCl·H-L-Val-L-Phe-L-Phe-ONSu AND HCl·H-L-Phe-L-Val-L-Phe-ONSu

Monomer concn mol/l	(L-Val-L-Phe-L-Phe)$_n$ Yield[a] %	Yield[b] %	Yield[c] %	$\eta_{sp/c}$[d]	(L-Phe-L-Val-L Phe)$_n$ Yield[a] %	Yield[b] %	Yield[c] %	$\eta_{sp/c}$[d]
1.0	99	92	90	0.095	93	91	89	0.148
0.50	97	87	88	0.106	92	91	90	0.153
0.33					90	86	—	0.157
0.25	87	83	—	0.119	88	85	84	0.148
0.14					84	82	—	0.161
0.13	83	80	76	0.140	85	81	79	0.142

a) The yields are the values by the first isolation procedure. b) The yields are the values by the second isolation procedure. c) The yields are the values by the third isolation procedure. d) The viscosities are the values of the polypeptides isolated by the second isolation procedure.

cedure, because we have found that the solubility of peptides in solvents such as *N,N*-dimethylformamide differs greatly with the sequences of amino acids.[10] The results of the polycondensation of HCl·H-L-Val-L-Phe-L-Phe-ONSu and HCl·H-L-Phe-L-Val-L-Phe-ONSu are shown in Table 2. For the first isolation procedure, the polymerization system was diluted with diethyl ether. This treatment precipitates from the polymerization system the polymer, an oligomer with a very low molecular weight, and also triethylammonium chloride. The precipitate was collected by filtration and washed with methanol to remove the salt. The yields by the first procedure are shown by the values with the superscript a. The polymer isolated by the first procedure was suspended in *N,N*-dimethylformamide and reprecipitated by the addition of methanol. By this treatment, the oligomer with very low molecular weights and cyclic oligomers were removed from the polymer.[11] The yields by the second procedure are shown with the superscript b. In the third procedure the polymer was precipitated from the polymerization system by the addition of methanol. The yields by the third procedure are shown with the superscript c.

A comparison of the yields of the two polymers suggests an interesting feature of polycondensation. The HCl·H-L-Val-L-Phe-L-Phe-ONSu monomer with the *N*-terminal L-valine residue, gave, by the first isolation procedure, the (L-Val-L-Phe-L-Phe)$_n$ polymer in higher yields than did another HCl·H-L-Phe-L-Val-L-Phe-ONSu monomer with *N*-terminal L-phenylalanine. However, the yields of the (L-Val-L-Phe-L-Phe)$_n$ polymer decreased after the second treatment to become similar to the values of the yields of (L-Phe-L-Val-L-Phe)$_n$. These results suggest that the first procedure of isolation precipitated not only the polymer but also oligomers with very low molecular weights, which were then removed by the second isolation procedure. This is demonstrated by the fact that the second procedure gave the yields similar to those of the third isolation procedure, which does not precipitated the lower oligomers.

We should also discuss the results of the polycondensation in connection with the values of the yields obtained by the second and third procedures for the isolation of the polymer. Two polymers with the same sequence, (L-Val-L-Phe-L-Phe)$_n$ and (L-Phe-L-Val-L-Phe)$_n$, were isolated in similar yields. This is also true for other sequential polypeptides. The results of the polycondensation of another pair of monomers, HCl·H-L-Val-L-Ala-L-Ala-ONSu and HCl·H-L-Ala-L-Val-L-Ala-ONSu, are shown in Table 3. Table 3 also contains the yields of the polymers isolated by the third procedure. These sequential polypeptides, (L-Val-L-Ala-L-Ala)$_n$ and (L-Ala-L-Val-L-Ala)$_n$, were obtained in the same yields by three different isolation

Table 3. Results of polycondensation of HCl·H-L-Val-L-Ala-L-Ala-ONSu and HCl·H-L-Ala-L-Val-L-Ala-ONSu

Monomer concn	(L-Val-L-Ala-L-Ala)$_n$			(L-Ala-L-Val-L-Ala)$_n$		
	Yield %	$\eta_{sp/c}$	$\overline{DP}$ $\bar{n}$	Yield %	$\eta_{sp/c}$ %	$\overline{DP}$ $\bar{n}$
0.50	100	0.157	8	100	0.192	20
0.33	100	0.178	12	100	0.192	20
0.25	99	0.186	14	97	0.224	27
0.20	99	0.161	10	97	0.186	17
0.17	98	0.157	8	96	0.182	16
0.14	98	0.140	6			
0.13	98	0.152	8	94	0.178	15

procedures, in contrast to the results for the sequential polypeptides containing L-phenylalanine residues. The polymer containing L-alanine residues, therefore, may contain lower oligomers. This is consistent with the fact that the yields of the polypeptides containing L-alanine residues are higher than those containing L-phenylalanine residues. Another fact which is analogous for the polymers containing L-phenylalanine is that the polymers containing L-alanine residues were isolated in the same yields and no effect of the *N*-terminal amino acid residue appeared. It may be conclusively stated from the above results that the effect of the *N*-terminal amino acid of the monomer in polycondensation, if it is present, cannot detected by comparing the yields of the resulting sequential polypeptides.

Contrary to the above results for the yields, the viscosity of the resulting sequential polypeptides clearly shows the influence of the *N*-terminal amino acid of the monomers on the polycondensation. The sequential polypeptide, (L-Val-L-Phe-L-Phe)$_n$ obtained from a monomer with the *N*-terminal L-valine has lower viscosities than the polypeptide, (L-Phe-L-Val-L-Phe)$_n$, obtained from a monomer with *N*-terminal L-phenylalanine (Table 2). An analogous result was obtained by the polycondensation of another pair of monomers containing L-alanines (Table 3). The polymer with *N*-terminal L-valine (L-Val-L-Ala-L-Ala)$_n$ has lower viscosities than those of the polymer with *N*-terminal L-alanine (L-Ala-L-Val-L-Ala)$_n$. We determined the number-average degree of polymerization ($\overline{DP}$) of these polymers by measuring the NMR peak area of the *N*-terminal amino protons and the internal amide protons. These results are also shown in Table 3. The polymer with *N*-terminal L-alanine has $\overline{DP}$s from 15 to 27, about ten repeating units longer than those of the polymer with *N*-terminal L-valine, which has $\overline{DP}$s from 6 to 14. This finding is consistent with the results of the viscosity measurement.

As the pairs of the monomers studied in this study give the same polypeptides, the growing polymer chains may have similar conformations in the polycondensation system. Therefore, the most important factors responsible for the molecular weight of the resulting polymers is the *N*-terminal amino acid of the monomers. The above results can best be explained by the presence of the steric hindrance of the side chain of L-valine at the *N*-terminal position of the monomers. Though the detailed mechanism of polycondensation of peptide active esters has not yet been demonstrated because there are many complicated factors resulting from the use of the very concentrated polymerization system containing the salt, the monomer-active ester may polymerize in a manner similar to that of the monomers of conventional polycondensation; *i.e.*, the monomers are condensed to give dimers, which are then again condensed to give tetramers. The resulting oligomers with the *C*-terminal active ester is condensed with the amino group of another oligomer-active ester to give a higher oligomer. The reactivity of the terminal functional groups may not vary with the chain lengths, but the rate of polycondensation decreases greatly as the peptide chains become longer, because the molecular motion is prevented, especially in a solid state or in gelatinous systems. As the sterical effect of the L-valine residue may not be very large, the presence of L-valine at the *N*-terminal position does not influence the condensation of short peptide chains, but does hinder the condensation of larger peptides. Thus, the polycondensation of monomers with *N*-terminal L-valine was terminated at a lower peptide stage; the polymer thus obtained had lower molecular weight than those with *N*-terminal L-phenylalanine and L-alanine. This explanation points out the importance of stirring the polycondensation system containing the monomer with *N*-terminal L-valine. In fact, the sequential polypeptide, (L-Val-L-Phe-L-Phe)$_n$ with the highest viscosity was obtained in a polycondensation system with the lowest concentration of the monomer-active ester, which was a gelatinous liquid state which could be well stirred (Table 2). In a more gelatinous polycondensation system containing a higher concentration of the monomer, where effective stirring becomes difficult, the molecular motion and the reaction of the growing peptide chains may be prevented to form a lower-molecular-weight polymer. This is demonstrated by the conformational study in the solid state (see below).

In order to demonstrate further the presence of the steric hindrance of L-valine in polycondensation, we polymerized another monomer with L-valines, HCl·H-L-Val-L-Val-Gly-ONSu. The results are shown in Table 4. The (L-Val-L-Val-Gly)$_n$ polymer has $\overline{DP}$s of about 10 over the range of concentration of the monomer used in this study. These $\overline{DP}$s are

Table 4. Results of polycondensation of HCl·H-L-Val-L-Val-Gly-ONSu

Monomer concn mol/l	Yield %	$\eta_{sp/c}$	$\overline{DP}$ $\bar{n}$
0.50	95	0.185	10
0.33	92	0.167	8
0.25	91	0.180	10
0.17	90	0.185	10
0.13	89	0.172	8

similar to those of (L-Val–L-Ala–L-Ala)$_n$ with the *N*-terminal L-valine and are lower than those of (L-Ala–L-Val–L-Ala)$_n$ with *N*-terminal L-alanine. This comparison may not be reasonable because these polymers are different compounds, and so many effects other than the steric hindrance of the *N*-terminal amino acid must be taken into consideration in discussing the results. However, the fact that the *N*-terminal L-valine decreases the molecular weight of the resulting polypeptides suggests the presence of the steric hindrance of the amino acid.

Conformations of the Polypeptides in the Solid State.

The conformations of the sequential polypeptides were studied by means of far-infrared spectroscopy. Itoh *et al.*[12] found some characteristic bands of amino acid residues with the α-helical and β-form structures, and showed that the far-infrared spectroscopy is very useful for elucidating the conformations of polypeptides in the solid state. We analyzed the far-infrared spectra of the sequential polypeptides according to the assignments established by Itoh *et al.* Figure 1 shows the far-infrared spectra of the sequential polypeptide (L-Ala–L-Val–L-Ala)$_n$. This polypeptide showed a strong band at 441 cm^{-1} characteristic of the L-alanine residue with the β-structure and weak bands at 540, 520, 409, and 375 cm^{-1} characteristic of the L-alanine and L-valine residues with the α-helix. The spectra suggest that the polypeptide takes predominantly the β-structure and contains a portion of the α-helix. Some polypeptides are known to transform their conformation from the β-structure to the α-helix upon treatment with dichloroacetic acid. The polypeptide (L-Ala–L-Val–L-Ala)$_n$ with $\overline{DP}$ 16, when treated with dichloroacetic acid, showed the infrared bands at 540, 524, 409, and 374 cm^{-1}, while the band at 441 cm^{-1} almost disappeared. This suggests that the polypeptide transformed the conformation to the α-helix upon treatment. Figure 2 shows the spectra of (L-Val–L-Ala–L-Ala)$_n$. The polypeptide isolated showed the band at 441 cm^{-1} characteristic

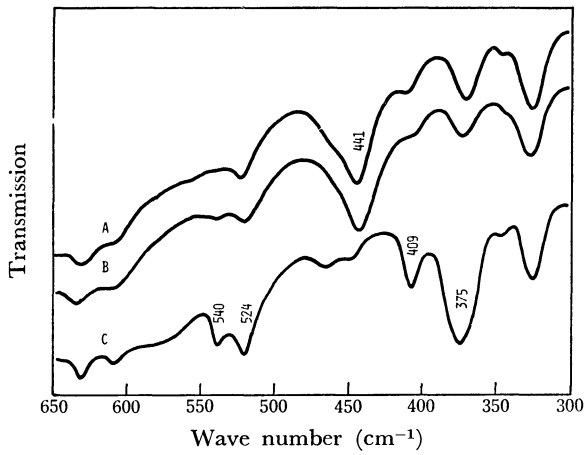

Fig. 1. Far-infrared spectra of the sequential polypeptide (L-Ala–L-Val–L-Ala)$_n$. A: The polypeptide with $\overline{DP}$ 27, B: The polypeptide with $\overline{DP}$ 16, C: The polypeptide with $\overline{DP}$ 16 after treatnemt with dichloroacetic acid.

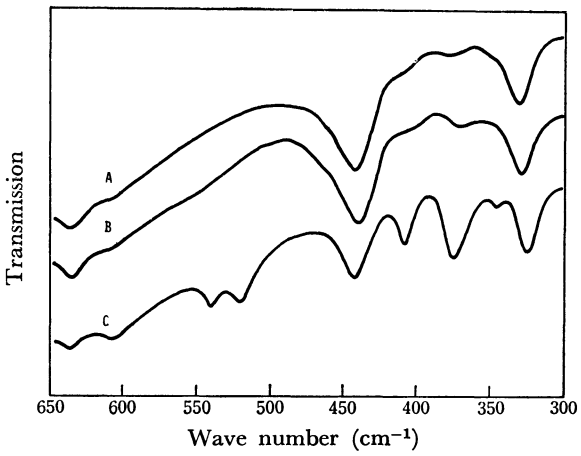

Fig. 2. Far-infrared spectra of the sequential polypeptide (L-Val–L-Ala–L-Ala)$_n$. A: The polypeptide with $\overline{DP}$ 8, B: The polypeptide with $\overline{DP}$ 14, C: The polypeptide with $\overline{DP}$ 14 after treatment with dichloroacetic acid.

of the β-structure, while the sample with $\overline{DP}$ 14 treated with dichloroacetic acid showed the bands at 540, 524, 409, and 375 cm^{-1} characteristic of the α-helix, and also that at 441 cm^{-1} characteristic of the β-structure. These results suggest that the polypeptide with *N*-terminal L-valine takes not only the α-helix but also the β-structure after the treatment. We suppose that the β-structure of the latter polypeptide may result from the presence of the oligomers with low molecular weights, perhaps five or lower repeating tripeptide units, because we have shown in another series of studies that some sequential tripeptide oligomers begin to take the α-helix at pentadecapeptides in the solid state.[13] Thus, the above results demonstrate that the sequential polypeptide with *N*-terminal L-valine (L-Val–L-Ala–L-Ala)$_n$ contains a fair portion of the lower oligomers, which cannot form the α-helical conformation. This is consistent with the results of polycondensation described above.

Some interesting results were obtained by the conformational study of the polypeptides, (L-Phe–L-Val–L-Phe)$_n$, (L-Val–L-Phe–L-Phe)$_n$, and (L-Val–L-Val–Gly)$_n$, though no evidence for elucidating the sterical

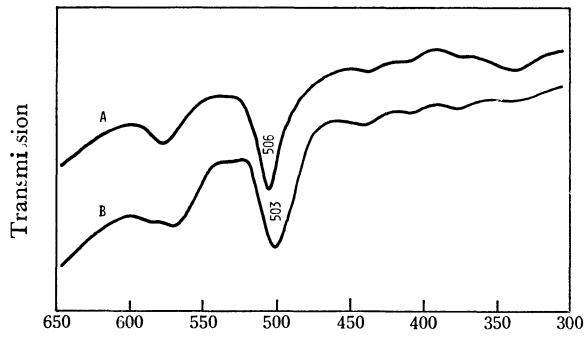

Fig. 3. Far-infrared spectra of the sequential polypeptide (L-Phe-L-Val-L-Phe)$_n$ with η_{sp}/c 0.161. A: Before treatment with dichloroacetic acid, B: After the treatment.

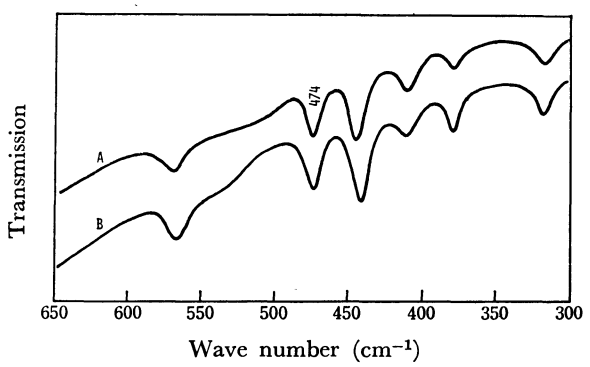

Fig. 4. Far-infrared spectra of the sequential polypeptide (L-Val-L-Val-Gly)$_n$. A: Before treatment with dichloroacetic acid, B: After the treatment.

effect of the *N*-terminal L-valine in polycondensation was obtained. Fig. 3 shows the far-infrared spectra of the polypeptide (L-Phe–L-Val–L-Phe)$_n$ before and after treatment with dichloroacetic acid. The spectrum of (L-Val–L-Phe–L-Phe)$_n$ was similar to those shown in Fig. 3. The spectra have the band near 505 cm^{-1} which has been assigned as characteristic of the L-phenylalanine residue with the β-structure. It is known that poly(L-phenylalanine) can take the α-helical conformation, but the stability of the α-helix is not so large as that for poly(L-alanine) and poly(L-leucine).[14] The β-structure of the sequential polypeptide in this study shows that it may not have an adequate molecular weight to form the α-helical conformation. Figure 4 shows the spectra of (L-Val–L-Val–Gly)$_n$ before and after the treatment. The polypeptide has already been studied by Itoh *et al.*[15] They found the band at 470 cm^{-1} characteristic of the poly(L-valine) structure, which differs from the β-structure.[3] Though the spectra of our sample have some new bands at 568, 412, and 380 cm^{-1} which were not found in the spectrum reported by Itoh, they have the characteristic band at 474 cm.$^{-1}$ This shows that the polypeptide takes the structure characteristic of poly(L-valine) before and after the treatment with dichloroacetic acid.

Experimental

Synthesis of Monomers. Nps-tripeptide ONSu esters were prepared by the stepwise synthesis of the Nps-tripeptide ethyl ester, the saponification of the tripeptide esters, and the active esterification of the tripeptide free acid. A typical synthesis of the monomers is illustrated by the following preparation of Nps–L-Phe–L-Val–L-Phe–ONSu.

Nps–L-Val–L-Phe–OEt. L-Phenylalanine ethyl ester hydrochloride (46.0 g (0.2 mol)) was suspended in 400 ml of tetrahydrofuran, and then 28 ml of triethylamine was added. The resulting crystals of the salt were removed by filtration. To the filtrate were added 59.2 g (0.2 mol) of Nps–L-valine NCA, after which the solution was stirred for 3 h at room temperature. The solvent was evaporated under reduced pressure. The residue was dissolved in 600 ml of ethyl acetate. The solution was washed with 5% citric acid, 5% sodium hydrogencarbonate, and water, and dried over sodium sulfate. The solution was concentrated under reduced pressure. To the residue hexane was added to crystallize the product, which was then recrystallized from warm ethyl acetate to give 81.1 g (91%) of a pure dipeptide; mp 149—150 °C, $[\alpha]_D$ −11.4 (*c* 1.0, *N,N*-dimethylformamide). Found: C, 59.25; H, 6.19; N, 9.40. Calcd for $C_{22}H_{27}N_3O_5S$: C, 59.31; H, 6.11; N, 9.43%.

Nps–L-Phe–L-Val–L-Phe–OEt. The above Nps-dipeptide ester (80.2 g (0.18 mol)) was dissolved in 180 ml of 2 M hydrochloric acid in ethanol. To the solution were added 500 ml of diethyl ether and 300 ml of hexane to precipitate the dipeptide ester hydrochloride. The precipitate was collected by filtration, washed with diethyl ether, and reprecipitated from methanol. The product was dissolved in 600 ml of tetrahydrofuran, and then 26 ml of triethylamine was added. The resulting crystals were removed. To the solution were added 63.7 g (0.18 mol) of Nps–L-phenylalanine NCA, after which the solution was stirred for 2 h at room temperature. The solution was treated analogously to the case of the dipeptide synthesis. The obtained Nps-tripeptide ester was recrystallized from tetrahydrofuran; 99.2 g (93%); mp 165—166 °C, $[\alpha]_D$ +41.9 (*c* 1.0, *N,N*-dimethylformamide). Found: C, 62.76; H, 6.21; N, 9.48. Calcd for $C_{31}H_{36}N_4O_6S$: C, 62.82; H, 6.12; N, 9.45%.

Nps–L-Phe–L-Val–L-Phe–OH. The Nps-triprptide ethyl ester (59.3 g (0.1 mol)) was dissolved in 200 ml of acetone, and then 100 ml of 1 M sodium hydroxide was added. The system was stirred for 1 h to give a clear solution, which was then concentrated under reduced pressure at 30 °C: the residual aqueous solution was diluted with 200 ml of water. The solution was extracted with 300 ml of diethyl ether and acidified with 15% citric acid. The system was extracted with ethyl acetate. The extract was washed with water and dried over sodium sulfate. The solution was concentrated under reduced pressure. Hexane was added to crystallize the product. The crude product was purified by recrystallization from tetrahydrofuran; 48.5 g (86%); mp 111—113 °C, $[\alpha]_D$ +50.5 (*c* 1.0, *N,N*-dimethylformamine). Found: C, 61.60; H, 5.82; N, 9.98%. Calcd for $C_{29}H_{32}N_4O_6S$: C, 61.68; H, 5.71; N, 9.93%.

Nps–L-Phe–L-Val–L-Phe–ONSu. The Nps-tripeptide free acid (56.5 g (0.1 mol)) was dissolved in 500 ml of tetrahydrofuran. To the solution were added 12.7 g (0.11 mol) of *N*-hydroxysuccinimide, after which the system was stirred for 10 min to give a clear solution. The solution was cooled at −10 °C, and 20.9 g (0.11 mol) of dicyclohexylcarbodiimide was added. The solution was then stirred for 5 h at −10 °C and allowed to stand for 20 h at 0 °C. The crystals of the urea were removed by filtration. A few drops of glacial acetic acid were added, and the solution was concentrated under reduced pressure at 10 °C. Hexane was added to the residue. The crystals were isolated and redissolved in tetrahydrofuran. The undissolved materials were removed, and the solution was concentrated. To the residue hexane was added to crystallize the product; 58.9 g (89%); mp 185—190 °C (dec). $[\alpha]_D$ +22.0 (*c* 1.0, *N,N*-dimethylformamide). Found: C, 59.97; H, 5.45; N, 10.46%. Calcd for $C_{33}H_{35}N_5O_8S$: C, 59.89; H, 5.33; N, 10.59%.

Polycondensation of Tripeptide ONSu Esters. Nps-tripeptide ONSu ester (0.03 mol) was dissolved in 30 ml of 2 M hydrochloric acid in dioxane, and 300 ml of diethyl ether were added. The resulting precipitate was isolated by filtration, washed with diethyl ether until the yellow color disappeared, and dried. The product was dissolved in a small amount of methanol. To the solution was added diethyl ether to precipitate the tripeptide ONSu ester hydrochloride. The hydrochloride (0.005 mol) was dissolved in *N,N*-dimethyl formamide at the concentrations listed in Table 2 to 4. To the solution was then added, with vigorous stirring, 0.84 ml of triethylamine. The system was stirred or allowed to

stand for 1 day at room temperature. After the polycondensation, the system was treated by the following three different procedures for the isolation of the polypeptide. First procedure: the polymerization system was diluted with 100 ml of diethyl ether. The precipitate was collected by filtration, washed with 200 ml of methanol and 50 ml of diethyl ether, and dried over P_2O_5. Second procedure: the product isolated by the first procedure was suspended with vigorous stirring in 30 ml of *N,N*-dimethylformamide for 6 h. The system was then diluted with 100 ml of methanol, and the resulting precipitate was isolated by filtration, washed with 50 ml of methanol and 50 ml of diethyl ether, and dried. Third procedure: the polymerization system was diluted with 100 ml of methanol. The resulting precipitate was treated in the same way as in the second procedure.

Measurements. The viscosity of the sequential polypeptides was measured at 25±0.1 °C with an Ostwald viscometer. The concentration of the polypeptide was adjusted to 0.5 g/100 ml of dichloroacetic acid. The NMR spectrum of the polypeptides was obtained with a JEOL JNM-PMX 60 NMR spectrometer at room temperature. The concentration of the polymer was adjusted to 10% in trifluoroacetic acid. The far-infrared spectrum was measured with a JASCO IR-F spectrophotometer. Nujol mulls were used.

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