Synthesis and Properties of High-Molecular-Weight Polypeptides Containing Tryptophan II. Copolypeptides of Tryptophan with Various Amino Acids[1)]

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High-purity *N*-carboxyl L-tryptophan anhydride (L-4-indoylmethyl-2,5-oxazolidinedione) was synthesized in good yield. Copolypeptides with a random sequence of L-trypophan (Trp) with glycine (Gly), L-alanine (Ala), L-α-amino-*n*-butyric acid (Abu), L-*n*-valine (Nval), L-*n*-leucine (Nleu), L-leucine (Leu), L-γ-methylglutamate (Glu(OMe)), and L-methionine (Met) were synthesized by the copolymerization of the corresponding *N*-carboxy-α-amino acid anhydrides (NCA) in solution. The rate of polymerization of Trp NCA was less than that of the other amino acid NCAs. The helix contents of the formed copolypeptides, estimated by the circular dichroism (CD) spectra, were remarkably decreased at a content of about 8% of Trp involved in poly(Trp-co-Nval) (poly(Trp,Nval)), poly(Trp-co-Leu) (poly(Trp,Leu)), poly(Trp-co-Nleu) (poly(Trp,Nleu)), and poly(Trp-co-Glu(OMe)) (poly(Trp,Glu(OMe))), but was not decreased at that percentage in poly(Trp-co-Met) (poly(Trp,Met)).

Tryptophan (Trp, **1i**) is widely found in the native proteins or several peptide hormones, such as snake-venom neurotoxin, ACTH, glucagon, and tyrocidine as a minor component of one or two percent. A very small amount of Trp residue influences the emergence of their toxicity or higher order conformations. Trp-29 residue is favorable for a conformational change of neurotoxin.[2,3)] The mutual interaction between various amino-acid residues in the proteins contributes to their higher order conformations or toxicity. The factors regarding the interaction between amino acids are so complicated that conformational studies for simplified synthetic polypeptides consisting of two amino acid residues, including Trp, would be interesting and important in both biological and conformational approaches to proteins.

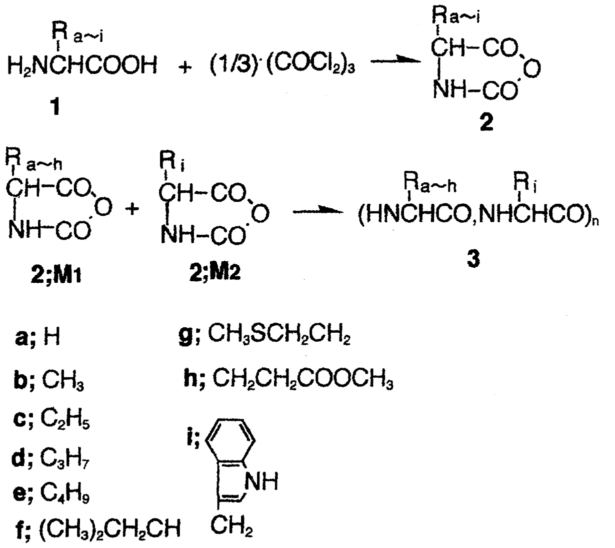
In a previous paper we reported on the synthesis and properties of high-molecular-weight copolypeptides of DL-tryptophan with L-alanine.[4)]

In this paper we report on the copolymerization of Trp NCA (**2i**) with various amino acid NCA (**2a**—**h**) to produce copolypeptides containing Trp (**3**). Also, the solubility and conformation of the copolypeptides were studied using the CD spectra. Further, the influence of the Trp residue on helix formation was compared with that of other amino acid residues having bulky pendant groups.

Experimental

Synthesis of NCA (2a—h). NCAs (**2**) were synthesized in high purity and good yield by a previously reported method (Scheme 1).[4)]

Copolymerization of NCAs. Trp NCA (0.92 g, 4 mmol) (**2i**) and Gly NCA (**2a**, 1.62 g, 16 mmol) were dissolved in acetonitrile (100 cm^{-3}). Butylamine (7.3 mg, 0.1 mmol) as an initiator was then added, and allowed to stand at 30 °C for 7 d. After the reaction the copolymer was separated on a sintered-glass filter by filtration, washed with a polymerization solvent, acetonitrile, and dried in vacuo at room temperature. The copolymer of Trp with Gly (**3a**, **i**) (0.66 g) was obtained. Other copolypeptides were prepared by an analogous method.



Scheme 1. Snthesis of Trp NCA and polypeptides containing Trp.

Copolypeptides Soluble in Solvents. The copolypeptide (0.1 g) was put in a solvent (10 cm^{-3}), i.e., *N*,*N*-dimethylformamide, dimethylsulfoxide, methyl alcohol, ethyl alcohol, and water, and kept for 24 h at 20—25 °C. An insoluble polymer was separated on a sintered-glass filter, and then washed with ethyl ether or water. The filtrate was dried in vacuo at room temperature.

Viscosity Measurement. Tne viscosity of the copolypeptides was measured in a solution of 1,1,1,3,3,3-haxafluoro-2-propanol (HFIP) using an ubbelohde viscometer at 25 °C.

Spectroscopic Measurement. Trp components in the copolymers were estimated from the UV-absorbance working curve of poly(DL-Trp) in solutions of HFIP at 268 nm. The CD spectra were recorded on a JASCO J-20 A at wavelengths of 250 nm to about 190 nm at 20 °C using a 0.1 mm path length cell at a concentration of about 0.03 mol dm^{-3} (residue basis).

The reduced mean residue ellipticity ($[\theta]_\lambda$), was defined as

$$[\theta]_\lambda = \theta/10C \times L,$$

where θ is the observed ellipticity in deg, L is the optical path length cell in cm, and C is the polymer concentration in mol dm^{-3} (residue basis).[5] The optical rotatory dispersion (ORD) of the polymerization systems was measured over the range of 650 to 250 nm at about 22 °C after completion of polymerization. The moffit parameter (b_0) was calculated with reference to the literature.[6]

Results and Discussion

Reactivity Ratio of NCA. Trp NCA was copolymerized with Gly NCA, Ala NCA, Abu NCA, Nval NCA, Nleu NCA, Leu NCA, Glu(OMe) NCA, or Met NCA in solution to produce copolypeptides. The polymerization conditions and properties of the copolypeptides are summarized in Table 1. The listed polypeptides have chain lengths capable of forming secondary structures.

The rate of the copolymerization of Trp NCA with the other (X) NCA can be expressed as

$$\begin{aligned} &d[M_1]/d[M_2] \\ &= ([M_1]/[M_2])\,(r_1[M_1]+[M_2])/([M_1]+r_2[M_2]), \end{aligned}$$

where $[M_1]$ and $[M_2]$ are the concentrations of Trp NCA and X NCA, respectively, and r_1 and r_2 are their respective monomer-reactivity ratios.

The monomer-reactivity ratios were determined from the general copolymerization equation by using the Mayo–Lewis integral method.[8,9]

In the polymerization of a mixture comprising of L-Trp NCA and other NCAs, the reactivity ratios r_1 were less than those of r_2. These reactivity ratios indicate that the reactivity of Trp NCA is very small.

Solubility. The copolypeptides containing Trp were mostly insoluble in water, ethyl alcohol and methyl alcohol. Some copolypeptides containing Trp at more than 5%, however, became soluble in *N*,*N*-dimethylformamide and dimethylsulfoxide. Although a part of poly(DL-Trp, L-Ala) was soluble in water,[4] poly(L-Trp, L-Ala) was insoluble; poly(L-Trp, Gly), -(L-Trp, L-Ala), and -(L-Trp, L-Leu), containing a small portion of Trp, were generally insoluble in HFIP.

Conformation. The CD spectra listed in Table 1 are given in Figs. 1, 2, 3, 4, 5, 6, 7, and 8. These ellipticities (between 190 and 250 nm) have been shown to be a sensitive indicator of the conformation of α-helix, β-sheet and randomly disordered polypeptides.[5] The CD spectra of polypeptides have demonstrated a positive strong peak ($[\theta]_{190}$=3000—52000) and a negative medium peak ($[\theta]_{216-218}$=−7700—−23000), which are assigned to a β sheet,[10—12] and a negative strong one at 190 nm and a positive weak one at 216 nm, which are assigned to randomly coiled conformation.[13,14] For low-molecular-weight poly(Ala) in a mixed solution of trifluoroethanol with water, two strong negative peaks ($[\theta_{207}]$=−36000) and ($[\theta]_{221}$=−31000), and a positive strong one ($[\theta]_{190}$=6600) were used to assign to an α-helix.[5] Since poly(Gly), poly(Ala), poly(Nbu), poly(Nval), poly(Nleu), and poly(Leu) having a high molecular weights were insoluble in HFIP, no CD spectra of those polypeptides were obtained. Many copolypeptides containing Trp were soluble in HFIP, but insoluble in 1,2-dichloroethane. Then, the conformation of those polypeptides containing Trp were evaluated based on the strength or the peaks at about 190, 208, 216, and 222 nm in HFIP solutions. Homopolypeptides of Ala, Nbu, Nval,

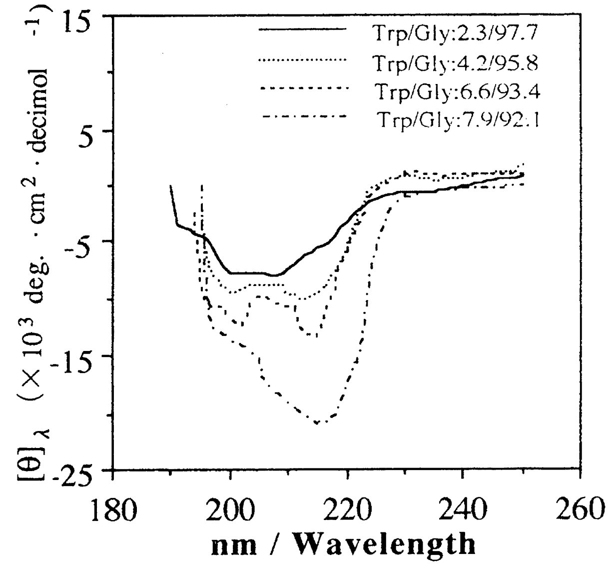


Fig. 1. CD spectra of poly(Gly, Trp) in HFIP solution. (Polypeptide concentration was 0.03 mol dm^{-3}).

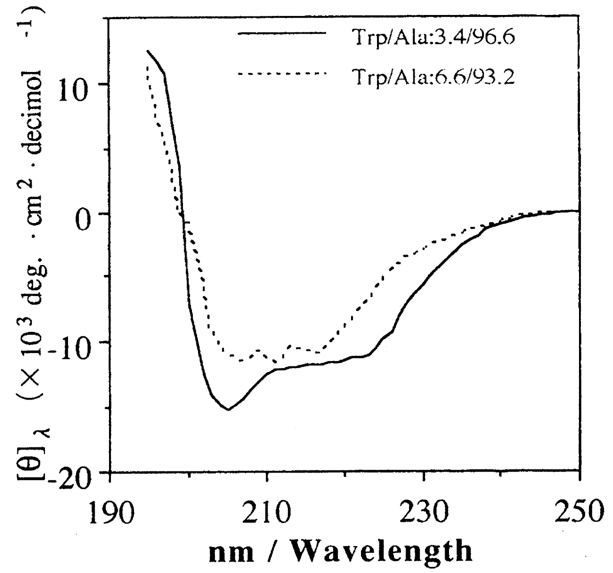


Fig. 2. CD spectra of poly(Ala, Trp) in HFIP solution. (Polypeptide concentration was 0.03 mol dm^{-3}).

Table 1. Synthesis and Properties of Copolypeptides Containing Tryptophan

Entry	NCA					Copolypeptides			
	(Trp/X)	Solvent[k)]		Concentration mol dm^{-3}	Conversion mol%	(Trp/X)	DP[a)] or η_{sp}/c[e)]	Solubility[m)]	Reactivity ratio
	Trp/Gly[b)]								
A1	5/95	ACN		0.23	54[l)]			C	
A2	10/90	ACN		0.22	59	2.3/97.7	118[a)]		r_1=0.3—1.0
A3	20/80	ACN		0.20	53	4.2/95.8	106[a)]		r_2=5.5—8.0
A4	30/70	ACN		0.18	49	6.6/93.4	98[a)]		
A5	40/60	ACN		0.17	56	7.9/92.1	112[a)]		
	Trp/Ala[j)]								
B1	21/79	DCE		0.23	79	3.4/96.6	158[a)]		
B2	40/60	DCE		0.25	63	6.8/93.2	126[a)]		
	Trp/Abu[c)]								
C1	5/95	C_6H_6/DCE;	90/10	0.15	89	1.1/98.9	178[a)]		r_1=0.2—1.6
C2	10/90	C_6H_6/DCE;	82/18	0.16	94	1.8/92.2	188[a)]		r_2=10—16.5
C3	20/80	C_6H_6/DCE;	65/35	0.17	75	5/95	150[a)]		
C4	60/40	C_6H_6/DCE;	65/35	0.11	62	7.8/92.2	124[a)]	A	
	Trp/Nval[d)]								
D1	5/95	C_6H_6/DCE;	87/13	0.17	93	2.1/97.9	1.20[e)]		r_1=0.05—0.6
D2	10/90	C_6H_6/DCE;	81/19	0.17	84	3.2/96.8	1.05[e)]		r_2=5.2—8.0
D3	30/70	C_6H_6/DCE;	53/47	0.13	72	7/93	0.45[e)]		
D4	40/60	C_6H_6/DCE;	41/59	0.13	64	9.7/90.3	0.23[e,4)]	AB	
	Trp/Nleu[f)]								
E1	5/95	ACN/DCE;	7/93	0.16	88	1.6/98.4	88[a)]		$r_1<0.5$
E2	10/90	ACN/DCE;	14/86	0.15	79	2.8/97.2	79[a)]		$r_2<7$
E3	20/80	ACN/DCE;	38/62	0.15	83	6/94	83[a)]		
E4	40/60	ACN/DCE;	49/51	0.13	57	8.8/91.2	57[a)]	AB	
	Trp/Leu[g)]								
F1	5/95	ACN		0.14	87[l)]		87[a)]	C	$r_1<0.9$
F2	10/90	ACN		0.15	91[l)]		91[a)]	C	$r_2<10$
F4	30/70	ACN		0.14	66	8.3/91.7	66[a)]	A	
F5	40/60	ACN		0.13	33	11.5/88.5	33[a)]	AB	
	Trp/Glu(OMe)[h)]								
G1	5/95	ACN		0.13	51	1.7/98.3	51[a)]		r_1=0.8—1.6
G2	10/90	ACN		0.13	58	3.7/96.3	58[a)]		r_2=5—16
G3	20/80	ACN		0.13	82	6.6/93.4	82[a)]		
G4	30/70	ACN		0.13	74	9.8/90.2	74[a)]	AB	
G5	40/60	ACN		0.13	66	11.8/88.2	66[a)]	AB	
	Trp/Met[i)]								
H1	10/90	ACN/DCE;	14/86	0.16	88	1.1/98.9	88[a)]		$r_1<0.8$
H2	20/80	ACN/DCE;	27/73	0.20	79	5.2/94.8	79[a)]	A	$r_2<12$
H3	30/70	ACN/DCE;	40/60	0.14	61	8.3/91.7	61[a)]	A	

Polymerizations were carried out at 30 °C. a) DP; Degree of polymerization=(Monomer/Initiator×Conversion %/100). b, c, d, f, g, h, i, j) Precipiteted polymer in the reaction system was filtered and dried. Polymerization was initiated with butylamine (BA) at an NCA-to-BA ratio of 200 (b,j) and 100 (f, g, h, and i), at 30 °C for 336 and 168 h (j), and initiated with triethylamine (TEA) at an NCA-to-TEA of 200 (c) and 100 (d) at 30 °C for 336 h. e) Concentration c: 0.5 g/100 cm^3 in dichloroacetic acid. Molecular weight of D4 was 4600, which calculated as $[\eta]=(\ln \eta_{rel})/c$ $[\eta]=5.1\times10^{-4}\times M^{0.73}$ (Ref. 7). k) ACN; Acetonitrile, DCE; Dichloroethane. l) Insoluble in HFIP. m) Solubility: Soluble in Dimethylformamide (A), Dimethylsulfoxide (B) at 25 °C. Insoluble in HFIP (C). Soluble in only HFIP; no mark.

Nleu, Leu, and Glu(OMe) have shown stable right-handed helix conformations.[15)] The ellipticity values $[\theta]$ at 208 and 222 nm generally decreased at a Trp content of more than 7% in the copolypeptides, suggesting that the Trp residue contained in the copolypeptides facilitate a decrease in the content of the right-handed helix in the copolypeptides.

Since Gly has no asymetric carbon, poly(Gly) ought not to have CD absorption. Copolypeptides comprising of 95.8% Gly and 4.2% Trp (poly(Gly: 95.8, Trp: 4.2)) and poly(Gly: 93.4, Trp: 7.9), however, gave two weak minimum peaks: The former gave $[\theta]_{201}=[\theta]_{212}=-1000$, and the latter gave $[\theta]_{202}=-1200$ and $[\theta]_{215}=-1300$, respectively, which exist

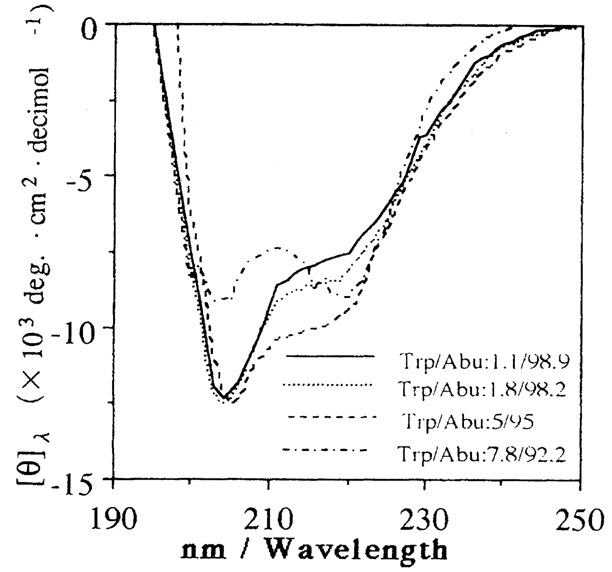


Fig. 3. CD spectra of poly(Abu, Trp) in HFIP. (Polypeptide concentration was 0.03 mol dm^{-3}).

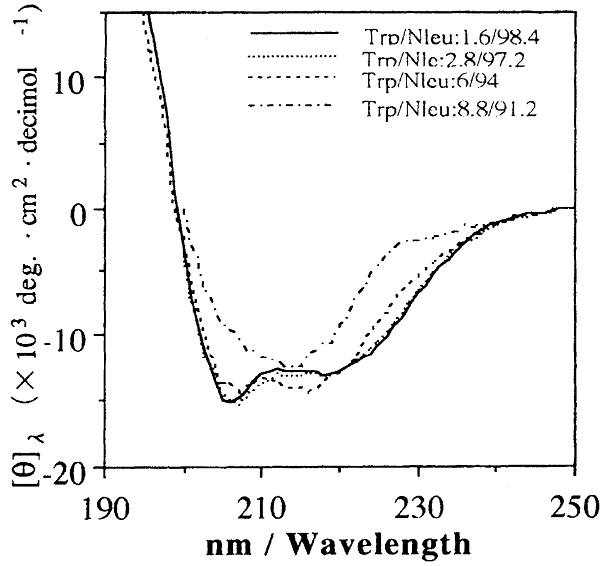


Fig. 5. CD spectra of poly(Nleu, Trp) in HFIP. (Polypeptide concentration was 0.03 mol dm^{-3}).

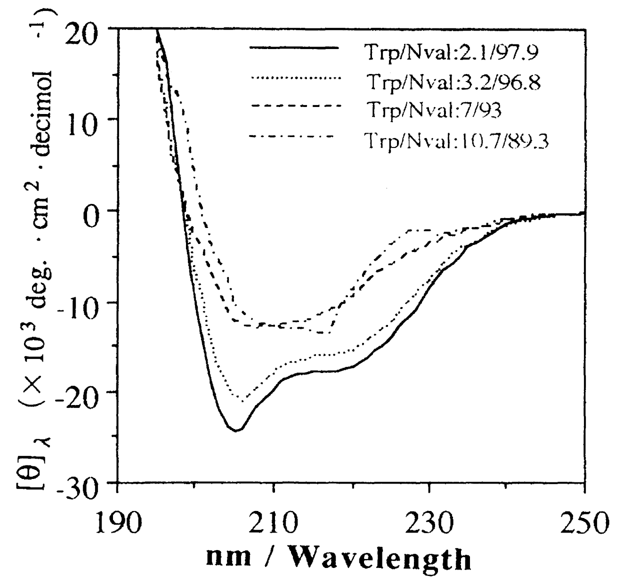


Fig. 4. CD spectra of poly(Nval, Trp) in HFIP. (Polypeptide concentration was 0.03 mol dm^{-3}).

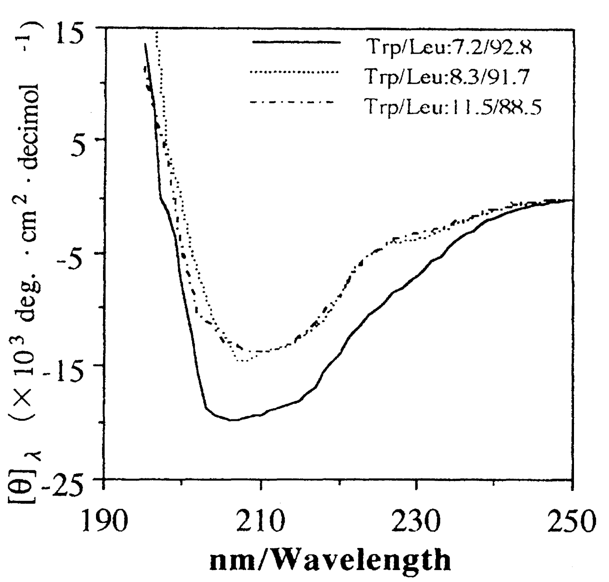


Fig. 6. CD spectra of poly(Leu, Trp) in HFIP. (Polypeptide concentration was 0.03 mol dm^{-3}).

in mixed conformations of random coils with a β-sheet. On the other hand, poly(Gly: 92.1, Trp: 7.9) gave a spectrum like a β-sheet (Fig. 1). Poly(Trp: 3.4, Ala: 96.9) gave the characteristic ellipticity of an α-helix ($[\theta]_{205}=-15200$ and $[\theta]_{222}=-11200$). Poly(Trp: 6.8, Ala: 93.2) gave the ellipticity of a mixture of an α-helix ($[\theta]_{207}=-11700$ and $[\theta]_{222}=-7500$) and a β ($[\theta]_{217}=-10900$) conformation (Fig. 2). Poly(Abu: 95, Trp: 5) gave an α-helical pattern ($[\theta]_{205}=-12500$ and $[\theta]_{222}=-8600$). On the other hand, poly(Abu: 92.2, Trp: 7.8) gave the pattern of a mixed conformation of an α-helix with a randomly coiled ($[\theta]_{205}=-9100$ and $[\theta]_{219}=-8900$) (Fig. 3). Regarding the infrared (IR) spectra, both of these polymers gave almost the same pattern in solid KBr disks after completing the polymerization: absorption amide I at 1655—1643 cm^{-1} and amide II at 1530—1515 cm^{-1}. IR spectra data are deposited as Document No. 69011 at the Office of the Editor of Bull. Chem. Soc. Jpn. Poly(Nval, Trp), poly(Nleu, Trp), and poly(Leu, Trp) disordered α-helix conformation by containing Trp resiues of 7, 8.8, and 8.3%, in the peptide chains, respectively (Figs. 4 and 5). Since poly(Leu) and poly(Trp, Leu) in the Leu content more than 93% were insoluble in HFIP, those CD spectra were difficult to measure. When the Trp content in the soluble polymers was increased from 7.2 to 8.3%, the ellipticity ($[\theta]_{208}$) was decreased to -14800 from -19600 (Fig. 6). The IR spectra of the insoluble poly(Trp, Leu) (F2) and soluble one (F5) showed almost the same pattern: absorption amide I at 1655—1543 cm^{-1} and amide II at

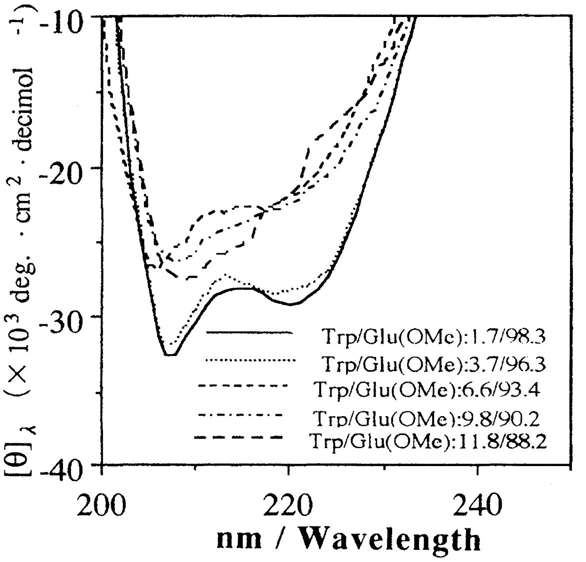


Fig. 7. CD spectra of poly(Gly(OMe), Trp) in HFIP. (Polypeptide concentration was 0.03 mol dm^{-3}).

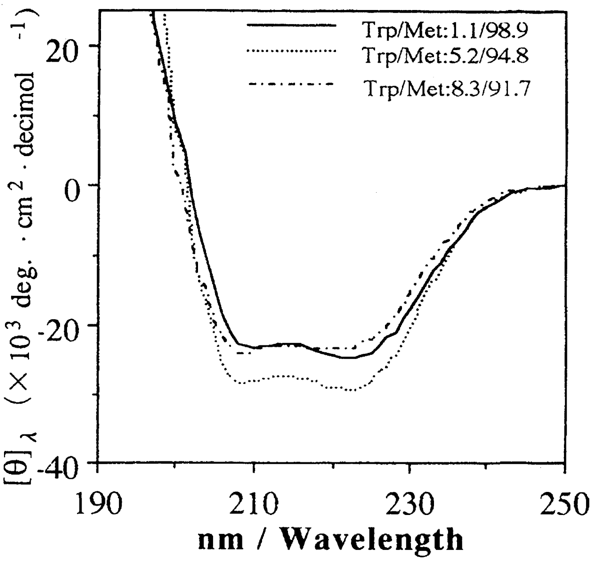


Fig. 8. CD spectra of poly(Met, Trp) in HFIP. (Polypeptide concentration was 0.03 mol dm^{-3}).

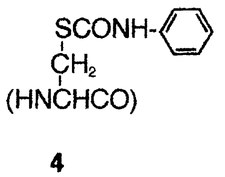
1530—1515 cm^{-1} in the solid state. These results, including that of the poly(Trp, Abu) described above, suggest that the conformation of the polymers is almost the same in the polymerization system after completing the polymerizations. The spectra of poly(Glu: 98.3, Trp: 1.7) and poly(Glu: 96.3, Trp: 3.7) had almost the same pattern, and showing two minimum peaks ($[\theta]_{208}=-32600$ and -32900, respectively, and $[\theta]_{222}=-29100$ and -28300, respectively). In poly(Trp: 6.6, Glu(OMe): 93.4) gave three minimum peaks ($[\theta]_{206}=-26900$, $[\theta]_{217}=-22800$, and $[\theta]_{219}=-22300$), which fit the mixed conformation of α-helix and β-sheet. Poly(Glu(OMe): 90.2, Trp(OMe): 9.8) and poly(Glu(OMe): 88.2, Trp: 11.8) showed one minimum-peak spectrum, suggesting that the peak at around 216 nm (β-sheet) became stronger (Fig. 7). Poly(Met: 94.8, Trp: 5.2) showed an α-helix pattern. In poly(Met: 91.7, Trp: 8.3), a peak at 216 nm (β-sheet) was increased and at 222 nm (α-helix) was decreased (Fig. 8).

Influence of Some Bulky Amino-Acid Residues on the Conformational Change in Polypeptides. Phenylcarbamoyl cystein (Cys(Pc)) (**4**) residue has a bulky pendant group (Scheme 2), the copolymer of which rarely took the α-helical conformation, similarly to poly(Trp), both of which should be predictable to influence the helix formation of copolypeptides. Poly(Cys(Pc): 3, Glu(OMe): 97) and poly(Cys(Pc): 8, Glu(OMe): 92) have two minimum peaks in HFIP ($[\theta]_{208}=-44000$ and -41500, respectively, and $[\theta]_{222}=-39500$ and -37000, respectively).[16] Both ellipticities suggest that those polypeptides are almost in the helix conformation in solution.[16]

HFIP has a strong tendency to form hydrogen bonding with basic groups.[17] The influence of protons on helix formation in a solvent of polypeptide is also well known. To evaluate the dependence of the Trp residue as well as the other bulky amino acid residues on the conformational change of polypeptides in an aprotic solvent, copolypeptides containing Trp, Phe, Pro, Ala, and Asp(OBzl) were synthesized by the copolymerization of NCAs in 1,2-dichloroethane. Since many aprotic solvents have absorptions below 227 nm, the CD spectra in the solutions can not be obtained. Therefore, the ORD spectra were studied for those copolyptides in the polymerization solution (Table 2).

The moffit parameters calculated in the ORD spectra ($b_0/-630$) reflect the helix content (%) of polypeptide in solution.[6] Poly(Phe) has a bulky phenyl pendant group, and is mostly insoluble in solvents. Some copolypeptides containing Phe as a component are, however, soluble in aprotic solvents, such as 1,2-dichloroethane, and benzene. ORD of poly(Phe: 50, Leu:50) (No. 1) has an α-conformation at a content of 84% ($b_0=-530/-630$).[6] Poly(Glu(OMe): 95, Phe: 5) (No. 2) gave -560 for its moffit parameter, which corresponds to a helix content of 89% ($b_0=-560/-630$).

It is well known that poly(β-benzyl-L-asparatate), having a bulky pendant group, takes a more stable conformation of a left-handed helix than a right-handed helix.[18] Poly(Ala) has mostly a stable conformation of a right-handed helix.[15] Poly(Asp(OBzl): 67, Ala: 33) (No. 3) and poly(Asp(OBzl): 50, Ala: 50) (No. 4) take a right-handed α-helix ($b_0=-400$ and -540) in a 1,2-dichloroethane solution.[19] The β-benzyl asparatate residue does not destroy the right-handed helical conformation of the poly(Ala). Poly(Pro) is not able to form a helix coformation in solutions. As described above, phenylalanine has a bulky pendant group. Although both residues should influence the conformation



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Scheme 2.

Table 2. Moffitt Parameter of Copolypeptides in 1,2-Dichloroethane

No.	Copolypeptides	b_0 of ORD	Concentration mol dm^{-3}
1.	Poly(Phe: 50, Leu: 50)[19]	−530	0.15
2.	Poly(Glu(OMe): 95, Phe: 5)	−560	0.15
3.	Poly(Asp(OBzl): 66.6, Ala: 33, 3)[19]	−400	0.15
4.	Poly(Asp(OBzl): 50, Ala: 50)[19]	−540	0.15
5.	Poly(Asp(OBzl): 70.5, Ala: 23.5, Pro: 6)	−330	0.1
6.	Poly(Asp(OBzl): 71, Ala: 23.9, Phe: 4.6)	−420	0.19
7.	Poly(Asp(OBzl): 66.8, Ala: 26.8, Trp: 6.4)	0	0.17

in the polypeptides, polypeptides No. 5 and No. 6 containing Pro 6% and Phe 6%, respectively, show a helix content of 52% ($b_0=-330/-630$) and 67% ($b_0=-420/-630$), respectively. Polypeptide No. 7, however, shows a disorder conformation at a Trp content of 6.4%.

Poly(Phe, Ala) with alanine content of 7 and 12% have taken 100% helix conformations based on Raman spectra in the solid state.[20]

This suggests that the Trp residue in the copolypeptide was influenced more than the bulky pendant groups, such as phenyl-, phenyl carbamoyl- or proline-residue, on the conformation of polypeptides. This is not only the influence of the bulk of the pendant group but also a characteritic of the indol group.

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