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Synthesis and Properties of High-Molecular-Weight Polypeptides Containing Tryptophan III.
Synthesis and Properties of Copolypeptides Containing 1-N_{in}-Formyltryptophan with Alanine[1]

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N-Carboxy-N_{in}-formyltryptophan anhydride (4-(L-1-N_{in}-formylindol-3-ylmethyl)-2,5-oxazolidine dione, Trp(Form) NCA) was synthesized by formylation of the indole group of tryptophan, followed by *N*-carboxylation. The reactivity of copolymerization of Trp(Form) NCA with *N*-carboxy-L-alanine anhydride (4-methyl-1-2,5-oxazolidinedione, L-Ala NCA) was higher than that of Trp NCA. The helix contents of the formed copolypeptides, estimated by CD spectra, were remarkably decreased at a content of about 27% of Trp(Form). The content of Trp(Form) residue needed to destroy helix conformation in the copolypeptides was more than that of Trp residue of 8%.

Tryptophan (**1**) is widely found in native protein or several hormonal peptides such as snake venom neutrotoxin, ACTH, and glucagon, it is a important amino acid for living activities.[2,3]

A very small amount of tryptophan residue influences the emergence of their living activities or higher order conformation. Mutual interaction between various amino acid residues in the proteins contributes to their higher order conformation or toxicity. The factors of interaction between amino acides are so complicated that conformational studied 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 mechanism of copolymerization of L-4-indolylmethyl-2,5-oxazolidine dione (Trp NCA, **2**) on producing polypeptides containing tryptophan.[4,5] Also, the influence of the Trp residue on helix formation of the polypeptides is reported. Trp residue less than 8% in the copolymers influenced their helix formation. Trp has a side chain comprising an indole nucleus, which is characteristic of both a hydrophobic aromatic group and a hydrophilic indol nitrogen capable of hydrogen bonding as a donor. It suggests that both the indole character of the proton as a donor and the bulkiness influence the rate of polymerization of Trp NCA and the secondary structures of the polymer formation. The reactivity of L-Trp NCA was so small that the copolypeptides containing more Trp residues are difficult to synthesize.

In order to synthesis the copolypeptides containing larger quantity of Trp and also to elucidate in detail the influence of both the bulkiness and the indol proton on the polymerization and secondary structures of the polymers formed, N_{in}-formyltryptophan (Trp(Form) **3**), masked indol N_{in}-hydrogen, as a donor by formyl group of a hydrogen acceptor, was synthesized and induced to *N*-carboxy-N_{in}-formyltryptophan anhydride (Trp(Form) NCA, **4**) to produce copolypeptides consisting of Trp(Form) and alanine (Ala, **6**, R; CH_3) by copolymerization with *N*-carboxy L-alanine anhydride (Ala NCA **5′**, R; CH_3).

Experimental

Synthesis of Trp(Form) NCA. Trp(Form) (**2**)[6,7] was synthesized by bubbling of HCl into a formic acid (100 cm^3) solution of tryptophan (Trp, 5 g). (Scheme 1); Yield, 86%. Found: C, 62.28; H, 5.28; N, 11.81%. Calcd for $C_{12}H_{12}N_2O_3$: C, 62.07; H, 5.17; N, 12.07%.

Trp(Form) NCA was synthesized by the analogous method of Trp NCA (**2**) reported previously ; Yield, 85%, mp 144—148 °C (decomp). Found: C, 60.95; H, 3.55; N, 10.34%. Calcd for $C_{13}H_{10}N_2O_4$: C, 60.47; H, 3.88; N, 10.85%. (IR spectra of both are deposited as Document No. 70024 at the Office of the Editor of Bull. Chem. Soc. Jpn.).

Polymerization and Copolymerization of Trp(Form) NCA with L-Ala NCA. Trp(Form) NCA (1 g, 3.87 mmol) and L-Ala NCA (1.34 g, 11.61 mmol) were dissolved in 1,2-dichloroethane (62 cm^3). Butylamine (5.65 mg, 0.077 mmol) as an initiator was then added, and the mixture was allowed 30 °C for 7 d. After the reaction, the polymerization solution was concentrated in vacuo to the half volume, and poured into acetonitrile (100 cm^3). The precipitated polymer was separated on a sintered-glass filter by filtration, washed with a solvent of NCA, acetonitrile, and dried in vacuo at room temperature. The copolymer of Trp(Form) with alanine (poly(Trp(Form)-co-Ala), 1.32 g) was obtained. Poly(Trp-

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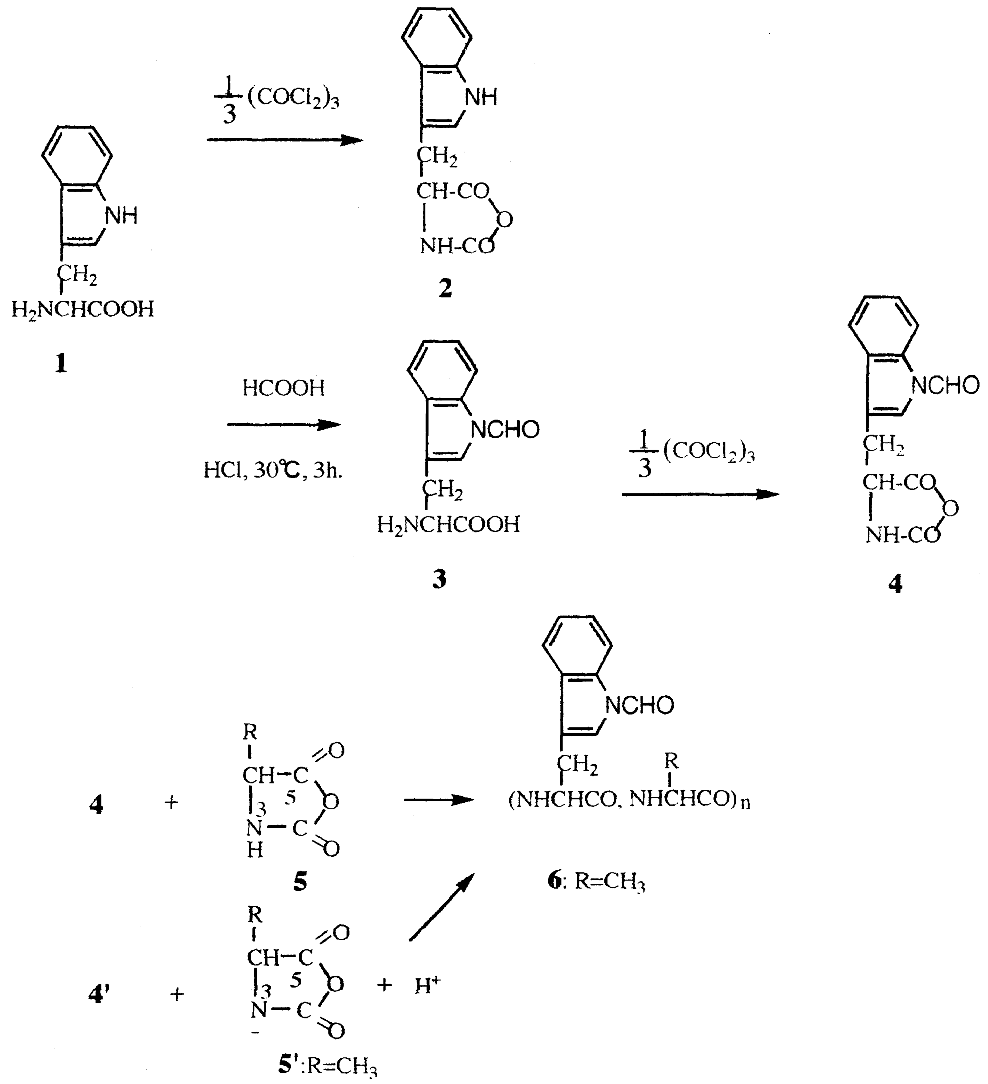


Scheme 1. Synthesis of Trp(Form) NCA and polypeptides containing Trp(Form).

(Form)) and other copolypeptides were prepared by an analogous method. The reactivity ratios were calculated by Lewis Mayo integral method.[8]

Viscosity Measurement. The viscosity of the copolypeptides was measured in a solution of dichloroacetic acid using an Ostwald viscometer at 30 °C. The intrinsic viscosity $[\eta]$ was defined as

$$[\eta] = (\ln \eta_{\mathrm{rel}})/c,$$

where η^{rel} is relative viscosity, c is polymer concentration of gram (0.25—0.5) in 100 cm^3.

Spectroscopic Measurement. Trp(Form) components in the copolymers were estimated from the UV absorbance working curve of poly(Trp(Form)) in a solution of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) at wavelength (λ) 248 nm at 22 °C using a 0.01 cm path length cell at a concentration of about 0.008 mol dm^{-3}. Infrared (IR) spectra were recorded on a Nippon Denshi JIR FX 6160 and JASCO 702 by KBr disks or Nujol® mull. The CD spectra were recorded on JASCO J-20A at weavelengths of 350 nm to about 190 nm at 22 °C using a 0.1 nm path length cell at a concentration of about 0.03 mol dm^{-3} (residue basis) in a solution of HFIP. The reduced mean residue ellipticity ($[\theta_\lambda]$), was defined as

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

where θ is the observed ellipticity in deg, L is the optical path length of the cell in cm, and C is the polymer concentration in mol dm^{-3} (residue basis).

^{1}H NMR Measurement. ^{1}H NMR spectra were recorded on Bruker ARX 400 in a solution of 1,1,1,3,3,3,-hexafluoro-2-propanol-d_2 at the same concentration as the CD measurements.

Results and Discussion

Reactivity of Trp(Form) NCA. Trp(Form) NCA was copolymerized with Ala NCA in 1,2-dichloroethane solution to produce poly(Trp(Form)) and poly(Trp(Form)-co-Ala). The polymerization conditions and properties of the copolypeptides are summarized in Table 1. The intrinsic viscosity of poly(Trp(Form)) obtained was higher that of Poly-(Trp) at almost the same condition.[4,5] The copolymerization of Trp(Form) NCA with Ala NCA proceeded to produce a alternating like poly(Trp(Form)-co-Ala). The reactivities of Trp(Form) NCA to Ala NCA were higher than those of Trp NCA to Ala NCA (Table 2). The mechanism of polymerization of NCA has been proposed as one of two routes: (1), attack on the C_5 carbon of NCA by amino residue of ami-

Table 1. Synthesis and Properties of Poly(Trp(Form)-co-Ala)

Ratio of monomer NCAs			Polymerization		Copolymer composition
No.	L-Trp(CHO)	L-Ala	Yield/%	η sp/c	L-Trp(CHO)/L-Ala
1	0	100			
2	5	95	89	1.52	4/96
3	15	85	86	0.77	13/87
4	25	75	88	0.65	16/84
5	40	60	78	1.22	27/73
6	100	0	93	0.71	100/0

Monomer, 1; Trp(Form) NCA, 2; Ala NCA. r1 = 0.2—0.3, r2 = 0.5—0.9.

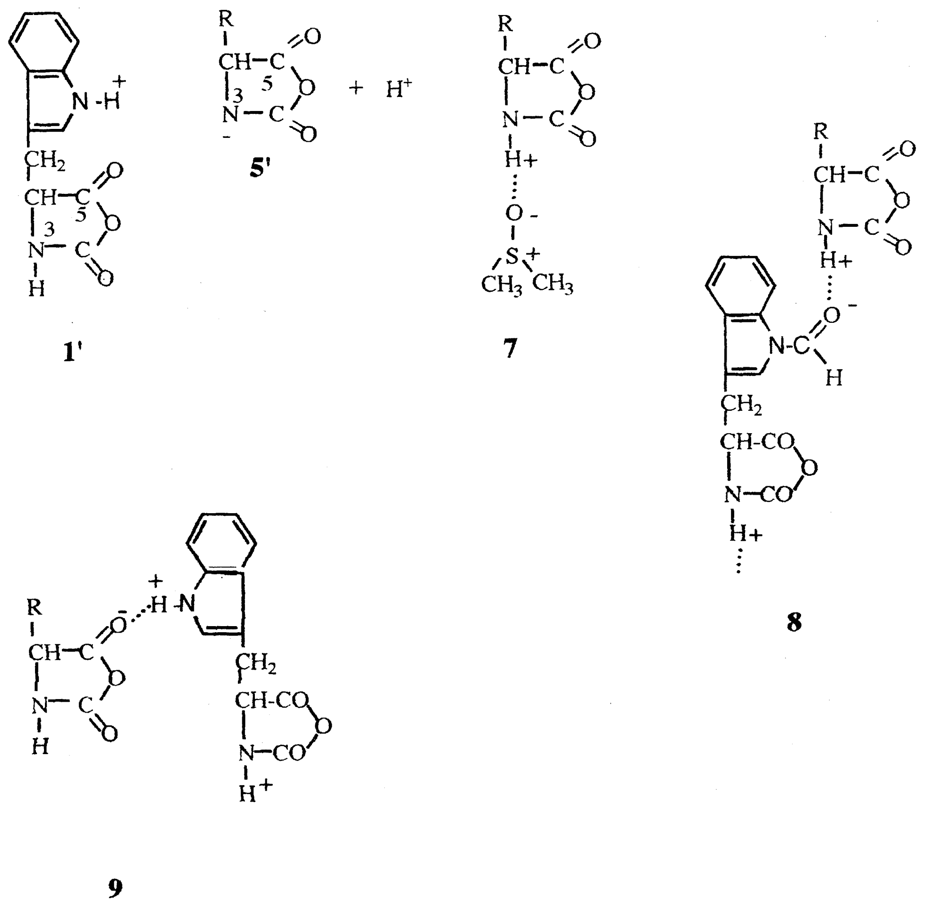
Table 2. Synthesis and Properties of Poly(L-Trp-co-L-Ala)

Ratio of monomer NCAs			Polymerization		Copolymer composition
	L-Tap	L-Ala	Yield/%	DP[a)]	L-Trp/L-Ala
1	10	90	80	160	1.9/98.1
2	21	79	79	158	3.4/96.6
3	40	60	63	126	6.8/93.2

a) DP; Degree of polymerization=(Monomer/Initiator) × (conversion- %/100). Polymerizations were initiated with butylamine (BA) at an NCA-to-BA ratio of 200 at 30 °C, 7 d, monomer concentration, 0.23—0.25 mol dm^{-3}. Monomer, 1; Trp NCA, 2; Ala NVCA. r1 = 0.5—0.6, r2 = 5.5—5.

no initiator or terminated amino residue, followed by loss of carbon dioxide, or (2), propagation via active NCA (**4′**; R=indol, and **5′**) produced by abstraction of 3N proton of NCA with strong bases.[9] The rate of second route (2:**4′**→**5′**) is faster and less steric hindrance of side chain on the NCA will occur than during that of (1:**4**→**5**).[10] These polymerizations proceed dependent on the initiator and solvents used.[11] It has been reported that the polymerizations of NCAs are accelerated in dimethyl sulfoxide proton acceptor solvent, because polymerization of NCA proceeds by the analogue mechanism of proton abstraction of hydrogen on 3N of NCA (**7**) (Scheme 2).[12] Polymerization of Trp(Form) NCA is suggested to accelerate by the complex formation between 3N proton and formylindole of the NCA (**8**). On the contrary, the polymerization of Trp NCA don't accelerate, since indole forms the complex **9** rather than **8**. The formed poly(Trp(Form)) and poly(Trp(Form)-co-Ala) have a chain length capable of forming secondary structures.

Solubility. The poly(Trp(Form)) and poly(Trp(Form)-co-Ala) were soluble in dichloroacetic acid and HFIP, respectively but insoluble in water. The former was highly swollen in pyridine and *N*,*N*-dimethylformamide. But the dichloroacetic acid solution became light blue and turned to dark violet within a few hours after making the solution. Since no precipitation was found in the solution, the viscosity of the solution could be observed. On the other hand, poly(L-Trp) was less soluble than poly(Trp(Form)) in HFIP. Poly(Trp)



Scheme 2. Interaction of Trp NCA and Trp(Form) NCA.

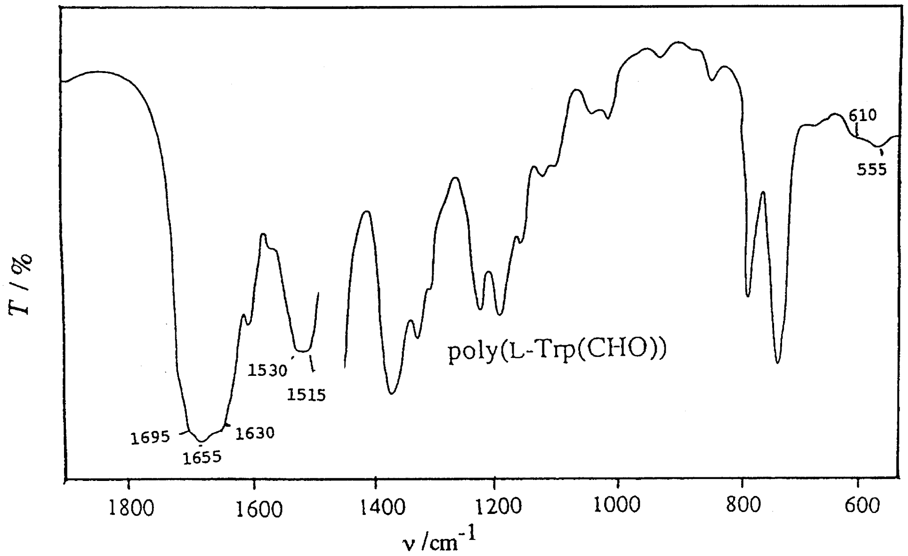


Fig. 1. IR spectra of poly(Trp(Form)).

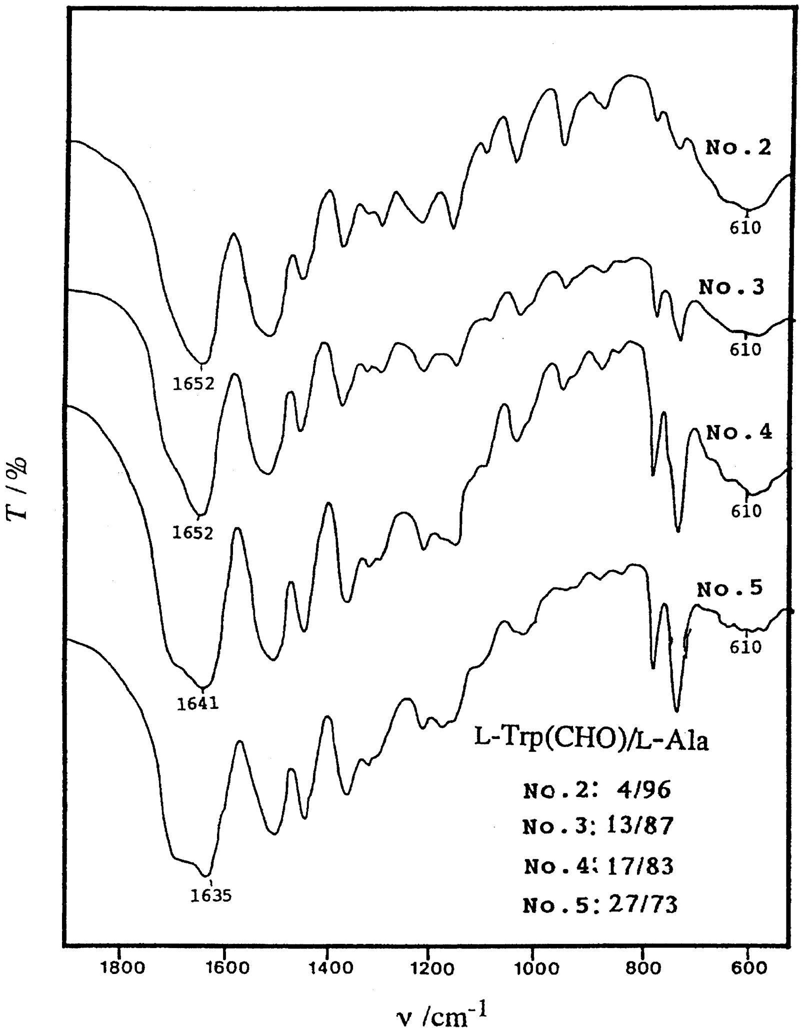


Fig. 2. IR spectra of poly(Trp(Form)-co-Ala).

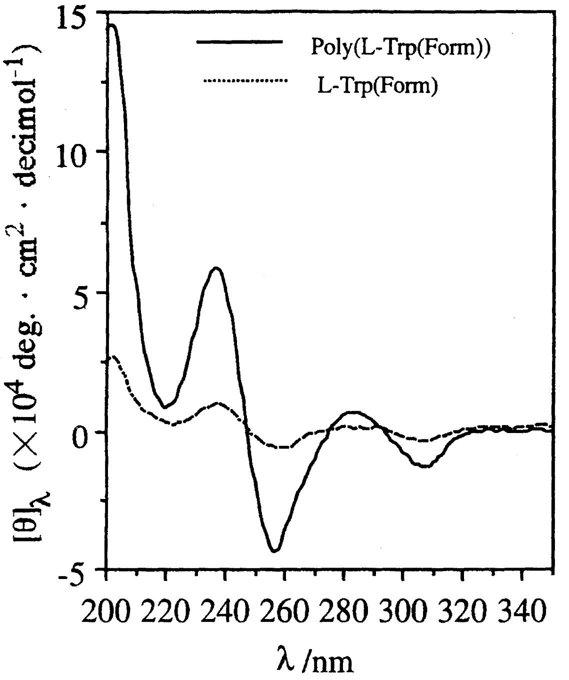


Fig. 3. CD spectra of Trp(Form) and poly(Trp(Fprm)) in HFIP.

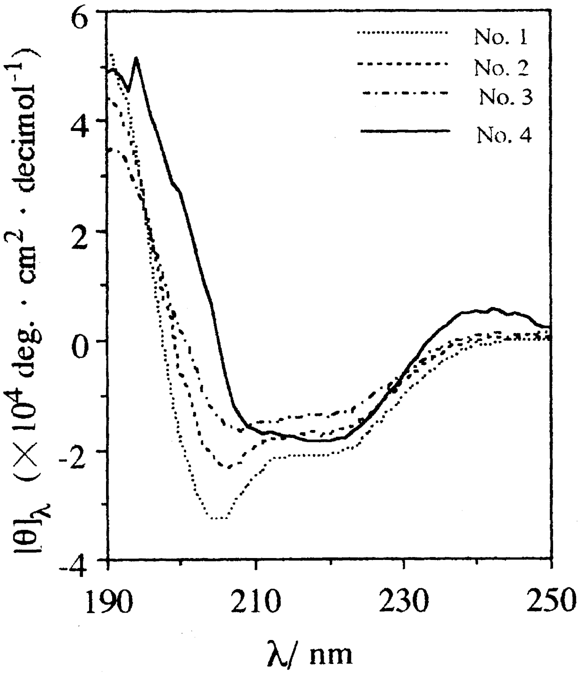


Fig. 4. CD spectra of poly(Trp(Form)-co-Ala) and poly-(Ala)[a] in HFIP.

a) Curve of poly(Ala) was obtained by extrapolation value of various components of poly(L-co-D-Ala), because poly(L-Ala) was insoluble.

and almost all copolypeptides containing Trp are soluble in dichloroacetic acid. However, some precipitations appeared in the solution after a while.

Conformation. The IR spectrum of poly(Trp(Form)) had broad and strong peaks at wavenumber (ν) 1630—1695 cm^{-1}, broad medium peaks at 1515—1530 cm^{-1}, and weak peak at 555 and 610 cm^{-1} (Fig. 1). It has been reported that the IR spectrum of Trp had a medium peak at 1672 and 1592 cm^{-1} which are corresponding to the side chain.[13] The IR spectrum of Trp(Form) had strong absorptions of N_{in}-formylindole at 1710 cm^{-1} and medium one at 1592 cm^{-1} (Deposited Document No. 70024 Fig. 1). The absorption at 1695 cm^{-1} for poly(Trp(Form)) may correspond to that at 1710 cm^{-1} of Trp(Form), some of the absorption of which may overlap with the amide I band of poly(Trp(Form)). The absorption about 1515 cm^{-1} (Amide II) corresponding to β-conformation and at 610 cm^{-1} (Amide V) of α-conformational peptides were observed.[14] The absorption at 555 cm^{-1} has been found in the β-conformation of silk fibroin (β-silk) and poly(Ala:50-co-Gyl:50).[14] In the copolypeptides, shown in Table 1, containing less than 30% Trp(Form), however, the amide I band would give useful structural information together with the amide II band which does not overlap the absorptions of Trp(Form). The amide I bands were shifted from 1651 cm^{-1} (α-) to 1635 cm^{-1} (β-) with increasing Trp(Form) in the polymer (Fig. 2). These results suggest that No. 1 is approximately in the α-, No. 2 and No. 3 in the mixed conformation of α- and β- or coiled, and No. 4 in the

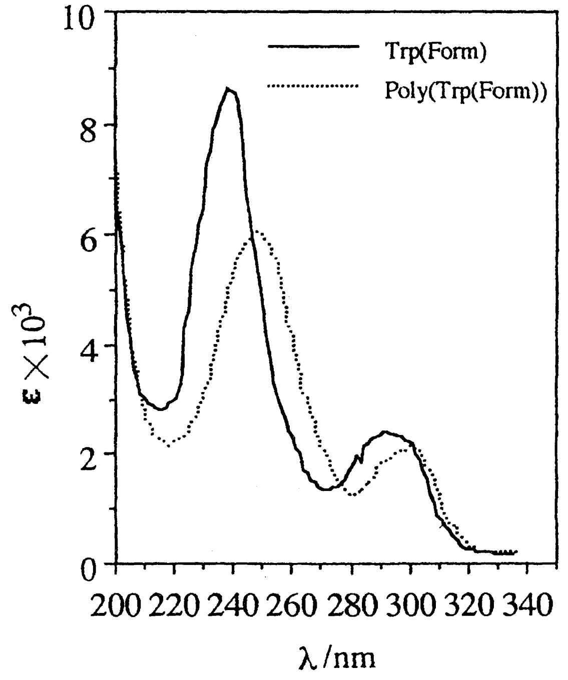


Fig. 5. UV spectra of Trp(Form) and poly(Trp(Form)). Concentration c; 0.00091 for Trp(Form), and 0.000979 for poly(Trp(Form)) mol dm^{-3} in HFIP at 22 °C.

Table 3. Fraction Helix in Poly(Trp(Form)-co-Ala)

No.	Copolymer composition	Helix% NMR	Helix% CD
1	4/96	93—79	85—91
2	13/87	55—52	61—63
3	17/83	50—43	44—45
4	27/73	40—38	20—32

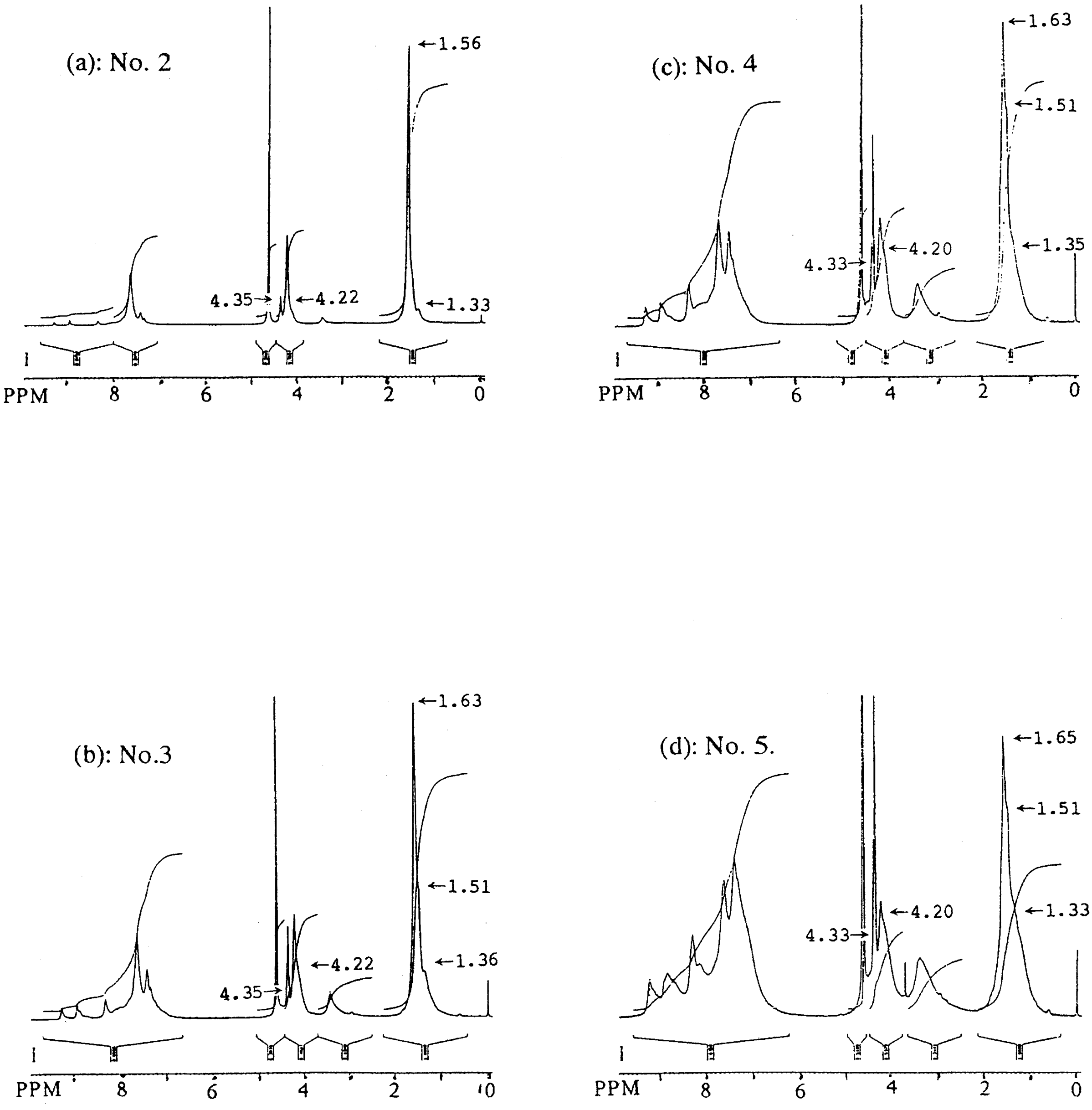


Fig. 6. NMR spectra of poly(Trp(Form)-co-Ala).

mixture of β- and coiled conformations.

The CD spectra of poly(Trp(Form)) and poly(Trp(Form)-co-Ala) listed in Table 1 are shown in Figs. 3 and 4 respectively. The conformations of those polypeptides were evaluated based on the strength of the peaks at about 190, 208, 216, and 222 nm, in HFIP solutions. These ellipticities have been shown to be a sensitive indicator of α-helix, β-sheet, and randomly disordered polypeptides.

But the CD spectra, like the IR one, characteristic of peptide backbones influence the absorptions of chromophore side-chains on poly(Trp), poly(Tyr), and poly(Phe).[15,16] The CD spectra of a low-molecular-weight poly(Trp) and a block copolypeptide ($(\text{Trp})_{30}$-$(\text{DL-Glu(OEt)})_{100}$) have been reported in a film and a solution, respectively.[17,18] The spectrum of poly(Trp) have given the maximum dichroism peaks at 190 nm ($[\theta]$=47200) and 225 nm ($[\theta]$=113000) and the minimum peak at 208 nm ($[\theta]$=82000) in the solution of 2,2,2-trifluoroethanol.[16—18] Poly(Trp(Form)) has UV strong absorptions at 247 nm (ε=6000) and 303 nm (ε=2150) (Fig. 5). These absorptions are presumed to influence the CD absorption of poly(Trp(Form)). The CD spectra of poly(Trp(Form)) gave a maximum strong peak at 201 nm ($[\theta]$=145000) and 237 nm ($[\theta]$=58600) and the minimum peak at 220 ($[\theta]$=8500) and 257 nm ($[\theta]$=−44100) (Fig. 4). Since the UV absorption of poly(Trp(Form)) at 216 nm (ε=2200) and 222 nm (ε=2300), respectively, were not so strong, the CD spectra of poly(Trp(Form)) seem to have minimum peaks at 216 nm of β-conformation and 222 nm of α-one. The results of CD spectra agreed with those of IR. In the UV spectra of poly(Trp(Form)-co-Ala), the maximum absorption (248 nm) did not shift with the components of Trp(Form). In the copolypeptides containing less than 30% Trp(Form), the CD spectra, as well as IR spectra, would give useful structural information. It has been reported that $[\theta]_{207}$ of low-molecular-weight poly(Ala) with helix conformation is 36000 in the mixed solution of 2,2,2-trifluroethanol (TFE) and water.[19] High-molecular-weight poly(Ala) was insoluble in the mixed solvent of TFE with water, TFE, and HFIP. Poly(L-Ala-co-D-Ala) were soluble in HFIP. Poly(Ala) with high-molecular-weight was presumed to have 36000 of $[\theta]_{207}$ from that of poly(L-Ala-co-D-Ala) (CD spectra are deposited as Documents No. 70024 at the Office of the Editor of Bull. Chem. Soc. Jpn.). The spectra of No. 2—4 and poly(Ala) had two negative peaks at 205 and 222 nm, and poly(Ala) and No. 5 had one negative peak at 216 nm and a maximum peak at 240 nm ($[\theta]=4800$) which was not observed in helical poly(Ala). The dichroism strength of poly(Trp(Form) : 27-co-Ala : 73) at 240 nm was only one twelfth of that of poly(Trp(Form)). The copolypeptides No. 2, 3, and 4 show the spectra like that of an α-helix and No. 5 like that of a β-sheet. These results agreed with those of IR spectra. The helix contains of the copolypeptides of Ala with Trp, estimated by CD spectra, have been remarkably decreased at a content of about 6% Trp in HFIP solution.[5] Poly(Ala : 16-co-Trp(Form) : 84) at a content of 16% Trp(Form) gave the CD spectrum α-helix conformation. The Trp(Form) residue in the copolypeptides was influenced less than the Trp residue of the helix conformation of polypeptides.

NMR spectra of the side-chain methyl proton on poly-L-alanine in trifluoroacetic acid have had three peaks resulting from the conformation: helix, random coil, and β-sheet.[20] In the helix structure of poly(Ala), the methyl group is trans to the carbonyl bond, and the methylene proton is cis to the carbonyl bond. Each resonance of methyl in poly(Ala) and methylene proton in peptide bonds, respectively, have been observed at higher fields when the groups are cis to the carbonyl bonds than when those are trans.

Poly(Ala-co-Trp(Form)) gave the triplet peaks between 1.33 and 1.65 ppm for methyl in Ala, and the doublet between 4.20 and 4.35 ppm (Fig. 6, 2—4). A comparison of observed methyl proton and methylene suggested that their peaks were increasingly shifted to lower frequency in the order helix, coil, and β, for the methyl proton, and β and the helix overlapped with coil for the methylene one. The conformational composition of the copolypeptides were determined from the peak intensity of each proton in the NMR spectrum (Table 3).

The spectra of poly(Trp(Form)-co-Ala) show that there are difference in strength of three peaks in the side-chain methyl proton ranging from 1.33 to 1.65 ppm; each strength is proportional to the contents of Trp(Form) residues (Fig. 6 (a—d)). These results also agreed with those of IR and CD spectra. The peaks of indole-hydrogens did not shift by the contents of Trp(Form) residues. This may indicate that there are no interaction of indole residue with amide bonds in the polypeptides.

These results suggest that both a hydrophobic aromatic group and a hydrophilic indol nitrogen capable of hydrogen bonding as a donor of Trp residue were affected by the rate of copolymerization of NCA and also by helix formation of the copolypeptide. N_{in}-formyl group masking indole one could be removed by 0.1 M aqueous piperidine or hydrazine in DMF solution ($1\ \mathrm{M}=1\ \mathrm{mol\,dm^{-3}}$).[6] This would be a new route of synthesis of polypeptides containing more Trp residue than that obtained by copolymerization of Trp NCA.

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1) Abbreviations of amino acids and their delivatives in this paper are those recommended by IUPAC-IUB Committee on Biochemical Nomenclature.

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