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On the Stability of Polyproline-I and II Structures of Proline Oligopeptides

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Summary

Proline oligopeptides composed of 13, 6 and 4 Pro residues were synthesized by solid-phase procedure. The circular dichroism spectra showed that the proline oligopeptides form the polyproline-II helix in water and in trifluoroethnol. However, it was shown that the oligopeptide composed of 13 Pro residues remarkably presents conformational transition from the polyproline-II to the polyproline-I helix in methanol and 1-propanol. This result is the first spectrum evidence that proline oligopeptides can form the polyproline-I helix in pure aliphatic alcohol such as methanol and 1-propanol. It was also shown that the propensity forming polyproline-I helix is more favorable in 1-propanol than in methanol, and also that the longer the chain-length is, the greater the stability of the polyproline-I helix is. Such a chain-length dependency of the conformational stability is also supported by the theoretical calculation using molecular mechanics.

Introduction

Poly(proline) is known to occur in two different helical conformations, i.e., polyproline-I (PPI) and polyproline-II (PPII) helices [1]. The PPI helix is a right-handed helix with an axial translation of 1.90Å composed of 3.3 residues per turn, and its all peptide bonds are in cis configuration with backbone dihedral angles of (ϕ , ψ , ω)=(-75°, +160°, 0°) [2]. The PPII helix is a left-handed helix with an axial translation of 3.20Å composed of 3.0 residues per turn, and its all peptide bonds are in trans configuration with (ϕ , ψ , ω)=(-75°, +145°, 180°) [3]. PPI is favored in aliphatic alcohols, and PPII is favored in water, trifluoroethanol, benzyl alcohol and organic acids. These two helices are distinguished by the spectroscopic or hydrodynamic measurements [4-21]. Especially, the circular dichroism (CD) spectrum is one of the most useful information for distinguishing two helices. PPI is generally characterized by the circular dichroism (CD) spectrum with a medium intensity

negative band at 198-200nm, a strong positive band at 214-215nm, and a weak negative band at 231-232nm. PPII is also characterized by the CD spectrum with a strong negative band at 202-206nm and a weak positive band at 225-229nm. The transition between PPI and PPII was investigated in water, organic acids, aqueous salt solutions, and mixed solvents such as propanol-water, aliphatic alcohol-organic acid [4, 6, 9, 13, 16-19]. It was shown that the PPI-PPII transition is much slower than the α-helix-coil transition in usual poly(amino acid)s and native proteins because of the difference in the elementary transition steps. The former transition is caused by the cis-trans isomerization of peptides bonds, but the latter one is the formation and rupture of hydrogen bonds.

It is pointed out that the polyproline-helices are very interesting conformational states to investigate the conformational character of unfold and molten globule states of the native protein, and also the relation between PPII and unordered conformation was investigated for the peptides having the specific amino-acid residues such as Pro, Ala and Lys as the major component [22-26]. Moreover, we have also found the experimental evidence that the periodic polypeptides having the Pro residue in their repetitive amino-acid sequences present the characteristic CD spectrum related to PPII or non-PPII conformation depending on the repetitive amino-acid sequence and solvents [27-35]. In this work, for investigating the conformational character of the polyproline helices, we synthesized proline oligopeptides composed of 13, 6 and 4 Pro residues (abbreviated as P13, P6 and P4, respectively), and investigated their conformations by CD measurements and molecular mechanics calculations.

Experimental

The proline oligopeptides were synthesized by the standard Boc solid phase procedure using Boc-Pro-PAM-resin (0.78mM/g), 1,3-diisopropylcarbodiimide (3eq.) as a coupling reagent (Watanabe Chemical Industries, Ltd., Hiroshima, Japan), then the peptide was cleavaged from PAM-resin using TFMSA. Crude polypeptides were purified by HPLC and characterized by MALDI-TOF-MS. Aqueous solution of purified polypeptides were freeze-dried, and then available polypeptides were obtained. CD spectra were measured at 5℃ with a JASCO J-720 spectropolarimeter (Jasco Co., Tokyo, Japan) using 0.1 cm path length cell. Concentrations were 0.25mM for all polypeptides. Conformational energy calculations were carried out for proline oligopeptides Ac-$(Pro)_n$-$N(Me)_2$(n=13, 6, 4) with the energy functions of ECEPP [36]. During minimization using the Powell algorithm [37], all ϕ and ω of Pro were allowed to vary, and ϕ of Pro was fixed to -75°.

Results and Discussion

The CD spectrum of P13 in water at 5℃ exhibited a strong negative band at 205nm and a weak positive band at 229nm as shown in Figure 1. These spectrum-patterns correspond to that of PPII helix as summarized in Table 1. The CD spectra of P13 did not show significant temperature-dependence in water in the range of 5℃ to 45℃, indicating that PPII conformation of P13 is stable in water. The CD spectrum of P6 in water at 5℃ exhibited a strong negative band at 202nm and a weak positive band at

226nm, and those of P4 in water at 5℃ exhibited a strong negative band at 200nm and a weak positive band at 226nm. That is, the negative bands are shifted to shorter wavelengths with a decrease in number of residues, suggesting that decreasing number of residues tends to decrease the stability of standard PPII helix because of molecular thermal fluctuation. These results also correspond to the previous results for the proline oligopeptides as summarized in Table 1.

Table 1. Summary of the CD parameters for polyproline-II helix.

Polypeptides (Averaged Number of Residues)	Solvent	λ ([θ])	λ([θ])	Reference
Poly(Pro) (570)	H_2O (5℃)	206(-46000)	227(+2000)	7
Poly(Pro) (140)	H_2O	207(-)	228(-)	6
Poly(Pro) (70)	H_2O	206(-35000)	228(+1600)	10
Poly(Pro) (60-80)	H_2O	206(-57600)	229(+2980)	12
Poly(Pro) (60-70)	H_2O	205(-)	226(-)	11
Poly(Pro) (20)	H_2O (20℃)	206(-31400)	229(+530)	15
H-$(Pro)_{40}$-OH	H_2O	206(-41000)	228(+3000)	9
Ac-$(Pro)_{11}$-OH	H_2O	206(-39000)	228(+2000)	13
H-$(Pro)_8$-OH	H_2O	206(-24000)	227(+3000)	5
H-$(Pro)_7$-OH	H_2O	201(-)	225(-)	11
Ac-$(Pro)_7$-Gly-Tyr-NH_2	H_2O (20℃, pH 7.0)	206(-34000)	228(+3000)	14
Ac-$(Pro)_7$-Gly-Tyr-NH_2	H_2O (5℃)	206(-40000)	228(+3500)	25
H-Gly-$(Pro)_5$-OH	H_2O	203(-22330)	228(+1540)	10
H-$(Pro)_{13}$-OH	H_2O (5℃)	205(-28800)	229(+1000)	This work
Poly(Pro) (60-80)	TFE	204(-73900)	227(+4910)	12
Poly(Pro) (140)	TFE	206(-)	226(-)	4
Poly(Pro)	TFE	204(-)	228(-)	8
H-$(Pro)_{13}$-OH	TFE (5℃)	203(-35500)	228(+1400)	This work

Table 2. Summary of the CD parameters for polyproline-I helix.

Polypeptides (Averaged Number of Residues)	Solvent	λ([θ])	λ([θ])	Reference
Poly(Pro) (60-80)	1-Propanol/H_2O (9:1)	200(-39800)	215(+47600)	12
H-$(Pro)_{40}$-OH	1-Propanol/H_2O (9:1)	198(-24000)	215(+61000)	9
Ac-$(Pro)_{11}$-OH	1-Propanol/H_2O (99.5:0.5)	198(-12000)	214(+20000)	13
H-$(Pro)_{13}$-OH	1-Propanol (5℃)	198(-8700)	214(+13500)	This work
H-$(Pro)_{13}$-OH	Methanol (5℃)	199(-16300)	214(+11100)	This work

In trifluoroethanol (TFE) at 5℃, the CD spectrum of P13 exhibited a strong negative band at 203nm and a weak positive band at 228nm, indicating that P13 forms the PPII conformation in TFE. The CD spectrum of P6 in TFE at 5℃ exhibited a strong negative band at 200nm and a weak positive band at 225nm, and those of P4 in TFE at 5℃ exhibited a strong negative band at 198nm and a weak positive band at 225nm. The negative bands are shifted to shorter wavelengths with a decrease number of residues in a similar manner as in water. The negative band in TFE has shorter

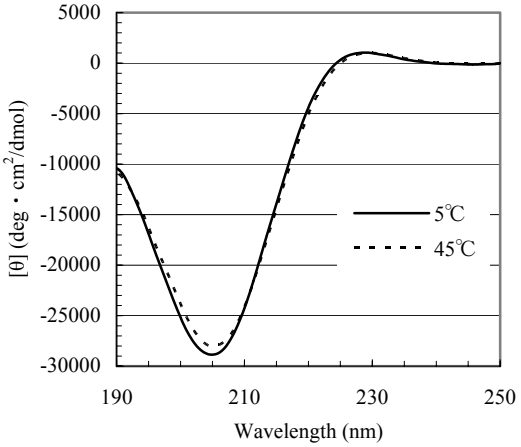

Figure 1. Circular dichroism spectra of P13 in water.

wavelength than that in water for each Pro-peptide. The temperature-dependence of the negative-band strength in TFE is slightly more significant than that of in water.

The CD spectra of P13 in methanol at 5℃ are shown in Figure 2, which exhibits remarkable dependence of CD spectra on the time after dissolving the peptide into methanol. Two minutes after, the CD spectrum exhibited a strong negative band at 204nm and a weak positive band at 228nm, which corresponds to the standard PPII spectrum. Then, 21 days after, it converted to the different spectrum exhibiting a strong negative band at 199nm, a strong positive band at 214nm and a weak negative band at 232nm, which correspond to the standard bands as shown in Table 2. These spectra indicate that P13 presents the conformational transition from PPII to PPI in methanol. It is well known that poly(proline) is insoluble in methanol. Then, it is also reported that P8 (Pro octapeptide) forms PPII helix in methanol by ORD measurement [5]. Figure 2 is the first spectrum evidence that proline oligopeptide can form PPI helix in methanol. The CD spectra of P6 in methanol at 5℃ exhibited a strong negative band at 201nm and a weak positive band at 224nm, and those of P4 in methanol at 5℃ exhibited a strong negative band at 200nm and a weak positive band at 224nm. They did not show any time dependence, indicating that PPII helix is stable for P6 and P4 in methanol. That is, an adequate number of residues is required to stabilize PPI helix for proline oligopeptides in methanol.

The CD spectra of P13 in 1-propanol at 5℃ are shown in Figure 3, which also exhibits remarkable dependence of CD spectra on the time after dissolving the peptide into 1-propanol. Two minutes after, the CD spectrum exhibited a strong negative band at 204nm and a weak positive band at 228nm, which corresponds to the standard PPII spectrum. Then, 14 days after, it converted to the different spectrum exhibiting a strong negative band at 199nm, a strong positive band at 214nm and a weak negative band at 232nm. The molar ellipticity at 214nm ($[\theta]_{214}$) was also plotted against the time in Figure 4. These results indicate that P13 also presents the conformational transition from PPII to PPI in 1-propanol as well as in methanol. The ratio of strength of the positive band at 214nm to that of the negative band at 199nm in 1-propanol is larger than that in methanol, which indicates that the propensity forming PPI helix is

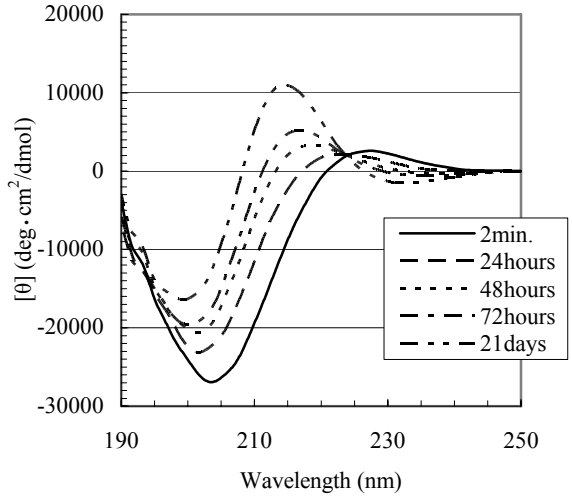

Figure 2. Circular dichroism spectra of P13 in Methanol at 5℃.

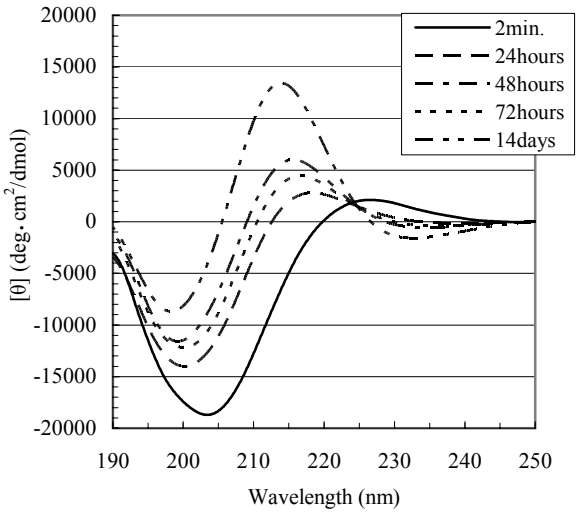

Figure 3. Circular dichroism spectra of P13 in 1-propanol at 5℃.

more favorable in 1-propanol than in methanol. The CD spectra of P6 in 1-propanol at 5℃ also presented the conformational transition from PPII to PPI in 1-propanol, and the ratio of strength of the positive band at 213nm to that of the negative band at 197 is smaller than that of P13. These results indicate that stability of PPI helix in 1-propanol decreases with a decrease in number of Pro residue, and also that 1-propanol is more PPI-favorable solvent than methanol. The CD spectra of P4 in 1-propanol at 5℃ exhibited a strong negative band at 200nm and a weak positive band at 225nm, and they did not show any time dependence, indicating that that PPI helix is not stable for P4 in 1-propanol.

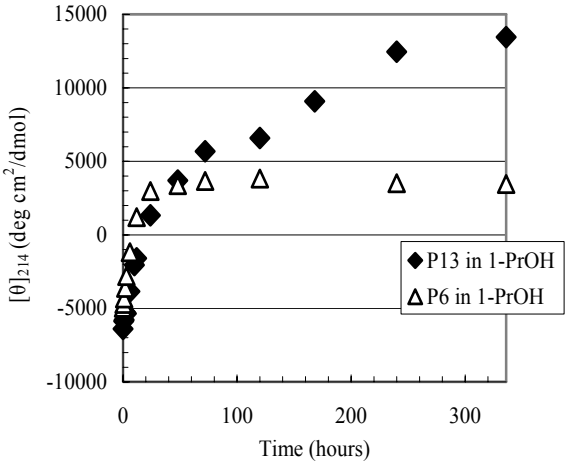

Figure 4. Time dependence of $[\theta]_{214}$ of P13 and P6 in 1-propanol.

Calculated results showed that PPI and PPII helices are found as stable conformations for 13-mer, 6-mer and 4-mer. The energy-difference between PPI and PPII helices per residues (abbreviated as $\Delta E_{PPI\text{-}PPII}$) is –0.20, +0.48 and +1.04 kcal/mol for 13-mer, 6-mer and 4-mer, respectively, indicating that PPI is more stable than PPII for 13-mer, but that PPI is less stable than PPII for 6-mer and 4-mer with intra-molecular interaction. These results remarkably reflect the difference of interaction mode between two helices, that is, inter-residue interaction is more favorable in PPI than in PPII. Calculated results correspond to the experimental results in methanol.

Conclusions

CD spectra showed that proline oligopeptides form the PPII helix in water and in TFE, and its stability decreases with a decrease in number of Pro residues. However, it was shown that P13 exhibited remarkable conformational transition from PPII to PPI in pure aliphatic alcohol such as methanol and 1-propanol. The propensity forming PPI was more favorable in 1-propanol than in methanol, and also increased with an increase in number of Pro residues, which corresponded to the theoretical results with molecular mechanics calculations.

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