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Syntheses and Circular Dichroism Studies of Oligopeptides Having Tri- or Tetra-L-prolyl Sequences

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Synopsis. CD spectra of many oligopeptides having tri- or tetra-L-prolyl sequences showed that a penta- or hexapeptide having a tetra-L-prolyl sequence took poly(L-proline) II helix irrespective of amino acid residues at N- or C-terminal of a L-prolyl cluster and that as to a size of the cluster, tri-L-prolyl sequence was enough to form the helix, when glycine was used as substituent.

It has been reported that oligo(L-proline)s begin to form poly(L-proline) II (PPII) helix at the tetramer, and that the pentamer or the greater has higher population of the helix.[1-4] However, there has been no report in detail on the PPII helix of oligopeptides containing a variety of amino acids other than L-proline. This paper describes syntheses and CD measurements of many oligopeptides having tri- or tetra-L-prolyl sequences (**1b**—**19b**, **12c**, and **14c**; see Table 1) to study effects of amino acid residues bound to the L-prolyl cluster on the formation of PPII helix.[5]

First, we synthesized sequential peptides **1b**—**6b**. Compound **1b** showed a negative CD band at 201 nm. Both **3b** and **5b** showed larger CD bands at longer wavelength of 203 nm (Table 1). Marked difference was not observed between **3b** and **5b**. Okabayashi *et al.* reported that Aoc-oligo(L-proline)s having some populations of PPII helix showed a CD minimum at longer wavelength than 203 nm.[2] So, the CD spectra suggests that both **3b** and **5b** have ordered conformation such as PPII helix and that **1b** has unordered one. All of Boc-[(Pro)$_4$-Gly]$_m$-OH showed large negative CD bands at 203.5 nm suggesting that all these peptides took also PPII helix. Therefore, prolyl peptides with more than five amino acid residues and at least one tri-L-prolyl sequence seem to take PPII helix. CD spectra of **7b** and **8b** support this consideration; **8b** showed a negative CD band at 203 nm similar to those of **3b** and **5b**, whereas **7b** showed a weak CD band at shorter wavelength of 200.5 nm.

TABLE 1. CD SPECTRAL DATA OF SYNTHETIC PEPTIDES[a)]

Peptides	$[\theta]^{\text{min}}_{\sim 200\,\text{nm}}$	$[\theta]^{\text{max}}_{\sim 225\,\text{nm}}$
	10^3 degree cm^2 dmol^{-1}	
Boc-(Pro)$_3$-Gly-OH (**1b**)	−23.3 (201.0)	0.9 (223.0)
Boc-(Pro)$_4$-Gly-OH (**2b**)	−26.2 (203.5)	1.3 (226.5)
Boc-[(Pro)$_3$-Gly]$_2$-OH (**3b**)	−28.9 (203.0)	0.9 (227.0)
Boc-[(Pro)$_4$-Gly]$_2$-OH (**4b**)	−31.8 (203.5)	0.9 (227.5)
Boc-[(Pro)$_3$-Gly]$_3$-OH (**5b**)	−31.1 (203.0)	0.6 (227.0)
Boc-[(Pro)$_4$-Gly]$_3$-OH (**6b**)	−32.8 (203.5)	0.7 (229.0)
Boc-Gly-(Pro)$_2$-Gly-OH (**7b**)	−17.0 (200.5)	not observed
Boc-Gly-(Pry)$_3$-Gly-OH (**8b**)	−23.1 (203.0)	0.6 (228.0)
Boc-Gly-(Pro)$_4$-OH (**9b**)	−27.6 (203.5)	2.0 (229.0)
Boc-Ala-(Pro)$_4$-OH (**10b**)	−25.9 (203.5)	1.6 (225.0)
Boc-Val-(Pro)$_4$-OH (**11b**)	−25.8 (204.0)	0.8 (229.0)
Boc-Ser-(Pro)$_4$-OH (**12b**)	−23.7 (204.0)	0.8 (230.0)
Boc-Thr-(Pro)$_4$-OH (**13b**)	−21.4 (206.5)	0.4 (230.0)
Boc-Tyr-(Pro)$_4$-OH (**14b**)	−20.8 (206.5)	4.3 (228.5)
Boc-Hyp-(Pro)$_4$-OH (**15b**)	−28.8 (203.5)	2.6 (226.0)
Boc-Ala-Ser-(Pro)$_4$-OH (**16b**)	−25.0 (203.0)	0.4 (235.0)
Boc-(Pro)$_4$-Ser-OH (**17b**)	−25.2 (203.0)	1.1 (226.0)
Boc-(Pro)$_4$-Val-OH (**18b**)	−29.8 (203.5)	0.4 (227.0)
Boc-Ser-(Pro)$_4$-Gly-OH (**19b**)	−25.7 (203.0)	1.1 (227.5)
Boc-Ser-(Pro)$_4$-NH$_2$ (**12c**)	−25.9 (204.0)	1.0 (227.0)
Boc-Tyr-(Pro)$_4$-NH$_2$ (**14c**)	−22.7 (206.0)	4.2 (228.0)

a) Figures in parentheses are wavelength at $[\theta]^{\text{extrema}}$

To study the relation between amino acid sequences and CD spectra, many peptides having a variety of amino acids at either or both of N- and C-termini of tetra-L-prolyl sequence (**9b**—**19b**, **12c**, and **14c**) were synthesized. N-terminal substitution caused various patterns of CD spectra (**9b**—**15b**), however, all these peptides showed CD bands at longer wavelength than 203 nm suggesting that they took PPII helix. A tendency was observed; the longer wavelength a CD band shifted the weaker its intensity decreased. Close similarity of CD spectra between Boc-Gly-(Pro)$_4$-OH (**9b**) and Boc-(Pro)$_5$-OH (which showed a CD minimum of -31.0×10^3 degree cm^{2}dmol^{-1} at 203.5 nm)[6] suggested that Gly residue was suitable for PPII helix. As to a C-terminal substitution, clear-cut tendency was not observed in the range of this experiment (**2b**, **17b**, and **18b**). Compounds **12b**, **16b**, and **19b**, which had the same Ser-(Pro)$_4$-sequence, showed similar CD spectra to each other suggesting that chain elongation from pentapeptide (**12b**) to hexapeptides (**16b** and **19b**) did not affect significantly to PPII helix. Amide derivatives (**12c** and **14c**) showed a little larger CD bands than the corresponding peptides with free carboxyl groups (**12b** and **14b**). It seems that charged group near L-prolyl cluster is not suitable for the helix.

From CD spectra of various prolyl peptides mentioned above, we concluded that a penta- or hexapeptide having a tetra-L-prolyl sequence took PPII helix irrespective of the amino acid residues at N- or C-terminal of a L-prolyl cluster, and that as to a size of the cluster tri-L-prolyl sequence was enough to form PPII helix, when glycine is used as substituent.

Experimental

Melting points were uncorrected. Optical rotations were measured on an Union automatic polarimeter PM-201. Yields and analytical data of synthetic peptides except for some oily products are summarized in Table 2; compounds **1a**—**19a** with benzyl ester group at C-termini are precursors of **1b**—**19b**.

Boc-(Pro)$_3$-Gly-OBzl (1a). To a chilled solution of Boc-Pro-OH (4.30 g, 20 mmol), H-(Pro)$_2$-Gly-OBzl·HCl (7.92 g, 20 mmol), and TEA (2.8 ml, 20 mmol) in CHCl$_3$ (80 ml) was added EDC·HCl (3.83 g, 20 mmol), and the reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature and evaporated. The residue was dissolved in EtOAc (120 ml), and the solution was washed successively with 10% citric acid, 4% NaHCO$_3$, and water, and then evaporated to a small volume after being dried over anhydrous Na$_2$SO$_4$. Addition of petroleum ether to the solution gave amorphous solid which was reprecipitated from EtOAc-petroleum ether; yield, 8.46 g (76%).

Compounds **2a**—**19a** were prepared as described for **1a**; in such cases that a product was soluble in water, column chromatographies with silica gel or Sephadex LH-20 were used in stead of acid and alkali washings.

Table 2. Yields and analytical data of synthetic peptides

Compound	Yield %	Mp θ_m °C	$[\alpha]_D^{22}$ [a] °	Found (%) C	Found (%) H	Found (%) N	Calcd (%) C	Calcd (%) H	Calcd (%) N
1a	76	72—73	−171.2	61.54	7.42	9.87	61.57	7.31	9.90[b]
2a	68	73—75	−215.4	62.23	6.95	10.71	62.46	7.25	10.70
3a	60	125—128	−243.6	59.52	7.33	11.70	59.28	7.25	12.02[c]
4a	54	155—157	−284.6	59.27	7.50	12.31	59.25	7.28	12.34[d]
5a	34	162—165	−273.6	57.52	6.98	12.91	57.87	7.24	12.86[e]
6a	34	191—193	−314.8	59.63	7.12	13.31	59.95	7.16	13.44[f]
10a	69	212—213	−264.6	63.18	7.38	10.54	62,95	7.40	10.49
11a	73	198—200	−259.6	63.99	7.54	9.99	63.86	7.68	10.06
12a	75	192—193	−249.6	61.36	7.05	10.12	61.48	7.22	10.24
13a	71	93— 95	−233.4	60.19	7.27	9.71	60.40	7.46	9.78[f]
14a	68	120—123	−196.2	63.52	6.92	9.24	63.30	7.13	9.00[f]
15a	57	118—124	−250.6	61.00	7.06	9.53	61.06	7.34	9.62[f]
16a	66	102—104	−232.4	58.46	7.05	10.86	58.37	7.35	10.75[c]
17a	69	105—108	−213.8	59.46	6.98	9.96	59.14	7.37	9.85[c]
19a	53	105—108	−221.6	56.89	6.91	10.95	57.20	7.27	10.82[d]
1b	93	115—117	−184.4	55.91	7.40	12.13	56.10	7.38	11.90[g]
2b	75	175—177	−208.4	59.06	7.60	11.37	55.75	7.45	12.04[f]
3b	87	178—180	−254.0	55.65	7.19	13.31	55.64	7.30	13.31[c]
4b	90	203—205	−303.0	56.57	7.29	13.34	56.31	7.33	13.40[d]
5b	84	208—210	−278.0	55.74	7.16	13.85	55.66	7.26	13.91[h]
6b	88	223—225	−312.2	56.25	7.21	13.78	56.52	7.28	13.93[e]
7b	80	90— 93	−142.0	51.12	6.97	12.45	51.34	7.26	12.60[f]
8b	61	123—128	−206.4	52.54	6.92	12.72	52.35	7.32	12.72[c]
9b	67	155—157	−224.2	53.96	6.82	11.52	54.08	7.56	11.68[d]
10b	58	157—161	−242.2	53.89	7.60	11.21	54.01	7.77	11.25[h]
11b	85	143—146	−248.8	56.67	7.53	10.66	56.95	7.97	11.07[c]
12b	67	173—175	−235.6	53.42	7.35	11.09	53.41	7.52	11.12[d]
13b	72	165—168	−230.0	52.97	7.49	10.67	52.64	7.77	10.58[e]
14b	69	186—191	−186.0	56.67	7.10	9.73	56.42	7.38	9.68[e]
15b	74	174—181	−251.8	54.86	7.43	10.53	54.94	7.53	10.68[d]
16b	80	156—159	−232.6	52.56	6.88	11.87	52.46	7.53	11.84[h]
17b	72	164—166	−216.0	53.18	7.25	11.04	53.41	7.52	11.12[d]
18b	85	143—146	−223.6	56.09	7.86	10.77	56.15	8.02	10.91[d]
19b	56	172—175	−244.6	52.66	6.93	12.20	52.47	7.34	12.24[d]
12c	77	147—151	−240.0	53.70	7.44	13.43	53.49	7.69	13.37[d]
14c	70	163—166	−193.4	57.27	7.42	11.71	57.21	7.48	11.77[c]

a) *c* 1, MeOH. b) $0.5H_2O$. c) $1.5H_2O$. d) $2H_2O$. e) $3H_2O$. f) H_2O. g) $0.25H_2O$. h) $2.5H_2O$.

Boc-(Pro)$_3$-Gly-OH (1b). Compound **1a** (5.57 g, 10 mmol) dissolved in MeOH (20 ml) was hydrogenated in the presence of 5% paradium carbon (*ca.* 2 g) for 4 h at room temperature. The product was recrystallized from EtOAc-petroleum ether; yield, 4.33 g (93%).

Compounds **2b—19b** were prepared as describe for **1b**.

H-(Pro)$_3$-Gly-OBzl·HCl. Compound **1a** (2.78 g, 5 mmol) dissolved in 0.1 mol dm^{-3} hydrogen chloride in formic acid (60 ml) was allowed to stand for 30 min at room temperature and evaporated. Addition of ether to the residue gave highly hygroscopic crystals, which were used for the next reaction without further treatment.

Boc groups in other peptides (**2a—4a**, and **12a**) were removed as described above.

Boc-Ser-(Pro)$_4$-NH$_2$ (12c). Compound **12a** (137 mg, 0.2 mmol) dissolved in MeOH (3 ml) saturated with NH_3 was allowed to stand at room temperature for 3 d. After the evaporation of solvent, the residue was purified by silica gel ($CHCl_3$-MeOH; 10:1, v/v) and Sephadex LH-20 (MeOH) column chromatographies; yield, 91 mg (77%).

Compound **14c** was prepared as described for **12c**.

CD Measurements. CD spectra were measured on a JASCO J-40A spectropolarimeter at 23 °C in H_2O solution with quartz cells of 1-mm path length. The ellipticity was expressed as mean residual molar ellipticity $[\theta]$ in degree cm^2 dmol^{-1}.

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5) The abbreviations used in this paper are those recommended by IUPAC-IUB: *J. Biol. Chem.*, **247**, 977 (1972). Additional abbreviations: Boc, *t*-butoxycarbonyl; OBzl, benzyl ester; Aoc, *t*-pentyloxycarbonyl; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; TEA, triethylamine. Symbols of amino acids denote the L-configuration.

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