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CD Spectra and Cyclization of Linear Pentapeptides as Gramicidin S Precursors with a Benzyloxycarbonyl Group on the Side Chain of Orn Residue

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Synopsis. Five pentapeptides related to gramicidin S, H–Val–Orn(Z)–Leu–D-Phe–Pro–OH, H–Orn(Z)–Leu–D-Phe–Pro–Val–OH, H–Leu–D-Phe–Pro–Val–Orn(Z)–OH, H–D-Phe–Pro–Val–Orn(Z)–Leu–OH, and H–Pro–Val–Orn-(Z)–Leu–D-Phe–OH, were cyclized with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and 1-hydroxybenzotriazole. The pentapeptide with a Pro residue at the C-terminal formed predominantly the cyclic dimer (diZ-gramicidin S), while the pentapeptide with an Orn(Z) residue at the C-terminal produced exclusively the cyclic monomer (Z-semigramicidin S). The reaction mode of those pentapeptides was correlated with their CD spectra.

Gramicidin S (GS),[1] cyclo(–Val–Orn–Leu–D-Phe–Pro–$)_2$, is an antibiotic cyclodecapeptide consisting of two identical pentapeptide sequences.[2] In 1957, Schwyzer and Sieber reported that the cyclization of the H–Val–Orn(Tos)–Leu–D-Phe–Pro *p*-nitrophenyl ester produced cyclic decapeptide (the ditosyl derivative of GS) instead of cyclic pentapeptide.[3] Since then, various analogs of GS have been synthesized by this cyclic dimerization method.[4] To search for favorable precursors for the chemical synthesis of GS, Minematsu et al. cyclized the succinimide ester (–ONSu) and azide ($-N_3$) of five pentapeptides related to GS, and reported that the H–Pro–Val–Orn(Z)–Leu–D-Phe–N_3 formed most predominantly the cyclic dimer (diZ-GS), while preferential formation of cyclic monomer (Z-semiGS) was observed in H–D-Phe–Pro–Val–Orn(Z)–Leu–ONSu.[5] On the other hand, the direct formation of GS by the cyclization of free precursor peptide is also possible with an excess of coupling reagents. For example, diZ-GS[6] and its analogs[7] have been prepared from the direct cyclization of the corresponding H–decapeptide–OH using (5-nitropyridyl)diphenyl phosphinate, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCD·HCl)/1-hydroxybenzotriazole (HOBt), respectively. The cyclization of H–Val–Orn(Boc)–Leu–D-Phe–Pro–OH by the phosphite method also produced Boc–semiGS and diBoc–GS in a 62:38 ratio and the total yield was 87%.[8] However, the effect of the alteration of the pentapeptide sequences for the direct cyclization has not been established.

In this paper, we report the total yields and the ratio of diZ-GS and Z-semiGS in the products of the direct cyclization of five pentapeptides **1**—**5** (Table 1) related to GS using WSCD·HCl and HOBt as coupling reagents. We also discuss the relationship between the cyclization and circular dichroism (CD) spectra of pentapeptides **1**—**5**.

The H-pentapeptide-OH's were prepared by a similar method to those described in a previous paper of this series.[9] The yields, physical properties, and analytical data on the newly synthetic peptides are summarized in Table 2. These precursor pentapeptides **1**—**5** were dissolved in pyridine at a concentration of 3×10^{-3} M, and cyclized by the treatment with WSCD·HCl (10 molar amounts) and HOBt (10 molar amounts) for 1 d at 25 °C. The products were isolated by the removal of the solvent and precipitation with water. The total yields and ratios of Z-semiGS and diZ-GS in the products were measured by HPLC. The results of the cyclization of the five pentapeptides are summarized in Table 1. The greatest formation of diZ-GS was observed in the cyclization of peptide **1** with a Pro residue at the C-terminal, and its yield was 64%. The cyclization of peptides **2**, **4**, and **5** with a Val, Leu, or D-Phe residue at the C-terminal, respectively, gave Z-semiGS and diZ-GS in about a 6:4 ratio. On the other hand, the cyclization of peptide **3** with an Orn(Z) residue at the C-terminal produced exclusively Z-semiGS in 56% yield. These results indicate that the direct cyclization of the linear pentapeptides with Pro and Orn(Z) residues at the C-terminal is an effective method for the syntheses of GS and semiGS, respectively.

The favorable pentapeptide sequences for the syntheses of GS and semiGS in the direct cyclization of pentapeptides **1**—**5** were different from those in the cyclization of pentapeptides preactivated by –ONSu or $-N_3$.[5] In the cyclization of the pentapeptide–N_3 or active ester related to GS, the ratios of the cyclic monomer and the cyclic dimer in products were greatly influenced by the amino acid sequence and concentration of the activated precursors.[4] The inconsistency between these two cyclization methods might be caused by the difference of the concentration of the activated precursors existing in pyridine, since the pentapeptides used in these two cyclizations have the same amino acid sequences.

Next, direct cyclization was done in ethanol at 25 °C for 1 d (concentration of peptides in ethanol: 3×10^{-3} M, M=mol dm^{-3}), using triethylamine (10 molar amounts) as a base. A sequence dependence on the ratio of Z-semiGS and diZ-GS in cyclization products was similar to that in the case of the cyclization of peptides **1**—**5** in pyridine, although the total yields were lower than those in pyridine. That is, the modes of the cyclizations of peptides **1**—**5** in both solvents seem to

Table 1. Cyclization of Precursor Pentapeptides Related to GS

	Precursor pentapeptides	Ratio of cyclic products		Yield(%) of total cyclic peptides
		Z–semiGS	diZ–GS	
1.	H–Val–Orn(Z)–Leu–D-Phe–Pro–OH	10	90	72
2.	H–Orn(Z)–Leu–D-Phe–Pro–Val–OH	56	44	76
3.	H–Leu–D-Phe–Pro–Val–Orn(Z)–OH	97	3	58
4.	H–D-Phe–Pro–Val–Orn(Z)–Leu–OH	65	35	62
5.	H–Pro–Val–Orn(Z)–Leu–D-Phe–OH	58	42	35

Table 2. Yields and Analytical Data of Synthetic Peptides

	Compounds	Yield	Mp	$[\alpha]_D^{25}$ (°)			Elemental analysis (%)		
		%	°C	(*c* 1, DMF)			C	H	N
1	Boc–Orn(Z)–Leu–D-Phe–Pro–Val–OBzl[a)]	56	63—67	−50.0	$C_{50}H_{68}O_{10}N_6\cdot H_2O$	C: F:	64.50 64.79	7.58 7.59	9.03 8.99
2	Boc–Leu–D-Phe–Pro–Val–Orn(Z)–OBzl[a)]	42	65—68	−42.6	$C_{50}H_{68}O_{10}N_6\cdot H_2O$	C: F:	64.50 64.88	7.58 7.64	9.03 9.04
3	Boc–D-Phe–Pro–Val–Orn(Z)–Leu–OBzl[a)]	72	65—68	−51.0	$C_{50}H_{68}O_{10}N_6\cdot 1/2H_2O$	C: F:	65.13 64.95	7.54 7.57	9.11 9.10
4	Boc–Pro–Val–Orn(Z)–Leu–D-Phe–OBzl[a)]	49	189—192	−22.0	$C_{50}H_{68}O_{10}N_6\cdot H_2O$	C: F:	64.50 64.71	7.58 7.49	9.03 9.08
5	Boc–Orn(Z)–Leu–D-Phe–Pro–Val–OH[b)]	84	91—96	−50.2	$C_{43}H_{62}O_{10}N_6\cdot H_2O$	C: F:	61.41 61.47	7.67 7.59	9.99 9.94
6	Boc–Leu–D-Phe–Pro–Val–Orn(Z)–OH[b)]	52	90—93	−45.0	$C_{43}H_{62}O_{10}N_6\cdot H_2O$	C: F:	61.41 61.20	7.67 7.63	9.99 9.99
7	Boc–D-Phe–Pro–Val–Orn(Z)–Leu–OH[b)]	70	92—97	−48.5	$C_{43}H_{62}O_{10}N_6\cdot H_2O$	C: F:	61.41 61.61	7.67 7.66	9.99 9.99
8	Boc–Pro–Val–Orn(Z)–Leu–D-Phe–OH[b)]	78	188—191	−28.3	$C_{43}H_{62}O_{10}N_6\cdot H_2O$	C: F:	61.41 61.47	7.67 7.59	9.99 9.94
9	H–Val–Orn(Z)–Leu–D-Phe–Pro–OH·HCl[c)]	93	139—142	−23.2	$C_{38}H_{54}O_8N_6\cdot HCl\cdot H_2O$	C: F:	58.71 58.68	7.39 7.67	10.81 11.08
10	H–Orn(Z)–Leu–D-Phe–Pro–Val–OH·HCl[c)]	90	133—137	−48.0	$C_{38}H_{54}O_8N_6\cdot HCl\cdot 3H_2O$	C: F:	56.11 56.21	7.56 7.47	10.33 10.45
11	H–Leu–D-Phe–Pro–Val–Orn(Z)–OH·HCl[c)]	86	145—150	−26.3	$C_{38}H_{54}O_8N_6\cdot HCl\cdot 2H_2O$	C: F:	57.38 57.31	7.48 7.70	10.57 10.83
12	H–D-Phe–Pro–Val–Orn(Z)–Leu–OH·HCl[c)]	85	143—146	−89.0	$C_{38}H_{54}O_8N_6\cdot HCl\cdot 1.5H_2O$	C: F:	58.04 58.09	7.43 7.40	10.69 10.61
13	H–Pro–Val–Orn(Z)–Leu–D-Phe–OH·HCl[c)]	89	153—154	−19.8	$C_{38}H_{54}O_8N_6\cdot HCl\cdot 1.5H_2O$	C: F:	58.04 58.08	7.43 7.24	10.69 10.97
14	Boc–(Val–Orn(Z)–Leu–D-Phe–Pro–)$_2$–OH[d)]	86	188—190	−65.0 (*c* 0.5)	$C_{81}H_{114}N_{12}O_{17}\cdot HCl\cdot H_2O$	C: F:	62.93 62.90	7.56 7.55	10.87 11.27
15	H–(Val–Orn(Z)–Leu–D-Phe–Pro–)$_2$–OH·HCl[c)]	85	167—171	−73.0 (*c* 0.5)	$C_{76}H_{106}N_{12}O_{15}\cdot HCl\cdot 2H_2O$	C: F:	60.85 60.58	7.46 7.37	11.20 11.17

a) Compounds **1**—**4** were synthesized from each C-terminal amino acid benzyl ester by a stepwise elongation using WSCD·HCl and HOBt. (Hold-in-solution method[10)]) These yields were calculated on the basis of the amount of each C-terminal amino acid benzyl ester as a starting material. b) Compounds **5**—**8** were obtained by the saponification of the corresponding Boc-pentapeptide benzyl esters **1**—**4**. c) Compounds **9**—**13** were prepared from the corresponding Boc-pentapeptide-OH's **5**—**8** by the treatment with 4 M HCl/dioxane, and compound **15** from the corresponding Boc-decapeptide-OH **14**. d) This compound was synthesized by the coupling of the succinimide ester derived from Boc–Val–Orn(Z)–Leu–D-Phe–Pro–OH with H–Val–Orn(Z)–Leu–D-Phe–Pro–OH.

be similar.

To investigate the relationship between the cyclization process and the conformation of precursor pentapeptides, the CD spectra of the five pentapeptides **1**—**5** and Z-semiGS were measured in ethanol at room temperature (Fig. 1). The CD spectrum of Z-semiGS (Fig. 1-B) showed a trough at 208 nm and a shoulder at 220 nm. The spectra of five pentapeptides **1**—**5** can be classified into two types (Fig. 1-A and 1-B). Since all of peptides **1**—**5** are constituted with the same amino acid residues, the difference among these spectra must be due to the distinction of their backbone conformations. The spectra of peptides **2**—**5** showed one or two negative troughs in the region of 200—220 nm (Fig. 1-B), and its patterns resembled that of Z-semiGS. Peptides **2** and **3**, which contain a –D-Phe–Pro– sequence inside of the peptide chains, show a comparatively deep trough and the trough of peptide **3** was deepest. These results suggest that the conformations of the precursors **2**—**5** resemble more or less to that of Z-semiGS in ethanol. In these studies, the structures of peptides **2**—**5** may be favorable for the monomeric cyclization. On the other hand, the CD spectrum of peptide **1** showed a curve (Fig. 1-A) that slopes steadily without any trough at

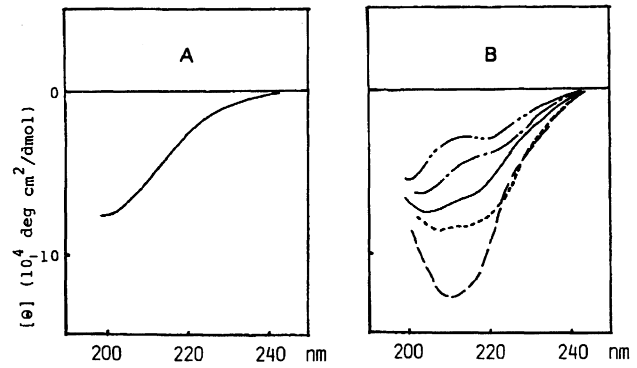

Fig. 1. CD spectra of five pentapeptide precursors and Z-semiGS in ethanol. (A) peptide **1**; ——. (B) peptide **2**; ——, peptide **3**; - - -, peptide **4**; — · —, peptide **5**; — ·· —, Z-semiGS; ·····.

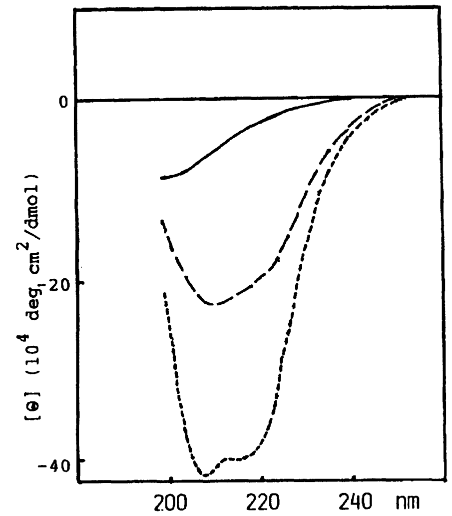

Fig. 2. CD spectra of H-Val-Orn(Z)-Leu-D-Phe-Pro-OH (——), H-(Val-Orn(Z)-Leu-D-Phe-Pro-)$_2$-OH (- - -), and diZ-GS (····) in ethanol.

200—220 nm, indicating that the conformation of peptide **1** is significantly different from that of Z-semiGS.

Further, the CD spectra of H-(Val-Orn(Z)-Leu-D-Phe-Pro-)$_2$-OH and diZ-GS were measured in ethanol (Fig. 2), indicating that the conformation of this linear decapeptide is similar to that of diZ-GS. The results appear to suggest that peptide **1**, which has a conformation different from that of Z-semiGS, slowly produces Z-semiGS by the intramolecular cyclization, while H-decapeptide-OH, formed by coupling of two molecules of peptide **1**, takes the diZ-GS-like conformation and cyclizes smoothly to afford diZ-GS.

Thus, a correlation between the cyclization products and CD spectra in ethanol of pentapeptides **1**—**5** was found. However, further detailed conformational analyses of precursor pentapeptides and cyclic products in pyridine should be needed for better understanding of the mode of cyclization.

Experimental

The yields, physical properties, and analytical data of the synthetic linear peptides are summarized in Table 2. All melting points are uncorrected. HPLC was done using an 800 series system (JASCO, Tokyo, Japan). A Finepak SIL C_{18} column (250 mm×4.6 i.d., 10 μm particle size, JASCO) was used: the flow-rate, 1 ml min^{-1}; the mobile phase, methanol–water (6 : 1, v/v); the wavelength of detection, 220 nm. The peak area was recorded using a Chromatopac C-R3A integrator (Shimadzu, Kyoto, Japan). The CD spectra were obtained using a J-500 spectropolarimeter (JASCO, Tokyo, Japan) in ethanol at room temperature.

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2) Amino acid residues with no prefix are of L-configuration unless otherwise noted. The abbreviations for amino acids and peptides are in accordance with rules of IUPAC-IBU Commission of Biological Nomenclature [*Eur. J. Biochem.*, **138**, 9 (1984).] Additional abbreviations used are as follows: Tos-, tosyl; Z-, benzyloxycarbonyl-.

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