Cyclization of Penta- and Hexapeptide Active Esters Related to Gramicidin S and Gratisin

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Synopsis. Four pentapeptide *N*-hydroxysuccinimide esters [X-Orn(Z)-Leu-Y-Pro-ONSu (X=Val or D-Val, and Y=Phe or D-Phe)] related to gramicidin S were allowed to cyclize in pyridine. The peptides with a L-Phe residue preceding Pro tend to form various cyclic polymers, while ones with a D-Phe residue exclusively yielded a cyclic monomer or cyclic dimer. Peptides with a D-Val residue at the N-terminal produced monomerization products. The observed tendencies were similar for cyclization of four hexapeptide *N*-hydroxysuccinimide esters [X-Val-Orn(Z)-Leu-Y-Pro-ONSu (X=Tyr(Bzl) or D-Tyr($BzlCl_2$), and Y= Phe or D-Phe)] related to gratisin.

An antibiotic peptide, gramicidin S (GS),[1] is a cyclic decapeptide consisting of two identical pentapeptide sequences of -Val-Orn-Leu-D-Phe-Pro- (Fig. 1).[2] In 1957, Schwyzer and Sieber reported that the cyclization of the H-Val-Orn(Tcs)-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] In an investigation of the influence of substitution of each amino acid residue in the H-$\overset{1}{\text{Val}}$-$\overset{2}{\text{Orn}}$(Z)-$\overset{3}{\text{Leu}}$-D-$\overset{4}{\text{Phe}}$-$\overset{5}{\text{Pro}}$ *p*-nitrophenyl ester to the cyclization, Izumiya et al. reported that the steric hindrance of the side chains of amino acid residues at the N-terminal and at position 4 in the active esters greatly affects the reaction.[4] However, the effects of an alteration of the configurations of the constituent amino acid have been little studied.

In present studies, four pentapeptide *N*-hydroxysuccinimide esters related to GS, X-Orn(Z)-Leu-Y-Pro-ONSu (X= Val or D-Val, and Y=Phe or D-Phe) (Fig. 2), were cyclized in order to examine the influence of the configurations of amino acid residues at the N-terminal and position 4. In addition, a similar investigation was carried out concerning four hexapeptide *N*-hydroxysuccinimide esters related to gratisin (GR) (Fig. 1),[5,6] X-Val-Orn(Z)-Leu-Y-Pro-ONSu [X=Tyr(Bzl) or D-Tyr($BzlCl_2$), and Y=Phe or D-Phe] (Fig. 2).

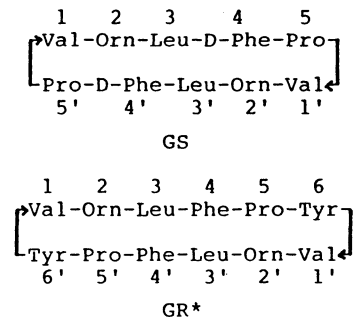

Fig. 1. Primary structures of GS and GR.
*The configuration of each amino acid residue has not been established yet.

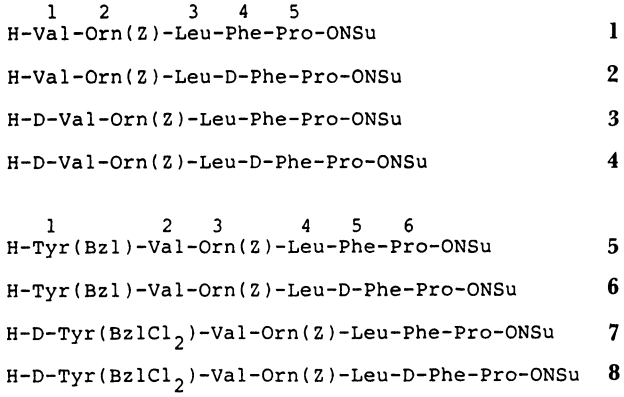

Fig. 2. Primary structures of penta- and hexapeptide active esters.

Boc-penta- and -hexapeptides were prepared by a conventional method.[6,7] Boc-penta- and -hexapeptides were converted into the penta- and hexapeptide-ONSu trifluoroacetates, which were cyclized in pyridine for 1 d at 25 °C (concentration of peptides in pyridine: 3×10^{-3} M). The ratio of the cyclic products was determined by high-performance liquid chromatography (HPLC) analysis. The molecular weight of each cyclic product isolated by semipreparative HPLC was determined by fast atom bombardment (FAB) or secondary ion mass spectrometry.

HPLC profiles of crude cyclic products are shown in Figs. 3 and 4. Cyclic peptides were eluted in order of the ring sizes.

Data of the cyclization are summarized in Table 1. Compounds **1** and **3**, both of which contain a L-Phe residue preceding Pro, gave cyclic products with widely distributed molecular weights. The former yielded the cyclic dimer, trimer, tetramer, and pentamer (roughly evenly), but not the cyclic monomer. The latter produced the cyclic monomer, dimer, trimer, and tetramer. Compound **2** yielded exclusively the cyclic dimer, diZ-GS, while Izumiya et al. reported that compound **2** gave a considerable amount of the cyclic monomer under similar conditions.[4,8] Compound **4** gave the cyclic monomer as a sole product. In the cases of the hexapeptide esters (**5**—**8**), each gave its cyclic monomer and dimer regardless of the configurations of the constituent amino acids. The ester con-

† 1 M=1 mol dm^{-3}.

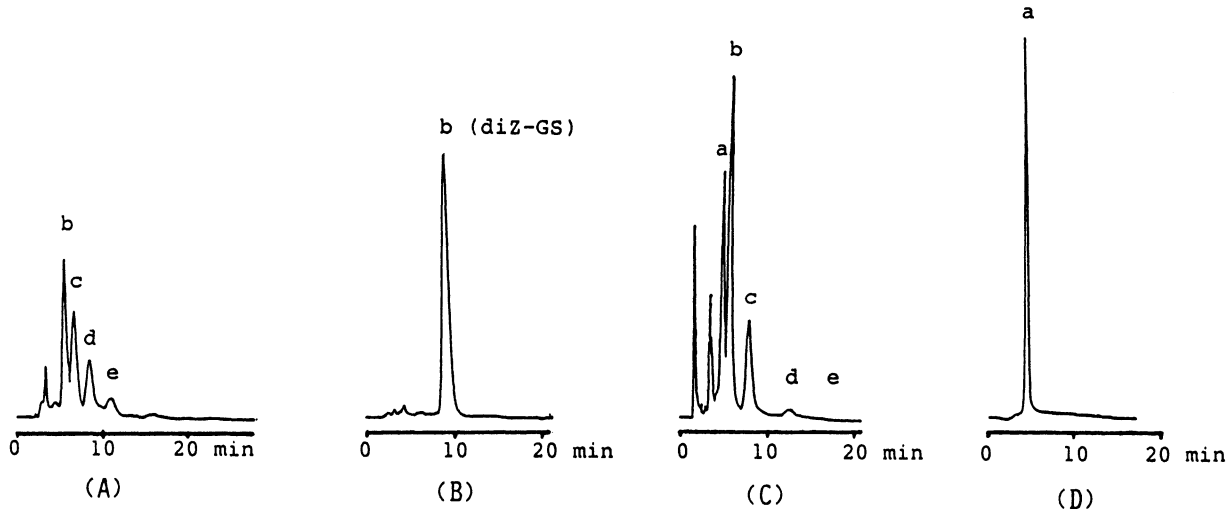

Fig. 3. HPLC profiles of crude products in cyclization of active esters related to GS.
The starting compounds are: (A), **1**; (B), **2**; (C), **3**; (D), **4**.
(a: cyclic monomer, b: cyclic dimer, c: cyclic trimer, d: cyclic tetramer, e: cyclic pentamer)

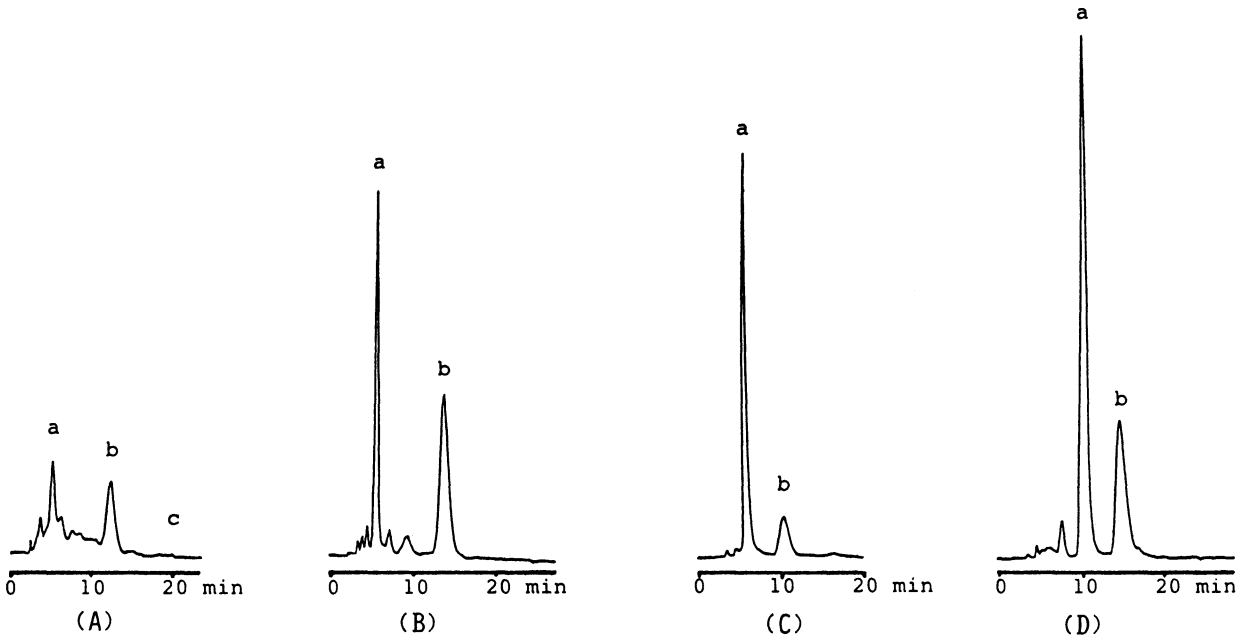

Fig. 4. HPLC profiles of crude products in cyclization of active esters related to GR.
The starting compounds are: (A), **5**; (B), **6**; (C), **7**; (D), **8**.
(a: cyclic monomer, b: cyclic dimer, c: cyclic trimer)

Table 1. Products of Cyclization of Penta- and Hexapeptide Active Esters[a)]

Starting compounds	Yield(%)[b] of cyclic product	Ratios of cyclic peptides				
		Monomer	Dimer	Trimer	Tetramer	Pentamer
1	30	0	34[1409]	32[2115]	24[2820]	10[3524]
2	57	0	100[1409]	0	0	0
3	40	30[704]	46[1409]	21[2115]	3[2819]	0[3523][c)]
4	48	100[704]	0	0	0	0
5	26	43[958]	57[1916]	0[2875][c)]	0	0
6	38	45[958]	55[1916]	0	0	0
7	53	78[1027]	22[2053]	0	0	0
8	70	67[1027]	33[2053]	0	0	0

a) Numbers in brackets are mass numbers observed as the molecular ion peaks. b) The yield based on cyclic peptides separated from reaction mixture by HPLC. c) Small amount of the corresponding cyclic trimer or pentamer was separated using HPLC.

taining a L-Phe residue preceding Pro (**5**) yielded some cyclic products with higher molecular weights, though their amounts were negligible. The data indicated that the configurations of the constituent amino acids in the active esters and the lengths of the esters affect the course of cyclic polymerization. In both cases, the penta- and hexapeptide active esters, the configuration of the N-terminal amino acids affects cyclization. Esters with D-amino acids (**3**, **4**, **7**, **8**) clearly produce products of cyclic monomerization or cyclization at lower molecular weights.

Experimental

Synthesis of Boc-penta- and -hexapeptides was performed as manner described in our previous papers.[6,7]

Boc-Tyr(Bzl)-Val-Orn(Z)-Leu-D-Phe-Pro-OH. Yield, 79% from the corresponding benzyl ester: mp 181—184 °C; $[\alpha]_D^{20}$, −29.3° (*c* 1.0, DMF): Found: C, 64.80; H, 7.24: N, 8.95%. Calcd for $C_{59}H_{77}N_7O_{12} \cdot H_2O$: C, 64.76; H, 7.27; N, 8.96%.

Boc-D-Tyr(BzlCl$_2$)-Val-Orn(Z)-Leu-D-Phe-Pro-OH. Yield, 90% from the corresponding benzyl ester; mp 164—165 °C; $[\alpha]_D^{20}$, −14.5 (*c* 1.1, DMF); Found: C, 60.71; H, 6.63; N, 8.04%. Calcd for $C_{59}H_{75}N_7O_{12}Cl_2 \cdot H_2O$: C, 60.92; H, 6.67; N, 8.43%. Cyclization via an active ester was performed in a similar manner to that described in our previous paper.[7] HPLC analysis was carried out using a Twincle system (Jasco) consisting of a Twincle pump, a VL-611 injector and a UVIDEC 100-III detector. A Finepak SIL C18 column (10 μm, 250×4.6 mm I.D., or 250×6.7 mm I.D., Jasco) was used: flow rate, 1 ml min^{-1}; solvent, methanol-water (9 : 1); monitoring wavelength, 220 nm. The peak area was recorded using a Chromatopac C-R3A integrator (Shimadzu Co.). Molecular weights of the cyclic products were determined by FAB mass spectrometry using a JEOL JMS-D300 mass spectrometer (in Toyo Jozo Co., Ltd.) and a JEOL JMS-HX110 mass spectrometer (in JEOL Ltd.), and/or secondary ion mass spectrometry using a Hitachi M-80 mass spectrometer (in Meijo Univ.).

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References

1) A. R. Battersby and L. C. Craig, *J. Am. Chem. Soc.*, **73**, 1887 (1951).

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 the rules of IUPAC-IBU Commission of Biological Nomenclature. Abbreviations used are as follows: Boc, *t*-butoxycarbonyl; Z, benzyloxycarbonyl; Tos, tosyl; Bzl, benzyl: BzlCl$_2$, 2,4-dichlorobenzyl: -ONSu, hydroxysuccinimide ester; DMF, *N*,*N*-dimethylformamide.

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