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Studies of gramicidin S analogues having various ring sizes by reversed-phase high-performance liquid chromatography

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Abstract

Many gramicidin S analogues containing six to fourteen amino acid residues were synthesized and their chromatographic behaviour was investigated using reversed-phase high-performance liquid chromatography. Cyclo(–Val–Leu–Orn–Leu–D-Phe–Pro–Val–Orn–Leu–D-Phe–Pro–) gave double peaks in the chromatogram, whereas the other peptides gave a single peak. The influences of the concentration of this peptide, column temperature and flow-rate on the chromatographic separation were examined. The isomeric conformers were separated from each of the double peaks and were in equilibrium with each other at low temperatures. These results suggested that the presence of the additional L-Leu residue preceding the Orn residue gives rise to moderate stabilization of their conformers.

1. Introduction

Gramicidin S (GS) (Fig. 1) [1] is an antibiotic cyclodecapeptide with a rigid β-pleated sheet conformation [2,3]. A characteristic feature of this conformation is the orientation of the side-chains in such a way that the two charged Orn side-chains are situated on one side of the molecule and the four hydrophobic Val and Leu side-chains on the other. (Note: amino acid residues with no prefix are of L-configuration unless stated otherwise. The abbreviations for amino acids and peptides are in accordance with the rules of the IUPAC–IUB Commission of Biochemical Nomenclature.) This arrangement is apparently maintained by a rigid ring structure containing two type II′ β-turns composed of D-Phe–Pro.

In order to investigate the contribution of the ring size to the secondary structure and the antibiotic activity of this antibiotic, we synthesized numerous GS analogues containing six to fourteen amino acid residues (Fig. 1) [4–6]. These GS analogues have the D-Phe–Pro sequence in each molecule. A Leu residue having a hydrophobic side-chain was used to enlarge the ring size. In the high-performance liquid chromatographic (HPLC) studies of these synthetic peptides using a reversed-phase column, we found that one of the GS analogues containing eleven amino acid residues gives double peaks in the chromatogram.

Recently, in studies of the HPLC behaviour of a cyclic dodecapeptide, gratisin (GR), we reported that the analogues having D-X–D-Y–L-Pro

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Fig. 1. Primary structures of synthetic peptides and gramicidin S. [a] Number of amino acid residues in each cyclic peptide.

or L-Pro–D-X–D-Y sequences (X and Y = amino acid residue) and strong antibiotic activity gave double peaks on the chromatogram, but that GR analogues containing D-X–L-Pro–L-Y sequences did not show the presence of conformers [7].

In this paper, we report the HPLC behaviour of GS analogues that have various ring sizes and contain the D-Phe–L-Pro sequence at the β-turn part, and discuss the effect of the ring structures on the formation of stable conformers.

2. Experimental

2.1. Synthesis of peptides

Cyclic peptides related to GS were synthesized by a conventional liquid-phase method [4,5]. Their primary structures are shown in Fig. 1 [4–6]. Syntheses of cyclo(–Val–Orn–Leu–D-Phe–Val–Orn–Leu–D-Phe–Pro–) and cyclo(–Val–Orn–Leu–Pro–D - Phe–D - Tyr–Val–Orn–Leu–D-Phe–Pro–) have not previously been reported. Cyclo(–Val–Orn–Leu–D-Phe–Val–Orn–Leu–D-Phe–Pro–) · 2HCl, m.p. 248–250°C (decomp.); $[\alpha]_D^{25}$ – 128.1° (*c* 0.3, EtOH). MS [fast atom bombardment (FAB)], *m/z* 1044 ($C_{55}H_{85}O_9N_{11}$; M + H^+). Amino acid analysis: Val, 2.00; Orn, 2.05; Leu, 2.10; Phe, 2.20: Pro, 0.95. Found: C, 56.45; H, 8.34; N, 13.21%. Calculated for $C_{55}H_{85}O_9N_{11} \cdot 2HCl \cdot 3H_2O$: C, 56.40; H, 8.00; N, 13.15%. Cyclo(–Val–Orn–Leu–Pro–D - Phe–D - Tyr–Val–Orn–Leu–D - Phe–Pro–) · 2HCl, m.p. 225°C (decomp.); $[\alpha]_D^{25}$ – 131.0° (*c* 0.5, EtOH). MS (FAB), *m/z* 1304 ($C_{69}H_{102}O_{12}N_{13}$; M + H^+). Amino acid analysis: Val, 2.10; Orn, 2.00; Leu, 2.20; Phe, 2.10: Pro, 1.90; Tyr, 1.00. Found: C, 57.72; H, 7.87; N, 12.63%. Calculated for $C_{69}H_{102}O_{12}N_{13} \cdot 2HCl \cdot 3.5H_2O$: C, 57.45; H, 7.82; N, 12.6%.

The homogeneities of the synthetic peptides were confirmed by means of TLC, HPLC, elemental analysis, fast-atom bombardment mass spectrometry and amino acid analysis of their acid hydrolysates.

2.2. HPLC analysis

HPLC was carried out using an 800 Series system (Jasco, Tokyo, Japan) consisting of a model 880 intelligent HPLC pump, a Model 875-UV intelligent UV–Vis detector, a Model 860-CO column oven and a Model 7125 syringe-loading sample injector (Rheodyne, Cotati, CA, USA). A Finepak SIL C_{18} column (250 × 4.6 mm I.D.) (Jasco) was used. The mobile phase was methanol–5% aqueous sodium perchlorate (4:1, v/v), the flow-rate was 1 ml/min and the

wavelength of detection was 220 nm. About 1 mg of each synthetic peptide was dissolved in 1 ml of the mobile phase and the resulting solution (10 μl) was injected into the chromatograph. The peak area was recorded using a Chromatopac C-R3A integrator (Shimadzu, Kyoto, Japan).

3. Results and discussion

The HPLC of peptides **1–23** and GS was usually performed at 20°C. Peptide **11**, containing eleven amino acid residues and possessing strong antibiotic activity, gave double peaks (Fig. 2), although other analytical results showed the homogeneity of the peptide. The other peptides with various ring sizes gave a single peak and did not show such phenomena, although these peptides also have the D-Phe–Pro sequence at the β-turn part.

The area ratio of the two peaks remained unchanged on diluting the sample injected, suggesting that the phenomena are not caused by intermolecular aggregation.

Next, the effect of column temperature (10, 20, 25, 30, 40 and 50°C) on the elution of peptide **11** was investigated (Fig. 2). With an increase in the column temperature, the two peaks gradually coalesced and resulted in a single peak.

The effect of flow-rate on the shape of the peak of peptide **11** is shown in Fig. 3. With a decrease in the flow-rate, the peptide was eluted as a broader peak.

The two isomers of peptide **11** isolated on a preparative scale by HPLC at 10°C were rechromatographed under the same conditions after storage for 1.5 h at 10°C (Fig. 4). The chromatograms of each isomer reverted to the original profile, suggesting that the two conformers slowly interconvert at low temperature.

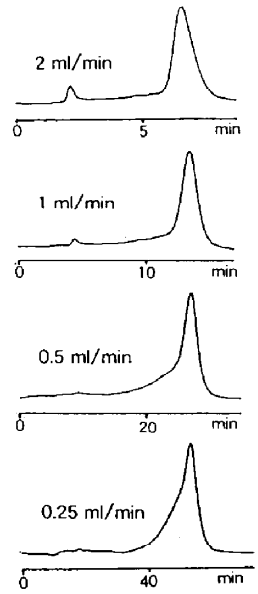

Fig. 3. Effect of flow-rate (0.25–2 ml/min) on the peak shape of peptide **11**. Column temperature, 20°C.

Similar examples of multiple peaks on HPLC separation have been reported for several biologically active peptides [7–11] and model dipeptides [12] which contain the Pro residue. These phenomena were ascribed to the slow interconversion by *cis–trans* isomerization of the X–Pro imide bond. The present HPLC results for peptide **11** may also be caused by *cis–trans* isomerization of the D-Phe–Pro imide bond.

Recently, in HPLC studies of a cyclic dodecapeptide, gratisin (GR), we reported that

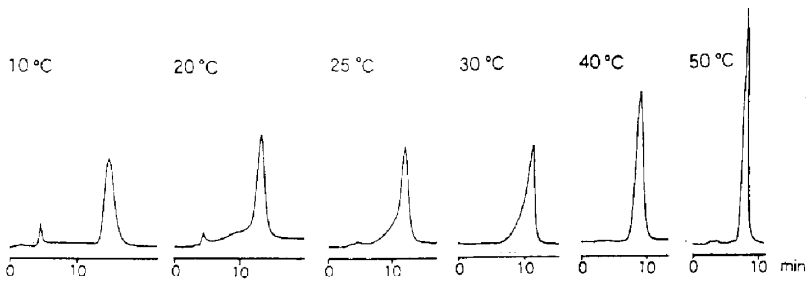

Fig. 2. Effect of column temperature (10–50°C) on the elution of peptide **11**. Flow-rate, 1 ml/min.

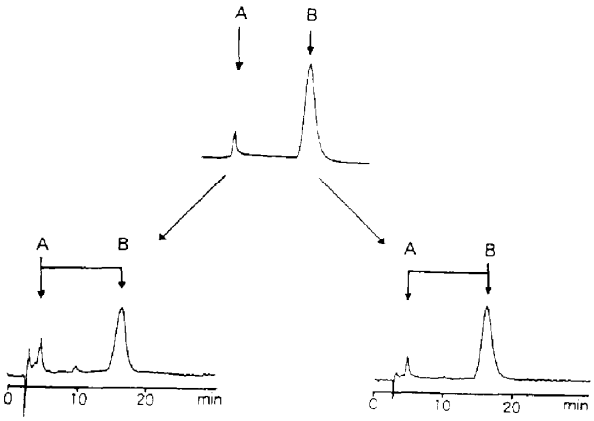

Fig. 4. Rechromatography of the isolated conformers (A, B) of peptide **11** after storage at 10°C for 1.5 h. Column temperature, 10°C; flow-rate, 1 ml/min.

GR analogues having D-X–D-Y–L-Pro or L-Pro–D-X–D-Y sequences (X and Y = amino acid residue) and strong activities gave double peaks in the chromatogram [7]. Peptides **15** and **16**, which contain eleven amino acid residues and one D-Phe–D-Tyr–L-Pro or L-Pro–D-Phe–D-Tyr sequence in the molecule, respectively, gave a single peak in the chromatograms. Further, peptide **17**, which contains twelve amino acid residues and the same hexapeptide sequence, Val–Leu–Orn–Leu–D-Phe–Pro, as peptide **11**, also gave a single peak. These results indicated that a special sequence resulting in the stabilization of conformers in cyclic undecapeptides on the chromatogram was different from that in cyclic dodecapeptides (GR-peptides). In addition, the presence of the additional L-Leu residue preceding the Orn residue in peptide **11** may be necessary to give rise to stabilization of their conformers.

The circular dichroism (CD) spectra of peptides **11–14** containing eleven amino acid residues at room temperature (ca. 20°C) in aqueous solution resembled each other, although the positions and the configurations of the additional Leu residue are different in each molecule, suggesting that the conformations of these peptides are similar to each other [6]. In the present studies, peptides **12–14** gave a single peak and peptide **11** gave double peaks in the chromatogram. On the basis of the chromatogram of the latter peptide, the conformer eluted slowly from the column was the main component. Therefore, the structure of the main conformer may be similar to those of peptides **12–14**.

The antibiotic activity of peptide **11** is slightly lower than those of peptides **13** and **14** [6]. In studies of the HPLC behaviour of peptides related to GS and GR, it was reported that the antibiotics with stronger activity exhibited a higher effective hydrophobicity, and were eluted more slowly from an octadecylsilica gel column [13]. Hence the lower activity of peptide **11** may be brought about by the presence of the conformational equilibrium.

Further detailed conformational analyses of these cyclic peptides are needed in order to understand clearly the phenomena mentioned above.

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