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Abstract

In order to investigate the effect of twofold symmetric structure of gramicidin S for antibacterial activity, [Leu^1, Val^3]-gramicidin S was synthesized by a solid phase peptide synthesis. The ORD curve of [Leu^1, Val^3]-gramicidin S was similar to that of gramicidin S. The antibacterial activity of [Leu^1, Val^3]-gramicidin S was equal to that of gramicidin S. The result shows that the twofold symmetric structure of gramicidin S does not influence the antibacterial activity of gramicidin S.

Key words: [Leu^1, Val^3]-gramicidin S, gramicidin S, antibiotics.

Gramicidin S (GS) is an antibiotics which is produced by certain strains of *Bacillus brevis* (*1*). GS is a decapeptide with a cyclic structure (-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu-D-Phe-Pro-) which consists of two repeated pentapeptide sequence and has a twofold symmetric structure (*2*). The conformation of GS was estimated by X-ray analysis as a β-pleated sheet structure (*3*). In this conformation, four hydrophobic side chains of valyl and leucyl residues are located in one side of a ring frame consisting of a cyclic peptide chain, while two hydrophylic side chains of ornithyl residues are located on the opposite side. Accordingly, GS seems to be a cationic surface active agent.

The strength of GS activity is related to the hydrophobicity of the side chains of valyl and leucyl residues and the number of cationic groups of ornithyl residues (*4*). Furthermore, it was reported that the analogs whose conformations were different from those of GS exhibit weak antibacterial activity (*4*). However, the effect of the twofold symmetric structure of GS for antibacterial activity is still not established.

In order to investigate the effect of twofold symmetric structure of GS for antibacterial activity, the analog of GS which does not have the twofold symmetric structure but have same hydrophobicity, same number of cationic groups and same conformation as GS must be synthesized and its antibacterial activity measured.

GS has valyl residues at positions 1 and 1′ and leucyl rseidues at positions 3 and 3′.

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Abbreviation: GS, gramicidin S; Boc-, t-butyloxycarbonyl; Z-, benzyloxycarbonyl; DCC, dicyclohexylcarbodiimide; AcOH, acetic acid; EtOH, ethanol; DMF, dimethylformamide; MeOH, methanol.

Then, the analog which has leucyl residue at position 1 and valyl residue at position 3 does not have the twofold symmetric structure, but the hydrophobicity is equal to that of GS. Then, we synthesized [Leu^1, Val^3]-GS and measured its antibacterial activity.

Experimental Procedures

All Boc-amino acids were synthesized by the literature (*5*). Chloromethylated polystyrene-divinylbenzene (2%) resin (0.66 mmol Cl/g) was purchased from Protein Research Foundation, Osaka, Japan. For amino acid analysis, peptides were hydrolyzed with 6 M HCl at 110℃ for 24 hr and analyzed on a Hitachi amino acid analyzer KLA-5. ORD measurements were performed with a JASCO spectropolarimeter J-20A.

Boc-D-Phe-Pro-Leu-Orn(Z)-Val-D-Phe-Pro-Val-Orn(Z)-Leu-resin. Boc-Leu-resin was prepared from chloromethylated polystyrene and Boc-Leu as described in the literature (*6*). Boc-Leu-resin (1.41 g, 0.5 mmol) was placed in a reaction vessel and swollen with AcOH (10 ml) for overnight. The following cycle was used for an addition of appropriate Boc-amino acid. The resin was rinsed with AcOH (10 ml × 3), shaken for 30 min in 1 M HCl in AcOH (10 ml), and rinsed successively with AcOH (10 ml × 3), EtOH (10 ml × 3), and DMF (9 ml × 3). The resin was shaken for 10 min in 10% NEt_3 in DMF (9 ml) and rinsed with DMF (9 ml × 3) and CH_2Cl_2 (8 ml × 3). The resin was suspended in CH_2Cl_2 (8 ml) containing 4-fold molar excess of Boc-amino acid and shaken for 10 min. DCC in equimolar amounts with Boc-amino acid was added and shaking continued for 20 hr. The coupling steps were terminated by rinsing with CH_2Cl_2 (8 ml × 3) and EtOH (10 ml × 3). After completion of the final coupling cycle, Boc-decapeptidyl resin was washed with CH_2Cl_2, EtOH, AcOH, and DMF (each 10 ml × 3), respectively.

Boc-decapeptide hydrazide. To the suspension of Boc-decapeptidyl resin in DMF (10 ml) was added $NH_2NH_2 \cdot H_2O$ (1 ml). The mixture was shaken for 2 days at room temperature, and the deprotected resin was filtered off and washed with DMF (10 ml × 3). The combined filtrates were evaporated in vacuo and the residue was treated with water (100 ml) to give white powder. The product was recrystallized from EtOH-petroleum ether; yield, 827 mg. Amino acid analysis gave the following ratio; Phe 2.00, Pro 2.23, Val 1.62, Orn 1.53, Leu 2.00.

Cyclic decapeptide. Boc-decapeptide hydrazide (150 mg, 0.1 mmol) was dissolved in 1 M HCl in AcOH (2 ml), and left for 1 hr at room temperature. The solution was evaporated in vacuo, and the residue (H-decapeptide hydrazide·2HCl) was dissolved in a mixture of DMF (2 ml) and AcOH (0.2 ml). To the solution were added 1 M HCl in AcOH (0.2 ml) and 1 M $NaNO_2$ (0.1 ml) at −20℃. The mixture was stirred at −10℃ for 15 min to produce the azide, and poured into pyridine (100 ml) at −5℃. The solution was stirred for 3 days at 5℃, evaporated in vacuo, and the residue dissolved in ethyl acetate (50 ml). The solution was washed successively with 4% $NaHCO_3$, water, 2% HCl and water, dried over Na_2SO_4, and evaporated. The product was used in the next step without purification.

[Leu^1, Val^3]-GS The obtained cyclic decapeptide was dissolved in a mixture of MeOH (10 ml) and 1 M HCl in AcOH (0.6 ml), and hydrogenated in the presence of palladium black for 5 hr. After removal of the catalyst, the filtrate was evaporated in vacuo, and the obtained crude [Leu^1, Val^3]-GS was purified by droplet countercurrent chromatography. The apparatus consisted of 150 columns. The solvent used was a mixture of cyclohexane-$CHCl_3$-MeOH-0.1 M HCl (3:10:10:5, by vol.), and an upper phase of the mixture was loaded in the column as a stationary phase. Crude [Leu^1, Val^3]-GS was dissolved in the lower phase and placed at the top of the first column. The lower phase as the moving phase was pumped with N_2 pressure through the top of the first column, and fractions (5 g) were collected. To each fraction was added MeOH (1 ml), and their absorbances at 250 nm were measured. The major peak (fraction numbers, 50-75) was collected and evaporated in vacuo. The solid was collected with the aid of ether; yield of [Leu^1, Val^3]-GS·2HCl, 58 mg (43%); mp. above 275℃. Amino acid analysis gave the following ratio; Phe 2.00, Pro 1.92, Val 2.09, Orn 2.07, Leu 2.00.

Antibacterial assay The bacteria (*Bacillus subtilis* IFO 3007) were grown in a medium of polypeptone-meat extract-yeast extract at 30℃. The cell culture was diluted with medium containing 10% of MeOH to 2.7×10^8 cells/ml (cell suspension). GS analog was dissolved in the medium containing 10% of MeOH, and various amounts of GS analog solution were placed in test tubes, made up to 1 ml with the medium containing 10% of MeOH, and the cell suspension (1 ml) was added. After incubation for 8 hr at 30℃, distilled water (1 ml) was added, and the absorbance at 620 nm was measured.

Results and Discussion

The protected linear decapeptide sequence was built up by stepwise addition of

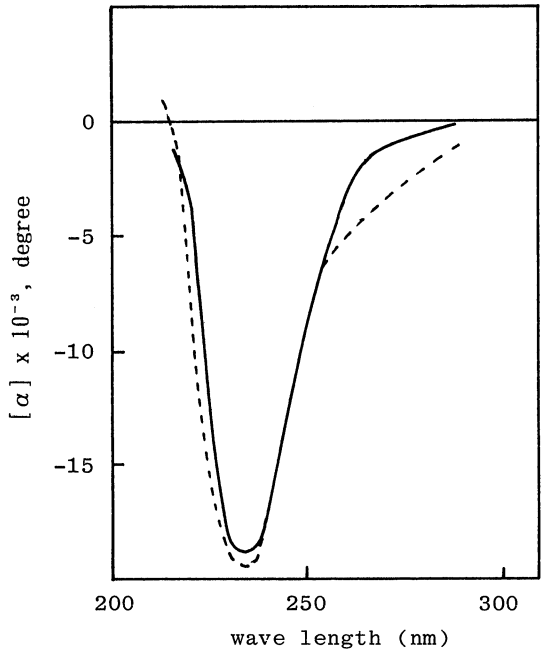

Fig. 1. ORD curve of GS and [Leu^1, Val^3]-GS. The ORD curves were measured in ethanol. (———), GS; (- - - - -), [Leu^1, Val^3]-GS.

Boc-amino acid to the Merrifield resin, starting with L-leucine as the C-terminal amino acid. The obtained Boc-decapeptidyl resin was treated with hydrazine hydrate to give Boc-decapeptide hydrazide. The Boc-decapeptide hydrazide was deblocked and converted to cyclic decapeptide by the azide method. After hydrogenolysis, the obtained crude [Leu^1, Val^3]-GS was purified by droplet countercurrent chromatography. The elution volume of [Leu^1, Val^3]-GS was nearly same as that of GS. The total yield of [Leu^1, Val^3]-GS was 43% calculated from Boc-Leu-resin.

As shown in Fig. 1, the ORD curve of [Leu^1, Val^3]-GS was similar to that of GS. The result suggests that the analog possesses a preferred conformation similar to that of GS. The antibacterial activity of GS and [Leu^1, Val^3]-GS were same (the minimum inhibitory concentration of these two compounds were 1.0 μg/ml). This shows that the twofold symmetric structure of GS is not necessary for antibacterial activity of GS.

References

1) G. F. Gause and M. G. Brazhnikova, *Am. Rev. Soviet Med.*, **2**, 134 (1944).
2) A. R. Battersby and L. C. Craig, *J. Am. Chem. Soc.*, **73**, 1877 (1951).
3) S. E. Hull, R. Karlsson, P. Main, M. M. Woolfson and E. J. Dodson, *Nature*, **275**, 206 (1978).
4) N. Izumiya, T. Kato, H. Aoyagi, M. Waki and M. Kondo "Synthetic Aspects of Biologically Active Cyclic Peptides" Kodansha, Tokyo (1979), p. 57.
5) L. Moroder, A. Hallett, E. Wunsch, O. Keller and G. Wersin, *Hoppe-Seyler's Z. Physiol. Chem.*, **357**, 1651 (1976).
6) A. Marglin, *Tetrahedren Lett.*, **1971**, 3145.