SYNTHETIC STUDIES ON GRATISIN. II

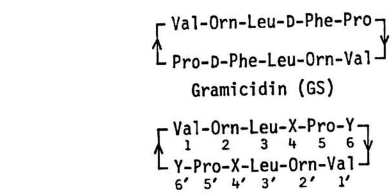
Sir:

An antibiotic peptide, gratisin, which is active toward *Bacillus subtilis* was isolated from *B. brevis* Y-33 by SILAEV *et al.*[1] It is a cyclododecapeptide composed of two each of Val, Orn, Leu, Phe, Pro and Tyr residues. The primary structure of gratisin was proposed as shown in Fig. 1,[2,3] but the configurations of the individual amino acid residues have not yet been established. We previously synthesized[4] all-L-gratisin containing only L-amino acid residues and [D-Phe$^{4,4'}$]-gratisin containing D-Phe residues in positions 4 and 4′ (Fig. 1), and examined the antibiotic activity of these peptides. The latter compound was synthesized because of the analogy of the primary structure of gratisin with that of gramicidin S (GS). [D-Phe$^{4,4'}$]-gratisin has antibiotic activity against all Gram-positive microorganisms tested, but is less potent than GS. On the other hand, all-L-gratisin showed little activity (Table 1). These results suggested that the Phe residue is present in the D-form in gratisin. The configurations of the amino acid residues following the Pro residues remained undetermined. Therefore, we synthesized [D-Phe$^{4,4'}$, D-Tyr$^{6,6'}$]-gratisin having -D-Phe-Pro-D-Tyr- partial sequence as shown Fig. 1, and investigated the relationship between the structure and antibiotic activity of these synthetic peptides.

[D-Phe$^{4,4'}$, D-Tyr$^{6,6'}$]-gratisin was synthesized by conventional methods. Boc-D-Tyr(BzlCl_2)-Val-Orn(Z)-Leu-D-Phe-Pro-OBzl (**1**) was obtained by the stepwise elongation from Pro-OBzl with the help of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (WSCD) and 1-hydroxybenztriazole. A part of **1** was converted to the hydrazide. Coupling of the Boc-hexapeptide hydrazide and hexapeptide ester derived from **1** was carried out by an azide method to afford Boc-(D-Tyr(BzlCl_2)-Val-Orn(Z)-Leu-D-Phe-Pro)$_2$-OBzl. This ester was converted into the corresponding hydrazide, which was treated with HCl/dioxane and then cyclized by the azide method in pyridine. The yield of cyclization was 63%. The protected groups of cyclododecapeptide were removed by hydrogenolysis to afford [D-Phe$^{4,4'}$, D-Tyr$^{6,6'}$]-gratisin. The homogeneity of [D-Phe$^{4,4'}$, D-Tyr$^{6,6'}$]-gratisin was confirmed by means of thin-layer chromatography, electrophoresis, amino acid analysis and elemental analysis.

The antibiotic activity of [D-Phe$^{4,4'}$, D-Tyr$^{6,6'}$]-gratisin was examined toward several microorganisms (Table 1). [D-Phe$^{4,4'}$, D-Tyr$^{6,6'}$]-gratisin had antibiotic activity against all Gram-positive microorganisms tested, and its activity was the same as that of GS against *B. subtilis* and stronger than that of [D-Phe$^{4,4'}$]-gratisin. These results suggest that -D-Phe-Pro- sequence plays an

Fig. 1.



All-L-gratisin X = L-Phe, Y = L-Tyr
[D-Phe$^{4,4'}$]-gratisin X = D-Phe, Y = L-Tyr
[D-Phe$^{4,4'}$, D-Tyr$^{6,6'}$]-gratisin X = D-Phe, Y = D-Tyr

Table 1. Antibiotic activities of GS and synthetic peptides.

Test organisms	Minimum inhibitory concentration (μg/ml)			
	GS	All-L-gratisin	[D-Phe$^{4,4'}$]-gratisin	[D-Phe$^{4,4'}$, D-Tyr$^{6,6'}$]-gratisin
Staphylococcus aureus ATCC 6538P	1.6	>50	50	6.3
Streptomyces pyogenes N.Y.5	1.6	>50	12.5	3.1
Micrococcus flavus ATCC 10240	0.8	25	12.5	3.1
Corynebacterium diphtheriae P.W. 8	0.8	25	3.1	3.1
Bacillus subtilis ATCC 6633	3.1	>50	50	3.1
Escherichia coli NIHJ-JC2	>100	>50	>50	>50
Proteus vulgaris OX 19	>100	>50	>50	>50

Abbreviations with no prefix show L-amino acid residue. The following are from J. Biol. Chem. 247: 977~983, 1972: Boc-, *t*-butoxycarbonyl; Z-, benyloxycarbonyl; BzlCl_2-, 2,6-dichlorobenzyl.

important role for antibiotic activity, and D-Tyr residue following Pro residue contributes to the activity.

In order to investigate the relationship between structure and antibiotic activity, the CD spectrum of [D-Phe$^{4,4'}$, D-Tyr$^{6,6'}$]-gratisin in aqueous solution was determined. This spectrum showed a trough at 200 nm and a shoulder at 215 nm, and the pattern was similar to that of random coils in proteins. On the other hand, the spectra of all-L- and [D-Phe$^{4,4'}$]-gratisin were similar to that of GS.[4]

We have had no opportunity to compare our synthetic preparations with natural gratisin. However, our investigation suggests that the synthetic peptide with D-residues in positions 4, 4', 6 and 6' is identical with the natural product.

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Makoto Tamaki
Michiaki Takimoto
*Shosuke Sofuku
*Ichiro Muramatsu

Department of Chemistry, Faculty of Science,
Toho University,
Miyama, Funabashi-shi, Chiba, Japan
*Department of Chemistry, College of Science,
Rikkyo (St. Paul's) University,
Nishi-ikebukuro, Tokyo, Japan

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