CHEMISTRY LETTERS, pp. 191-194, 1992.

Synthesis of [D-Pyrenylalanine$^{4,4'}$]gramicidin S by Solid-Phase-Synthesis and Cyclization-Cleavage Method with Oxime Resin

Ming XU, Norikazu NISHINO,* Hisakazu MIHARA, Tsutomu FUJIMOTO, and Nobuo IZUMIYA†

Department of Applied Chemistry, Faculty of Engineering, Kyushu Institute of Technology, Tobata-ku, Kitakyushu 804

†Department of Industrial Chemistry, Faculty of Engineering, Kyushu Sangyo University, Higashi-ku, Fukuoka 813

Cyclic decapeptide [D-pyrenylalanine$^{4,4'}$]gramicidin S has been efficiently synthesized *via* stepwise solid-phase-synthesis and cyclization-cleavage with the Kaiser's oxime resin in 45% total yield. This analog showed the similar conformation to the natural gramicidin S in MeOH. The pyrene ring introduced into the peptide is a useful fluorescent probe for the study of the mechanism of the antimicrobial activity.

The Kaiser's oxime resin method in the solid-phase-synthesis (SPS) of peptides utilizes an oxime ester as an anchoring linkage.[1] This is a kind of active ester and, therefore, allows the mild cleavage of the peptidyl linkage to the resin without accompanying the deprotection at side chains. During the application of the oxime resin SPS in the synthesis of long polypeptides, the rapid removal of cyclic dipeptides was often observed in a certain condition.[1a,f] We investigated this defect of the oxime resin in detail[2] and further it guided us to study the cyclization-cleavage (CC) method for the synthesis of cyclic peptides. We chose a fluorescent gramicidin S (GS)[3] analog containing D-pyrenylalanine (D-Pya)[4] instead of D-Phe for the demonstration of the facility of the SPS-CC method with the oxime resin (Fig. 1). The fluorescently probed GS analog will show the significant utility to investigate the progress of the action of the antibiotic to the biomembrane. Recently, Ösapay *et al.* reported the cyclization of tyrocidin A from the oxime resin in the same concept but with a little difference.[5]

Ohno *et al.*[6] applied SPS for GS starting with tert-butyloxycarbonyl (Boc)-Leu incorporated into Merrifield polymer[7] by the consideration of the biosynthesis. Accordingly, we also chose Boc-Leu-oxime resin (0.50 mmol/g resin) to start the stepwise SPS with the benzotriazol-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) reagent[8] to give Boc-(D-Pya-Pro-Val-Orn(Z)-Leu)$_2$-oxime resin (Z, benzyloxycarbonyl). The procedure is described in Table 1. After the deprotection of Boc group with 25% trifluoroacetic acid (TFA)/dichloromethane (DCM), a dimethylformamide (DMF) solution with 2 equivalents (equiv.) of NEt_3 and AcOH was added into the reaction vessel. AcOH was used as a catalyst for the cyclization-cleavage.[9] Time course of the liberation of protected [D-Pya$^{4,4'}$]GS from the resin is shown in Fig. 2. After shaking for 24 h, the cyclic decapeptide was isolated from the solution as solid in 85% yield and in 75% purity on reversed-phase (RP)-HPLC (MS-GEL C18 120Å (Asahi Glass, Co.), 4.6 mm x 120 mm, eluted at 25.0 min by a linear gradient of 64-100% acetonitrile/0.1% TFA over 30 min). After the purification

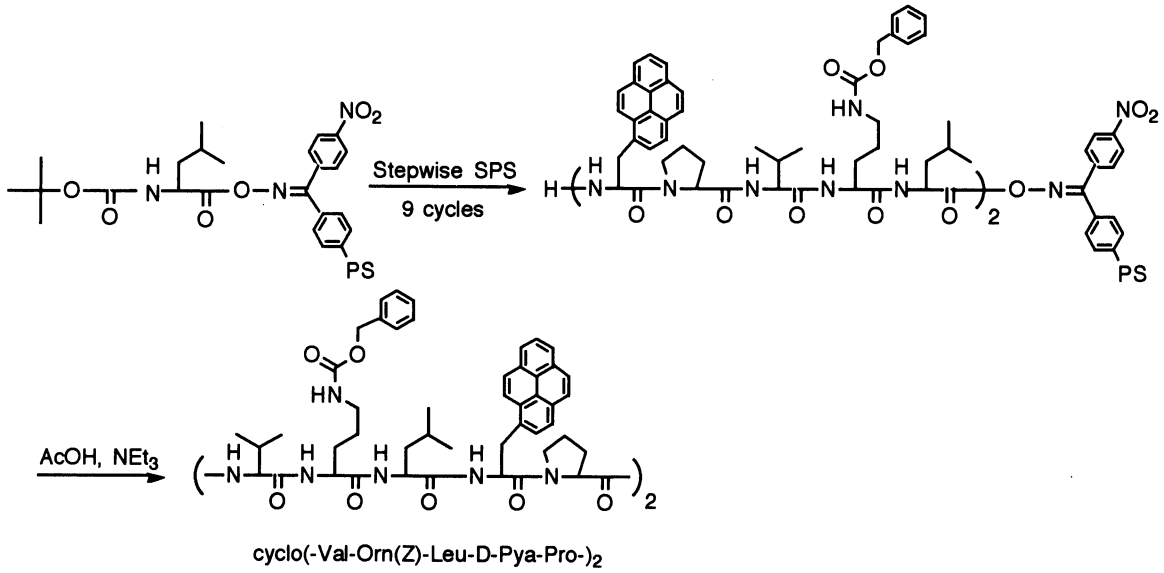

Fig. 1. Solid-phase-synthesis and cyclization-cleavage (SPS-CC) for the synthesis of [D-Pya$^{4,4'}$]gramicidin S. PS, polystyrene-co-1% divinylbenzene resin; Z, benzyloxycarbonyl.

of the crude protected cyclic decapeptide by gel filtration (Sephadex LH-20, column size 2.0 cm x 90 cm, DMF), Z groups on Orn were removed by hydrogenation with 5% Pd-C as a catalyst. Purification by RP-HPLC (MS-GEL C18 120Å, 1.0 cm x 25 cm, eluted at 9.9 min by the same condition) gave the desired [D-Pya$^{4,4'}$]GS in 45% total yield (purity > 98%): FAB-MS, m/z = 1390 $(M+H)^+$; Anal ($C_{80}H_{100}N_{12}O_{10}\cdot 2CF_3COOH\cdot 11H_2O$) C, H, N; mp 225-227 °C (decomp.); $[\alpha]_D^{25}$ -242° (c 0.1, MeOH).

Usually, cyclic peptides are synthesized *via* corresponding linear peptide active esters in pyridine in high dilution condition ($\approx 10^{-3}$ mol·dm^{-3}) to avoid polymerization. The cyclization directly from the oxime resin, however, requires no high dilution , because the chance of polymerization is geographically diminished. Thus, the omission of the activation of linear precursor and high dilution condition facilitated so much the synthesis of cyclic peptides. Ösapay *et al.*[5] intentionally restricted the amount of the first amino acid (0.114 mmol/g resin) at about 20% of the maximum incorporation to the resin considering the dimerization during the cyclization reaction. However, the use of the oxime resin with the first amino acid in regular content (0.50 mmol/g resin) gave predominantly the cyclic monomer with rather small amount (8%) of the dimerized cyclic peptide. This should be attributed to the high tendency of β-turn formation

Table 1. Protocol for the peptide chain assembly on the oxime resin[a)]

Operation and reagents	Time / min
1. TFA deprotection	
25% TFA in DCM (x1)	1
25% TFA in DCM (x1)	30
2. Washes	
DCM (x2)	1
isopropyl alcohol (x1)	1
DCM (x2)	1
3. Coupling	
Boc-AA-OH and BOP (3.0 equiv., each) in DMF NEt_3 (6.5 equiv.)	45
4. Washes	
DMF (x4)	1
DCM (x2)	1
5. Kaiser test	

a) Solvent volume is 15 mL for 1 g resin.

of the linear decapeptide segment. The bent structure could preferentially bring the monomer cyclization. For the efficient synthesis of the cyclic peptide, of course, higher content of peptide on the resin (the condensed condition) is more favorable. The small amount of dimerized impurity was easily removed during passing through the column of Sephadex LH-20.

The circular dichroism (CD) and absorption spectra of [D-Pya$^{4,4'}$]GS in MeOH are given in Fig. 3. The profile and ellipticity in CD spectra at the carbonyl region (200 nm - 230 nm) indicate that the conformation of the framework resembles that of the natural GS.[3] The type II' β-turn and antiparallel β-sheet structure seemed to be maintained in spite of the introduction of bulky D-Pya at D-Phe positions. Though there observed negative Cotton effect for each absorption band corresponding to the pyrene ring, no exiton interaction between two pyrene rings was traced. The excimer formation was either not detected in fluorescence spectrum in MeOH (data not shown). Therefore, it was concluded that two pyrene rings are separated by rigid β-sheet conformation. The CD and fluorescence spectra in H_2O/MeOH (95/5, v/v) showed no significant difference from the results in MeOH, suggesting that the conformation of [D-Pya$^{4,4'}$]GS was not affected much by solvent or even the enhanced hydrophobic properties did not allow the formation of aggregates in aqueous solution.

To examine the interaction mode between [D-Pya$^{4,4'}$]GS and phospholipid bilayer, the GS analog was incubated with dipalmitoyl-DL-α-phosphatidylcholine (DPPC) vesicles in 10 mM (1 M=1 mol·dm^{-3}) Hepes/100 mM NaCl, pH 7.4, and then the mixture was applied to gel filtration on a Sepharose 4B column (1.5 cm x 25 cm) with the same buffer. Only 27% of the GS analog was retained in the fractions containing DPPC vesicles. The majority was included in lower molecular weight fractions where SDS micelles were eluted. These results indicate that the amphiphilic molecules of GS analog interact with the vesicles, and subsequently some remain in vesicles, but others take out phospholipid molecules from the vesicles to make micelle-like aggregates. This progress in interaction would be closely related to the antimicrobial activity of GS and its perturbation ability of phospholipid bilayer.[10] Furthermore, the ratio III/I (0.80) of the intensity of the

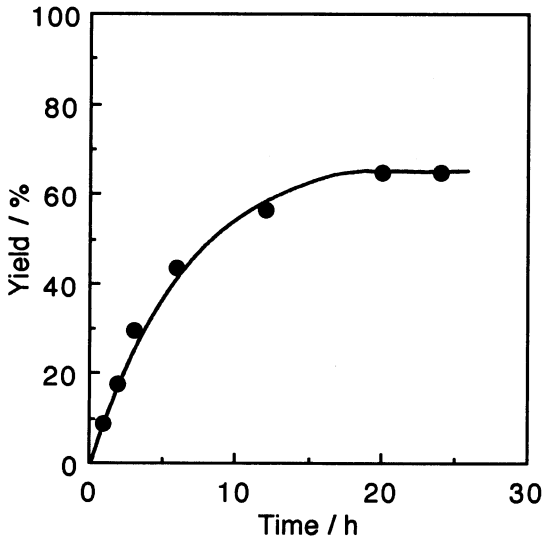

Fig. 2. Time course of the yield of protected GS analog by the cyclization cleavage. Yields are normalized with the results of HPLC.

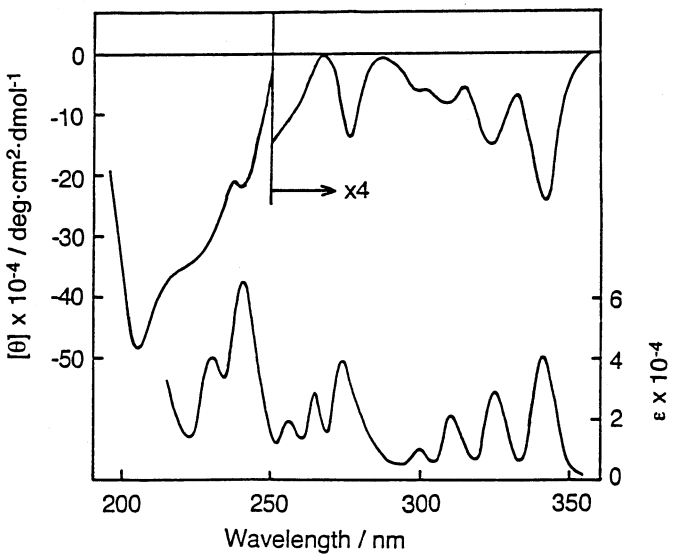

Fig. 3. CD and absorption spectra of [D-Pya$^{4,4'}$]GS in MeOH.

(0, 2) band III to that of the (0, 0) band I in the Pya-fluorescence spectrum in the presence of DPPC vesicles revealed that the peptide actually incorporated into the membrane environments.[11] The GS analog bearing pyrene rings, which is a useful fluorescent probe to detect the interaction with membrane circumstances, may be a good model to study the detailed mechanism of the antimicrobial activity.

References

1) a) W. F. DeGrado and E. T. Kaiser, *J. Org. Chem.*, **45**, 1295 (1980); b) W. F. DeGrado and E. T. Kaiser, *ibid.*, **47**, 3258 (1982); c) S. H. Nakagawa and E. T. Kaiser, *ibid.*, **48**, 678 (1983); d) S. H. Nakagawa and E. T. Kaiser, *J. Am. Chem. Soc.*, **107**, 7087 (1985); e) H. Mihara and E. T. Kaiser, *Science*, **242**, 925 (1988); f) T. Sasaki, M. A. Findeis, and E. T. Kaiser, *J. Org. Chem.*, **56**, 3159 (1991).

2) We observed that the elimination of cyclic dipeptides from the oxime resin was unexpectedly faster than reported in the literature.[1a] This study will be reported elsewhere.

3) N. Izumiya, T. Kato, H. Aoyagi, M. Waki, and M. Kondo, "Synthetic Aspects of Biologically Active Cyclic Peptides - Gramicidin S and Tyrocidins," Kodansha, Tokyo (1979).

4) S. Egusa, M. Sisido, and Y. Imanishi, *Chem. Lett.*, **1983**, 1307; H. Mihara, S. Lee, Y. Shimohigashi, H. Aoyagi, T. Kato, N. Izumiya, and T. Costa, *Int. J. Peptide Protein Res.*, **30**, 605 (1987).

5) G. Ösapay, A. Profit, and J. W. Taylor, *Tetrahedron Lett.*, **31**, 6121 (1990).

6) M. Ohno, K. Kuromizu, H. Ogawa, and N. Izumiya, *J. Am. Chem. Soc.*, **93**, 5251 (1971).

7) G. Barany and R. B. Merrifield, "The Peptides, Analysis, Synthesis, Biology," ed by E. Gross and J. Meienhofer, Academic Press, New York (1980), Vol. 2, pp. 1-284.

8) B. Castro, J.-R. Doromoy, G. Evin, and C. Selve, *Tetrahedron Lett.*, **1975**, 1219.

9) B. F. Gisin and R. B. Merrifield, *J. Am. Chem. Soc.*, **94**, 3102 (1972); G. Ösapay and J. W. Taylor, *ibid.*, **112**, 6046 (1990).

10) H. Yoshitomi, Y. Shimohigashi, S. Ono, M. Waki, and T. Inoue, "Peptide Chemistry 1988," ed by M. Ueki, Protein Research Foundation, Osaka (1989), pp. 317-320; Y. Yagi, S. Kimura, and Y. Imanishi, *Int. J. Peptide Protein Res.*, **36**, 18 (1990).

11) K. Kalyanasundaram and J. K. Thomas, *J. Am. Chem. Soc.*, **99**, 2039 (1977).

(Received September 21, 1991)