Spatially close porphyrin pair linked by the cyclic peptide Gramicidin S

Toru Arai,*[a] Naoki Maruo,[a] Yuko Sumida,[a] Chie Korosue[a] and Norikazu Nishino*[b]

[a] *Department of Applied Chemistry, Faculty of Engineering, Kyushu Institute of Technology, Tobata, Kitakyushu, Japan, 804-8550. E-mail: arai@che.kyutech.ac.jp*

[b] *Institute for Fundamental Research of Organic Chemistry, Kyushu University, Higashi, Fukuoka, Japan, 812-8581*

Received (in Cambridge, UK) 7th April 1999, Accepted 23rd June 1999

Two porphyrins were attached to the cyclic decapeptide Gramicidin S and its analogs *via* the side chain amide bonds and the solvent-dependent molecular structure was characterized by various spectroscopic methods.

Since the elucidation of a natural system with a number of tetrapyrroles,[1] such porphyrin assemblies have attracted much attention. To understand the function of the multi-porphyrin systems, many porphyrin arrays with different linker architecture have been studied as models.[2] So far, the porphyrin units have often been connected by tight linkers which determine their distance and orientation.[2b–d] However, a peptide-linked porphyrin array may be interesting because of its likeness to the natural system with a flexible peptide moiety.[3] The flexible linker may afford a porphyrin array wherein structural changes reflect its surroundings such as solvents.

Gramicidin S [*cyclo*(-Val-Orn-Leu-D-Phe-Pro-)$_2$, GS, Fig. 1] is a natural cyclic decapeptide with a pair of antiparallel β-sheets and two β-turns.[4] GS has often been modified without changing its rigid cyclic conformation in various solvents.[4b,c] The incorporation of porphyrins to the Orn residues facing each other may afford a defined porphyrin dimer. Here we report the syntheses and the spectroscopic characterisation of the porphyrin array attached on this rigid cyclic decapeptide and its analogs.

The cyclic decapeptides with side chain Z-protection (**1a**, **2a** and **3a**) were synthesized *via* the cyclisation–cleavage method on the oxime resin in 74–84% yield.[4c] After Z-deprotection with H_2–Pd/C, 5-(*p*-carboxyphenyl)-10,15,20-tritolylporphyrin (2 equiv.) was coupled with PyBOP–HOBt in 70–84% yield. The products with two porphyrins (**1b**, **2b** and **3b**) were characterized with FAB-HIMS and ^{1}H NMR (1D and 2D) spectra.

In the ^{1}H NMR spectrum of **2b** in DMSO-d_6, the α-amide NH signals appeared at δ 8.93 (D-Phe), 8.80 (Leu), 8.50 (Orn) and 7.30 (Val) at 303 K (data not shown). Both the chemical shifts and their temperature dependency (−8.3, −2.6, −4.5 and −2.0 ppb K^{-1}, respectively) were close to those of GS.[4c] These facts suggested the intramolecular hydrogen bonding between the NH and CO groups of Leu and Val residues (see Fig. 1), *i.e.* the conformational similarity of **3b** to GS. The attachment of porphyrins on the Orn positions did not change the peptide conformation. Such phenomena were also confirmed in the ^{1}H NMR study in $CDCl_3$.

The striking feature of the ^{1}H NMR spectrum of **2b** was that the tolyl groups of porphyrins appeared as two sets of doublets, δ 8.06/7.61 (4H each) and δ 7.79/7.23 (8H each) [Fig. 2(*a*)]. The methyl signals of the tolyl groups also appeared as two sets, δ 2.66 (6H) and 2.39 (12H). Compared with the monomeric porphyrin [Fig. 2(*b*)], apparently four tolyl groups among six in **2b** were high field shifted probably because of the ring current effect of the other porphyrin. The tolyl group near the other porphyrin (10-position, the inner tolyl groups in Fig. 3) may be high field shifted, but those at the 15- and 20- positions may be not. The tolyl signals at δ 7.79 and 7.23 were broad at 303 K [Fig. 2(*a*)] and were sharp at 333 K (data not shown), which implied restricted rotation of the porphyrin ring along the porphyrin–peptide axis. The signals of the 10- and 20-positions might be averaged and showed a broadened signal in the higher field. These facts suggest that the two porphyrins are spatially close. Fig. 3 shows the molecular structure of **2b** (bis-zinc complex) built by CAChe® MM2, adopting the crystal structure for the peptide moiety.[4a,5] This model also suggests that the two porphyrins are spatially close, and moreover, the left-twisted chiral orientation of the porphyrins (see below).

In CH_2Cl_2 **1b**, **2b** and **3b** (1.6 μM concentration, determined by the amino acid analysis) showed strong CD signals (data not shown), for instance **2b**, $[\theta]_{min}$ and $[\theta]_{max}$ at 424 and 416 nm ($[\theta]_M$ = −168 000 and 97 000 deg cm^2 dmol^{-1}, respectively). These CD signals were assigned to the split Cotton effects *via* the exciton coupling of the two porphyrins.[6] The (−+) sign of the couplets indicated the left-twisted orientation of the porphyrins, which was also suggested by the CAChe® calculation (Fig. 3). The intensity of the CD signals were **1b** < **2b** > **3b**. The drastic difference between **2b** (Orn) and **3b** (Lys) suggested that the flexible Lys side chains caused the loose structure of **3b**.

The Cotton effect for the porphyrins on GS was solvent-dependent (Fig. 4). The CD spectra of **2b(Zn$_2$)** in toluene (1.6 μM) showed a split Cotton effect at 435, 427 and 418 nm ($[\theta]_M$ = −951 000, 1275 000 and −234 000 deg cm^2 dmol^{-1}). The CD signals were weaker in other solvents, for instance in MeOH, $[\theta]_{430}$ = −803 000 and $[\theta]_{422}$ = 594 000 deg cm^2 dmol^{-1}. The intensity of the Cotton effect was in the order toluene > MeOH > trimethyl phosphate > TFE > CH_2Cl_2 > pyridine. These facts may indicate that the two

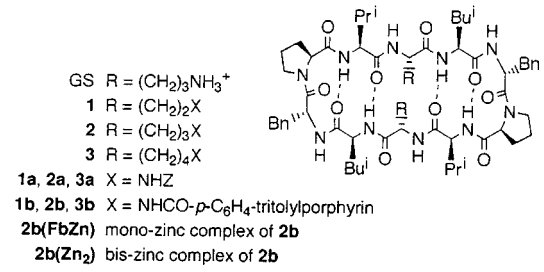

Fig. 1 Structure of Gramicidin S (GS) and its analogs.

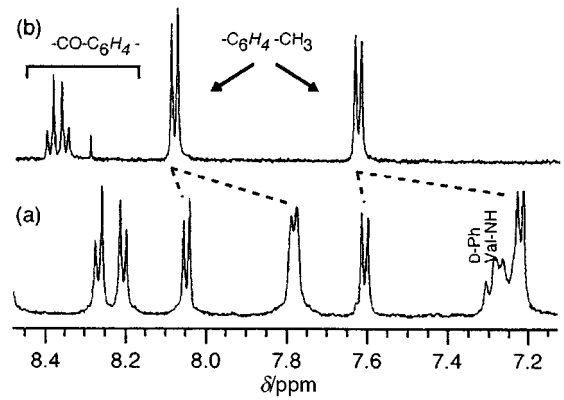

Fig. 2 ^{1}H NMR spectra of (*a*) **2b** and (*b*) 5-(*p*-methoxycarbonylphenyl)-10,15,20-tritolylporphyrin in DMSO-d_6 (303 K).

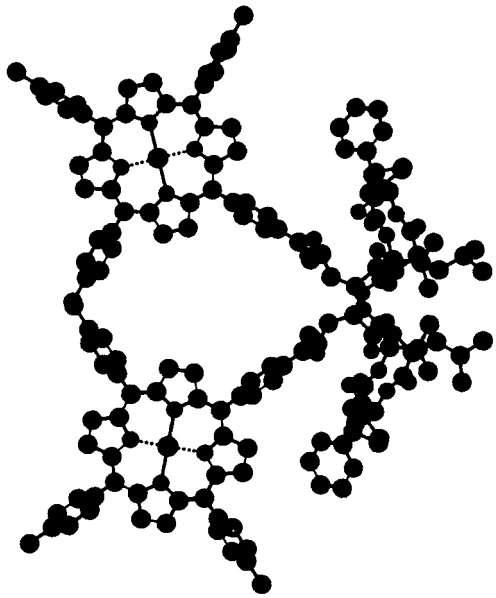

Fig. 3 CAChe® MM2 generated structure of **2b(Zn$_2$)**.

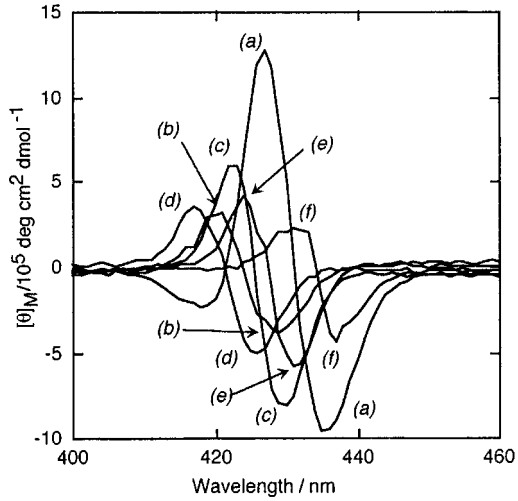

Fig. 4 CD spectra (1.6 μM for peptide) of **2b(Zn$_2$)** in (*a*) toluene, (*b*) CH_2Cl_2, (*c*) MeOH, (*d*) TFE, (*e*) trimethyl phosphate and (*f*) pyridine.

porphyrins in **2b(Zn$_2$)** were in close proximity in toluene, and the distance differed in the solvents. The different assembling ability of porphyrins in these solvents might cause different CD in the solvents, probably because of the different stabilization effect of the solvent for the π–π interaction. The details of the π–π interaction of the porphyrins are not clear yet, however, an electrostatic model may account for the tendency for porphyrin assembly in nonpolar solvents.[7] The UV spectra also supported the different orientation of the two porphyrins in these solvents. The λ_{max} of **2b(Zn$_2$)** in toluene (424 nm) was a little red-shifted from that of zinc tetratolylporphyrin (421 nm), which might be interpreted by the edge-to-edge interaction of the two π-systems.[8] However, such a shift of the λ_{max} was not observed in the other solvents investigated in the CD study, probably because of the weak interaction of the porphyrins.

Anyway, the porphyrins on GS showed different CD, *i.e.* different orientations in various solvents. This interesting fact was further confirmed by steady-state fluorescent spectrometry.[2a,3a] For this purpose, **2b(FbZn)** with one free-base porphyrin and one zinc porphyrin was synthesized. GS with different side-chain protection (Z and Fmoc) was synthesized in the same way as **2a**. After selective Z-deprotection by TFA–thioanisole–*m*-cresol (75% yield),[9] 5-(*p*-carboxyphenyl)-10,15,20-tritolylporphyrin was coupled using PyBOP–HOBt (quantitative). Then, Fmoc was removed with piperidine and zinc 5-(*p*-carboxyphenyl)-10,15,20-tritolylporphyrinate was coupled. After HPLC purification, **2b(FbZn)** was isolated in 60% yield (2 steps), and characterized with FAB-HIMS, HPLC and ^{1}H-NMR. The visible spectrum of **2b(FbZn)** (data not shown) was close to the average of **2b** (bis free-base) and **2b(Zn$_2$)**, suggesting no interaction of the two porphyrins in **2b(FbZn)** in the Q-state. However, the Soret band was somewhat broadened, suggesting a weak interaction in the B-state.[2a]

Upon exciting **2b(FbZn)** in toluene at 552 nm, the emission appeared at 608, 655 and 723 nm with an intensity ratio of 14:58:28. The ratio of ε_{552} of **2b** (bis free-base) and **2b(Zn$_2$)** was 40:60, *i.e.* 40% of the excitation light at 552 nm was absorbed by the free-base component of **2b(FbZn)** and 60% by the zinc porphyrin. From the comparison with the fluorescence spectra of **2b** (λ_{em} = 655, 719 nm, 74:26) and **2b(Zn$_2$)** (λ_{em} = 608, 652 nm, 46:54), intramolecular quenching of the excited state zinc porphyrin by the free-base porphyrin was suggested. The energy transfer from excited Zn porphyrin to free-base smoothly occurred intramolecularly, because these two porphyrins were in close proximity. The quenching efficiency was calculated to be *ca.* 82%. The same experiment performed in CH_2Cl_2 showed that 65% of the energy of the excited zinc porphyrin in **2b(FbZn)** was quenched by the free-base porphyrin. This semi-quantitative fluorescence experiment might suggest that the orientation of porphyrins differs in solvents, as suggested by CD and UV spectroscopy. However, the different energy transfer efficiencies in toluene and in CH_2Cl_2 could be due to a single solvent effect, as one of the reviewers pointed out.

We thank Professor Hideo Akisada at Kyushu Kyoritsu University for CD measurements. The molecular modeling study was partially done by Dr Masako Fujiwara at JOEL, which is gratefully acknowledged.

Notes and references

1 G. McDermott, S. M. Prince, A. A. Freer, A. M. Hawthornthwaite-Lawless, M. Z. Papiz, R. J. Cogdell and N. W. Isaacs, *Nature*, 1995, **374**, 517.

2 (*a*) R. L. Brookfield, H. Ellul, A. Harriman and G. Porter, *J. Chem. Soc., Faraday Trans. 2*, 1986, **82**, 219; (*b*) H. Kurreck and M. Huber, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 849; (*c*) A. Osuka, N. Mataga and T. Okada, *Pure Appl. Chem.*, 1997, **69**, 797; (*d*) S. I. Yang, R. K. Lammi, J. Seth, J. A. Riggs, T. Arai, D. Kim, D. F. Bocian, D. Holten and J. S. Lindsey, *J. Phys. Chem. B*, 1998, **102**, 9426 and references cited therein.

3 (*a*) H. Tamiaki, K. Nomura and K. Maruyama, *Bull. Chem. Soc. Jpn.*, 1993, **66**, 3062; (*b*) T. Arai, K. Takei, N. Nishino and T. Fujimoto, *Chem. Commun.*, 1996, 2133; (*c*) H. Y. Liu, J. W. Huang, X. Tian, X. D. Jiao, G. T. Luo and L. N. Ji, *Chem. Commun.*, 1997, 1575.

4 (*a*) G. Némethy and H. A. Sheraga, *Biochem. Biophys. Res. Commun.*, 1984, **118**, 643; (*b*) N. Nishino, T. Arai, J. Hayashida, H. I. Ogawa, H. Yamamoto and S. Yoshikawa, *Chem. Lett.*, 1994, 2435; (*c*) T. Arai, T. Imachi, T. Kato, H. I. Ogawa, T. Fujimoto and N. Nishino, *Bull. Chem. Soc. Jpn.*, 1996, **69**, 1383.

5 To construct the molecular model of **2b(Zn)$_2$**, the structure of GS [ref. 4(*a*)] was first taken into MolSkop®, then translated to the CAChe software.

6 X. Huang, B. H. Rickman, B. Borhan, N. Berova and K. Nakanishi, *J. Am. Chem. Soc.*, 1998, **120**, 6185 and references cited therein. See also ref. 4(*b*).

7 C. Hunter, *From Simplicity to Complexity in Chemistry–and Beyond*, ed. A. Müller, A. Dress and F Vögtle, Vieweg, 1996, pp. 113–126.

8 M. Kasha, H. R. Rawls and M. A. El-Bayoumi, *Pure Appl. Chem.*, 1965, **11**, 371; T. Nagata, A. Osuka and K. Maruyama, *J. Am. Chem. Soc.*, 1990, **112**, 3054.

9 H. Yajima, Y. Minamitake, S. Funakoshi, Y. Hirai and T. Nakajima, *Chem. Pharm. Bull.*, 1981, **29**, 1752.

Communication 9/02774B