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Environment-dependent conformation and antimicrobial activity of a gramicidin S analog containing leucine and lysine residues

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An analog of gramicidin S, cyclo(-L-Leu-L-Lys-L-Leu-D-Leu-L-Leu-)$_2$, in which four out of five amino acid components of gramicidin S were substituted, has been synthesized. This analog assumes a conformation similar to that of gramicidin S in acidic liposomes and a random conformation in neutral liposomes. The antimicrobial activity of this analog corresponded to one-fourth of that of gramicidin S. A possible mechanism for conformational changes in acidic liposomes is discussed.

Gramicidin S; Structure-activity relationship; Peptide synthesis; Liposome-induced peptide conformation

1. INTRODUCTION

Gramicidin S (fig.1) is a cyclic decapeptide antibiotic exhibiting high inhibitory activity against Gram-positive bacteria [1]. Its decapeptide backbone holds a rigid antiparallel β-sheet structure stabilized by four intramolecular hydrogen bonds, two of which form two type II' β-turns as shown in fig.1. Structure-activity relationship and conformational studies with various gramicidin S analogs have shown that the amphiphilic property formed by the β-sheet and β-turn structure is necessary for antimicrobial activity [2]. Higashijima et al. [3] reported that the activity of gramicidin S analogs correlated with the formation of a gramicidin S-like conformation upon binding to phospholipid membranes. Results of the studies on peptide-lipid interactions indicated that the activity of biologically active peptides correlates with the conformation of the molecules in biological membranes rather than that in aqueous solution [4]. Considering these observations, we designed and synthesized a peptide assuming a gramicidin S-like conformation in lipid bilayers and expected it to exhibit biological activity.

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Abbreviations: see IUPAC-IUB Commissions [(1984) Eur. J. Biochem. 138, 9-37]. Others: EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; HONSu, *N*-hydroxysuccinimide; TFA, trifluoroacetic acid

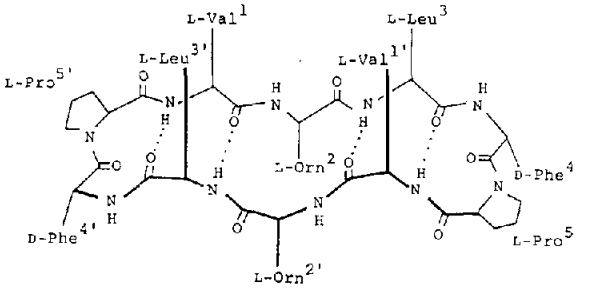

Fig.1. Structure of gramicidin S.

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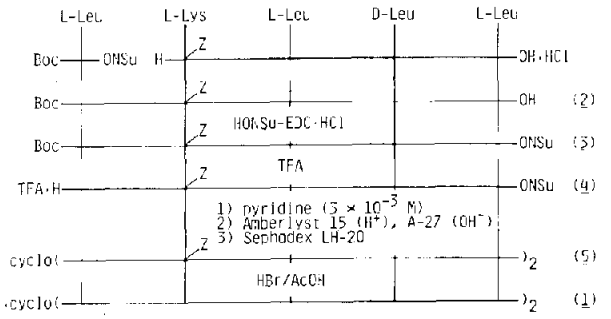

Fig.2. Synthesis of the modified gramicidin S (**1**).

We present here the synthesis and antimicrobial activity of a gramicidin S-like analog, cyclo(-L-Leu-L-Lys-L-Leu-D-Leu-L-Leu-)$_2$ (**1**) in which the neutral amino acid residues in gramicidin S, namely L-valine, L-phenylalanine and L-proline, were replaced by leucine and the basic residue L-ornithine was replaced by L-lysine. We also show that compound **1** assumes a gramicidin S-like conformation much more easily in acidic liposomes than in aqueous solution and neutral liposomes.

2. EXPERIMENTAL

Dipalmitoyl-DL-phosphatidylcholine (DPPC) and dipalmitoyl-DL-phosphatidylglycerol (DPPG) were purchased from Sigma.

Molecular mass was determined by fast atom bombardment mass spectroscopy (FAB-MS) on a JEOL JMS-DX 300 mass spectrograph with a JMA-3100 apparatus. CD spectra were recorded on a JASCO J-40A automatic recording spectropolarimeter. ^{1}H-NMR spectra in DMSO-d_6 were recorded on a JNM GX-400 spectrometer. Multilamellar vesicles were prepared as described previously [5].

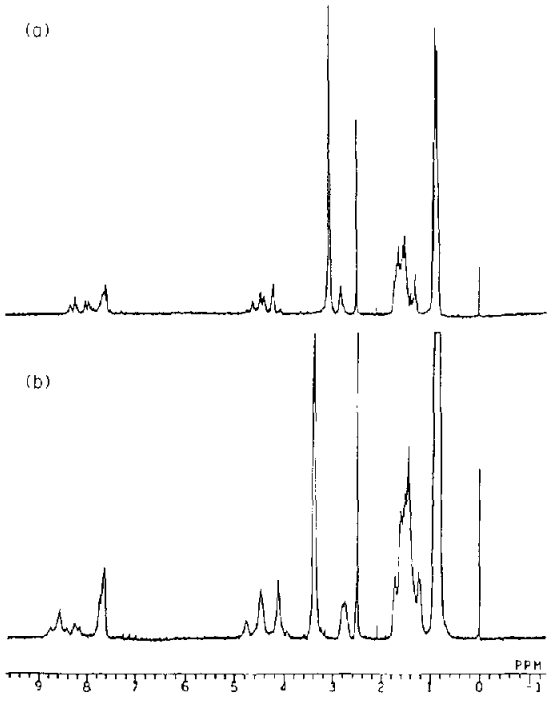

Fig.3. ^{1}H-NMR spectra of compound **1** in DMSO-d_6 at 90°C (a) and at 25°C (b).

The route for the synthesis of the modified gramicidin S analog **1** is shown in fig.2. The peptide chain was elongated stepwise by use of Boc-amino acid active ester and successive HCl-dioxane treatment until protected pentapeptide (**2**) was ob-

Table 1

Antimicrobial activity of gramicidin S and compound **1**

Organism	Minimum inhibitory concentration (μg/ml)	
	Gramicidin S	**1**
Staphylococcus aureus FDA 209P	3.13	12.5
Staphylococcus aureus 308A-1	3.13	25
Bacillus subtilis PCI 219	3.13	25
Shigella flexneri EW-10	3.13	25
Shigella sonnei EW-33	100	>100
Klebsiella pneumoniae DT	25	100
Escherichia coli NIHJ JC-2	>100	>100
Proteus vulgaris IFO 3988	>100	>100
Pseudomonas aeruginosa U31	>100	>100

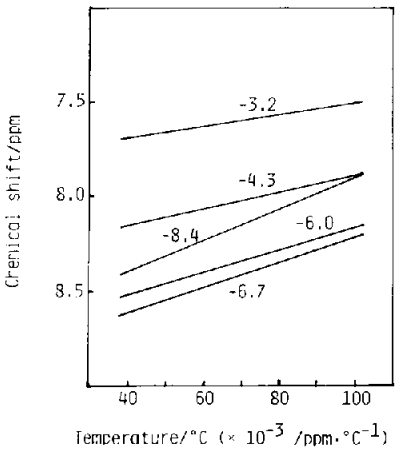

Fig.4. Temperature dependence of amide protons of the major conformer in compound **1** in DMSO-d_6.

tained. The pentapeptide **2** was converted to active ester (**3**), which was treated with TFA to obtain pentapeptide trifluoroacetate (**4**·TFA). The **4**·TFA was subjected to cyclization in pyridine at 3 mM **4**. Purification by successive column chromatography on Amberlyst 15, Amberlyst A-27 and Sephadex LH-20 yielded a protected cyclic decapeptide (**5**) (18% from **4**). Treatment of **5** with 30% HBr in acetic acid afforded the desired compound **1**·2HBr [98%, m.p. 249–252°C; M_r $(m+1)^+$1162 by FAB-MS, calcd $(m+1)^+$1161.9]. All crystalline compounds gave satisfactory elemental analyses.

3. RESULTS

Data of the antimicrobial assay are summarized in table 1. Compound **1** shows antimicrobial spectra similar to those of gramicidin S and has moderate activity, one-fourth to one-eighth of that of gramicidin S.

^{1}H-NMR spectra of **1** displayed overlapping signals in the amide and α-proton regions showing the presence of one major and probably a few minor conformers at 25°C (fig.3b). Both protons showed one set of well-resolved signals at 90°C (fig.3a). This suggests that compound **1** exists in an equilibrium of rapidly interconverting conformers at higher temperature. The temperature dependences of amide proton chemical shifts of the major conformer indicated that two NH protons with low temperature coefficients were solvent-shielded or hydrogen-bonded and three NH protons with high temperature coefficients were solvent-exposed (fig.4). The linear temperature dependences of all the NH chemical shifts indicate that the conformer

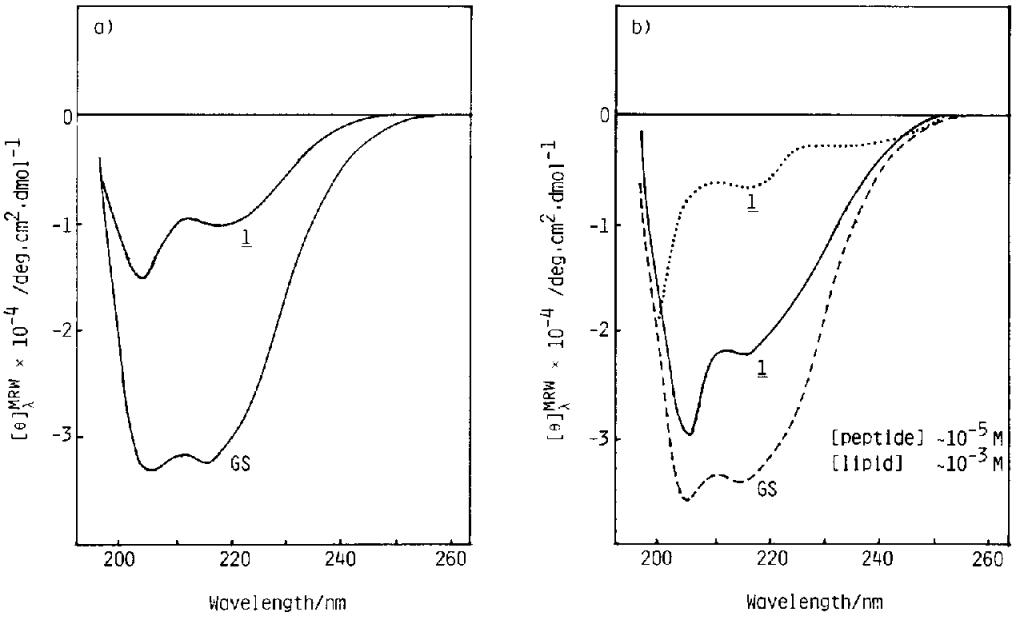

Fig.5. CD spectra of gramicidin S and compound **1** in 20 mM Tris-HCl buffer (pH 7.4) (a) and in the presence of liposomes in the same buffer: DPPC (···); DPPC-DPPG (3:1) (——) and DPPC or DPPC-DPPG (3:1) (– – –) (b).

equilibrium of **1** does not change with temperature.

Fig.5 shows CD curves of gramicidin S and **1**. characteristic feature (a double minimum of troughs at about 205 and 214 nm) for gramicidin S is essentially maintained for **1** in a buffer solution at pH 7.4, though the intensity of the troughs decreased dramatically. In liposomes containing acidic phospholipid, the intensity of the double minimum of **1** is much higher than that in the buffer solution, though still less than that of gramicidin S. Interestingly compound **1** shows a random-like CD curve in neutral liposomes. On the other hand, CD curves of gramicidin S do not show any difference in buffer, neutral and acidic liposomes.

4. DISCUSSION

^{1}H-NMR study shows that compound **1** assumes one major and a few minor conformations. The major conformation has two amide protons probably participating in hydrogen bonding as shown by temperature-dependence experiments. This suggests that **1** has four intramolecular hydrogen bonds like gramicidin S, if it assumes a C2 symmetry structure. CD spectra suggest that **1** assumes a gramicidin S-like conformation in buffer solution, though the troughs are considerably more shallow than those of GS. In the presence of DPPC-DPPG liposomes (3:1), the population of conformers having the gramicidin S-like conformation increased remarkably, indicating that the liposome induced the peptide to assume an amphiphilic structure by forming antiparallel β-sheet and type II′ β-turn structures. Higashijima et al. [3] reported that the active peptide analogs of gramicidin S which interact with phospholipids exist as a gramicidin S-like conformation in both neutral and acidic liposomes. However, compound **1** does not assume gramicidin S-like conformation in neutral liposomes but does in acidic liposomes. These results mean that acidic liposomes interact with compound **1** to increase the gramicidin S-like conformation by charge interaction, while neutral liposomes interact with the peptide **1** to rupture the gramicidin S-like conformation which is present in buffer solution albeit in a low concentration.

It is well-known that type II and type II′ β-turns formed by the L-X-D-Y and D-X-L-Y sequences (X and Y are amino acid residues), respectively, have low energies of conformations [6]. Compound **1** is able to assume both β-turns by the sequence of L-Lys-L-Leu-D-Leu-L-Leu or L-Leu-D-Leu-L-Leu-L-Leu. If compound **1** assumes a type II β-turn conformation, it is impossible for compound **1** to form an effective amphiphilic structure like gramicidin S because the basic amino acid and L-leucine residues direct to the same side of an antiparallel β-sheet. We previously reported that CD curves of type II and II′ β-turns are characteristic and have a double minimum in the 201–206 nm and 215–223 nm region and a minimum in the region of about 230 nm, respectively [7,8]. CD curves of **1** in buffer solution and neutral liposomes showed the double minimum due to the type II′ β-turn conformation. No troughs due to type II β-turn conformation were observed. The CD curve in neutral liposomes showed that compound **1** takes a random structure. A possible explanation is as follows: amphiphilic antiparallel β-structure is first induced through an electrostatic interaction with peptide and acidic liposomes, followed by formation of type II′ β-turn. On the other hand, charge interaction with cationic lysine residues in peptide and phospholipids in neutral liposomes is not as effective as in acidic liposomes. Consequently, neither the antiparallel β-sheet structure is induced at the surface of neutral lipids nor the type II′ β-turn conformation.

Membranes of microorganisms contain acidic phospholipids. It is likely that compound **1** shows activity in cell membranes by assuming a gramicidin S-like conformation.

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REFERENCES

[1] Gause, G.F. and Brazhnikova, M.G. (1944) Am. Rev. Soviet Med. 2, 134–138.
[2] Izumiya, N., Kato, T., Aoyagi, H., Waki, M. and Kondo, M. (1979) in: Synthetic Aspects of Bio-

logically Active Cyclic Peptides - Gramicidin S and Tyrocidines, Kodansha, Tokyo and Wiley, New York.
[3] Higashijima, T., Miyazawa, T., Kawai, M. and Nagai, U. (1986) Biopolymers 25, 2295–2307.
[4] Kaiser, E.T. and Kézdy, F.J. (1984) Science 223, 249–255.
[5] Lee, S., Mihara, H., Aoyagi, H., Kato, T., Izumiya, N. and Yamasaki, N. (1986) Biochim. Biophys. Acta 862, 211–219.
[6] Chandrasekaran, R., Lahshminarayanan, A.V., Pandya, U.V. and Ramachandran, G.N. (1973) Biochim. Biophys. Acta 303, 14–27.
[7] Lee, S., Mizuno, H., Nakamura, H., Kodera, Y., Kato, T., Go, N. and Izumiya, N. (1984) FEBS Lett. 174, 310–313.
[8] Bandeker, J., Evans, D.J., Krimm, S., Leach, S.J., Lee, S., McQuie, J.R., Minisian, E., Nemethy, G., Pottle, M.S., Scheraga, H.A., Stimson, E.R. and Woody, R.W. (1982) Int. J. Peptide Protein Res. 19, 187–205.