CHEMISTRY LETTERS, pp. 671-672, 1993.

Inactivation of Gramicidin S by Acylation of Two Ornithine Residues with Higher Fatty Acids

Yasuyuki SHIMOHIGASHI,* Shin ONO, Hiroshi SAKAMOTO, Haruko YOSHITOMI,
Michinori WAKI,† and Motonori OHNO
Laboratory of Biochemistry, Department of Chemistry, Faculty of Science,
Kyushu University, Fukuoka 812

Two δ-amino groups of ornithine$^{2,2'}$ of antibiotic gramicidin S (GS) were modified with a series of the acyl groups $C_nH_{2n+1}CO$ (n=0-17). None of these diacylated analogs exhibited any antimicrobial activities for fifty kinds of bacteria examined, being in contrast to the previous study in which the derivatives of GS carrying acyl groups of n=8-15 holded high activity (Uehara, *Chem. Abstr.*, **54**, 17279h (1960)). Careful comparisons of physicochemical properties between the analogs synthesized by us and by Uehara suggested that the reported high activities of diacylated GS might be attributable to contamination of unmodified GS.

Importance of ornithine (Orn) residues in gramicidin S (GS), cyclo(-Val-Orn-Leu-D-Phe-Pro-)$_2$, has been repeatedly emphasized by chemical modification experiments.[1] For instance, inactivity of diacetyl-GS (n=1) has been well established.[2] On the other hand, Uehara[3] has reported that, among a series of diacylated GS, [Orn(COC_nH_{2n+1})$^{2,2'}$]GS, the analogs with n=1–7 were inactive, whereas those with n=8–15 were highly active. The analog with n=10 was shown to be most active in the series. If GS analogs acylated with higher fatty acids were active as Uehara reported, these hydrophobic GS analogs might have a specific mechanism to elicit antimicrobial activities. There might be functional roles different from each other between longer acyl chains (n=8–15) and shorter ones (n=1-7) on the Orn residues. In order to clarify such a mechanism, we newly synthesized a series of diacylated GS with n=0–11, 13, 15, and 17 and assayed for their antimicrobial activities.

Acylation of GS (100 mg, 74 μmol) was carried out with acid chlorides in pyridine. When 2–6 equivalents of acid chlorides were used as Uehara reported,[3] the reaction was incomplete in all cases. It was found by monitoring on TLC that at least 50-fold molar excess of acid chlorides was needed for completion. After precipitation with water, the resulting solid was purified on a column (1.8 cm x 140 cm) of Sephadex LH-20 to remove the excess acid chloride: yield of purified GS analogs, 90-98%. The purity was verified by elemental analysis, high-performance TLC and HPLC. When melting points measured were compared with those reported by Uehara for each pair of analogs, large deviations were found between analogs with n=8–15. Melting points reported by Uehara were considerably lower (ΔT=35–125°C) than those by us in this study. Also, the reported values of specific rotation were much lower than ours ($\Delta[\alpha]_D$ = 125–180°) for compounds of n=12–15. These results strongly suggest that diacylated GS analogs prepared by Uehara were contaminated by

---

†Present address: Seikagaku Co., Tokyo Research Institute, Higashi Yamato, Tokyo 207.

some impurities, probably by native GS used as a starting material and/or acid chlorides. This is quite likely, since Uehara only crystallized from methanol without any chromatographic purification.

Antimicrobial activities of synthesized diacylated GS analogs were evaluated for fifty kinds of Gram-positive and negative bacteria. None of analogs exhibited activity against any bacteria. Although Uehara[3] reported that didecanoyl-GS (n=10) was as potent as native GS, our compound was completely inactive against all fifty kinds of bacteria. It is strongly suspected that the compounds prepared by Uehara contained non-acylated GS and thus the antimicrobial activities observed relied upon unmodified GS remained.

Reported high antimicrobial activities of GS analogs diacylated with higher fatty acids have long been inexplicable in the studies of structure-activity relationships of GS.[1,4] The long methylene chains on $Orn^{2,2'}$ of GS has been thought to give rise to specific interaction with bacteriobiomembranes. However, the present study showed that all the diacylated GS analogs are actually inactive as well as diacetyl-GS. The conclusion that diacylation of GS-ornithines inactivates GS completely appears to be compatible with the general understandings in the structure-activity studies on GS.

The authors are grateful to Yoshitomi Pharmaceutical Co., Ltd. for their biological assays.

References

1) N. Izumiya, T. Kato, H. Aoyagi, M. Waki, and M. Kondo, "Synthetic Aspects of Biologically Active Cyclic Peptides–Gramicidin S and Tyrosidines," Kodansha, Tokyo (1979).
2) Y. A. Ovchinnikov, V. T. Ivanov, V. F. Bystrov, A. I. Miroshnikov, E. N. Shepel, N. D. Abdullaev, E. S. Efremov, and L.B. Senyavina, *Biochem. Biophys. Res. Commun.*, **39**, 217 (1970).
3) T. Uehara, *Chem. Abstr.*, **54**, 19279h (1960), in which *Osaka-shiritsu Daigaku Igaku Zasshi*, **8**, 1499 (1959) (in Japanese) is reffered.
4) M. Waki and N. Izumiya, "Biochemistry of Peptide Antibiotics," ed by H. Kleinkauf and H. von Dohen, Walter de Gruyter & Co., Berlin (1990), p. 205.

(Received February 16, 1993)