Short Communication

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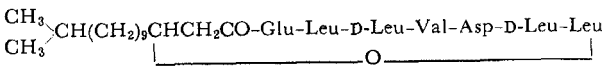
Confirmation of the Structure of Surfactin by Mass Spectrometry

Sir:

Surfactin is a crystalline peptidelipid surfactant (mp 140°C) isolated from the culture fluids of *Bacillus subtilis* as a potent clotting inhibitor in the thrombin-fibrinogen reaction.[1] In the previous reports, the main component of the surfactin-composing fatty acids was determined to be 3-hydroxy-13-methyl-tetradecanoic acid (C_{15} iso β-OH acid)[2,3] and C_{13} and C_{14} β-OH acids were also detected by gas chromatography as minor components.[3] The amino acid sequence of the heptapeptide linked to these fatty acids was determined chemically by the use of Edman's method.[4] Thus the total structure of surfactin was established as I.[3,5]



I

The present authors describe the confirmation of the structure of surfactin by mass spectrometry.

Recently mass spectrometry has found promising applications in the elucidation of the primary structure of oligopeptides. An important factor for the success of this technique is to prepare satisfactorily volatile derivatives of peptides. Lederer *et al.* reported a procedure for the permethylation of the -CONH- groups in peptides.[6] Thomas developed this technique[7] and, using the permethylation method described by Hakomori,[8] established a more rapid and complete methylation technique applicable to peptides containing aspartic or glutamic acid, which gave undesirable results under the condition of Lederer's reaction. We also have applied the permethylation method of Thomas to the alkali-treated derivative of surfactin, in which the lactone ring present in surfactin has opened, and have obtained an interpretable mass spectrum (Fig. 1).

The permethylated structure of this derivative is assumed as II.

In the spectrum of Fig. 1, the molecular ion peak (M, *m/e* 1207) is not recognized. The fragment peak (M-32, *m/e* 1175) at the high mass end is due to the loss of MeOH from the fatty acid moiety of II. The next peak at *m/e* 1144 is due to the further loss of OMe from the C-terminal amino acid, since the peak, *m/e* 986 (*m/e* 1144-MeLeuOMe), is not observed. Peaks in the lower mass region form a series of fragment peak groups. Each group shows a characteristic feature consisting of five peaks, P* (the most abundant peak,

1) K. Arima, A. Kakinuma and G. Tamura, *Biochem. Biophys. Res. Commun.*, **31**, 488 (1968).
2) A. Kakinuma, G. Tamura and K. Arima, *Experientia*, **24**, 1120 (1968).
3) A. Kakinuma, H. Sugino, M. Isono, G. Tamura and K. Arima, *Agr. Biol. Chem.*, **33**, 973 (1969).
4) A. Kakinuma, M. Hori, M. Isono, G. Tamura and K. Arima, *ibid.*, **33**, 971 (1969).
5) A. Kakinuma, M. Hori, H. Sugino, I. Yoshida, M. Isono, G. Tamura and K. Arima, *ibid.*, **31**, 1523 (1969).
6) B. C. Das, S. D. Gero and E. Lederer, *Biochem. Biophys. Res. Commun.*, **29**, 211 (1967).
7) D. W. Thomas, *ibid.*, **33**, 483 (1968).
8) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).

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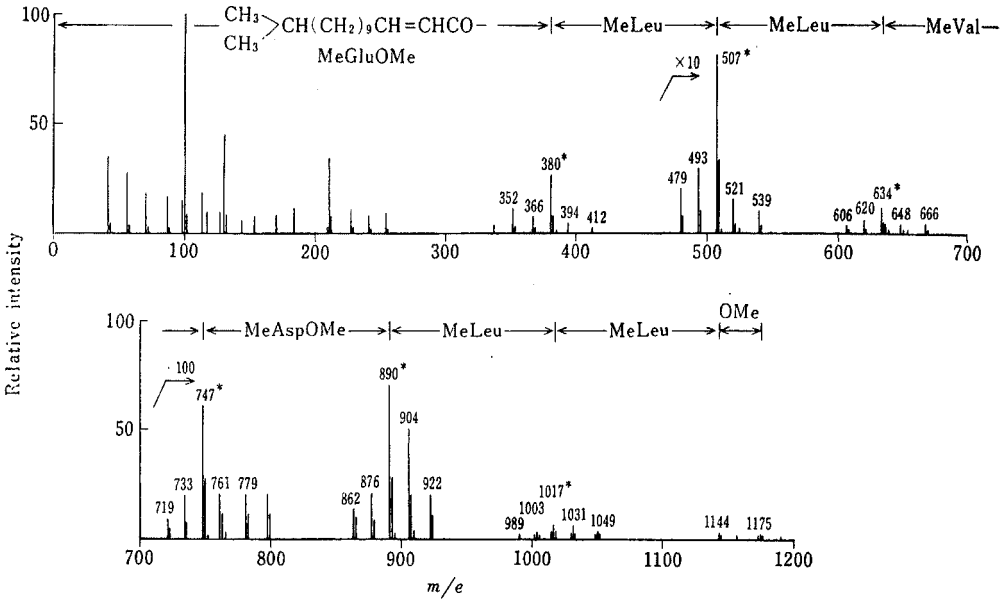
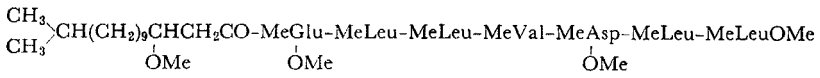


FIG. 1. Mass Spectrum of the Permethylated Derivative of Alkali-Treated Surfactin.

The spectrum was determined with a Hitachi RMS-4 mass spectrometer using a direct inlet system. The accelerating voltage was reduced so that the spectrum could be measured up to *m/e* 1250. Ionizing energy, 70 eV; ion source temperature, 200°C; evaporation temperature, 260°C. Alkali-treated surfactin was prepared as described previously.[5]



II

narked with an asterisk in Figure), P*+32, P*+14, P*−14, and P*−28. The mass difference of 127 between *m/e* 1144 and the highest P* (*m/e* 1017) corresponds to the loss of an N-methylated leucine (MeLeu) indicating that the C-terminal amino acid in surfactin is leucine. The P* peaks at *m/e* 890, 747, 634, 507 and 380 can be regarded to have derived by the further successive elimination of MeLeu, MeAspOMe, MeVal, MeLeu and MeLeu from *m/e* 1017, respectively. The remaining amino acid, Glu, is therefore directly linked to the fatty acid moiety. Thus, the amino acid sequence in surfactin determined chemically by Edman's method was confirmed mass spectrometrically. A series of peaks (P*+32) described above may be produced by the sequential cleavage of peptide bonds without the elimination of MeOH from II. P*−14 and P*−28 peaks, also accompanied by weak peaks 32 m.u. higher would mainly be due to the fragmentation of surfactin homologues having C_{14} and C_{13} fatty acids, respectively. Although peaks (P*+14) may suggest the possibility of the existence of a surfactin homologue with C_{16} fatty acid, they are interpreted as presumably produced from the dehydrated fragment of II,[9] since the peaks 32 m.u. higher are not apparently observed.

9) E. Bricas, J. van Heijenoort, M. Barber, W. A. Wolstenholme, B. C. Das and E. Lederer, *Biochemistry*, 4, 2254 (1965).

In the course of this work, Thomas and Ito[10] reported the result of a mass spectrometric reinvestigation on an antibiotic esperin (mp 238°C)[11] and proposed for it a revised structure, which is similar to that of our surfactin.

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10) D. W. Thomas and T. Ito, *Tetrahedron*, **25**, 1985 (1969).
11) T. Ito and H. Ogawa, *Bull. Agr. Chem. Soc. Japan*, **23**, 536 (1959).

Atsushi KAKINUMA
Akira OUCHIDA
Takashi SHIMA
Hiromu SUGINO
Masao ISONO
Gakuzo TAMURA*
Kei ARIMA*

Research and Development Division,
Takeda Chemical Industries, Ltd.,
Osaka, Japan
**Department of Agricultural Chemistry,*
The University of Tokyo, Tokyo, Japan

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