Short Communication

[Agr. Biol. Chem., Vol. 33, No. 10, p. 1523~1524, 1969]

Determination of the Location of Lactone Ring in Surfactin

Sir:

The structure of surfactin, a bacterial crystalline peptidelipid surfactant with a potent inhibiting activity against clot formation in the thrombin-fibrinogen reaction,[1] was elucidated in the previous communications.[2,3]

Though surfactin itself is not acetylated in pyridine-acetic anhydride, it is converted by mild alkali treatment to a structure which can be easily acetylated under the same condition. The generation of the acetylated derivative was proved both NMR spectrometrically and thin-layer chromatographically (Table I).

one carboxyl group of either of glutamic acid, aspartic acid or C-terminal leucine. The dibasic property of surfactin was also suggested by comparison of its equivalent weight, 520~540, obtained titrimetrically, with its molecular weight, 1030~1055 measured by the vapor pressure method or 1036 calculated from its structure in the presence of the lactone ring.

The present communication is concerned with the determination of the location of the lactone ring in surfactin.

In order to solve the problem which carboxyl group in the peptide binds to the hydroxyl group of the carboxylic acid, surfactin was reduced with $LiBH_4$ in tetrahydrofuran in three ways, namely, (1) without any pretreatment, (2) after methylation with diazomethane in ether, and (3) after treatment with 0.5 N NaOH for 1 hr at room temperature. The resulting product from each reaction was then completely hydrolyzed

TABLE I. GENERATION OF THE ACETYLATED DERIVATIVE FROM THE ALKALI-TREATED SURFACTIN

		Rf in thin layer chromatography (solvent : ethanol)	Signal corresponding to O-Acetyl (NMR, $(CD_3)_2SO$)
Surfactin	Not treated	0.58	—
	Treated with pyridine-acetic anhydride	0.58	—
Alkali-treated surfactin	Not treated	0.40	—
	Treated with pyridine-acetic anhydride	0.79	1.95 ppm (singlet, 3H)

Alkali-treated surfactin was prepared as follows: To 25 ml of ethanol solution containing 1 g of surfactin was added 0.5 ml of 2.5 N NaOH and the resulting crystals were collected and dissolved in water. The solution was brought to pH 3 with HCl and the generated precipitate was collected, washed with water and dried.

This seems to indicate the presence of a lactone ring in the surfactin molecule between the hydroxyl group of the carboxylic acid and

1) K. Arima, A. Kakinuma and G. Tamura, *Biochem. Biophys. Res. Commun.*, **31**, 488 (1968).
2) A. Kakinuma, M. Hori, M. Isono, G. Tamura and K. Arima, *Agr. Biol. Chem.*, **33**, 971 (1969).
3) A. Kakinuma, H. Sugino, M. Isono, G. Tamura and K. Arima, *ibid.*, **33**, 973 (1969).

with 6 N HCl or 6 N NaOH at 105°C for 24 hr and the amino acid composition was determined with an amino acid analyzer.

Results obtained from these experiments are summarized in Table II. When surfactin was reduced without any pretreatment, one of four leucine residues disappeared; when surfactin was methylated prior to reduction, glutamic acid, aspartic acid and one of four leucine residues disappeared; and when the lactone ring in surfactin was previously opened by alkali treatment, no change in amino acid composition occurred.

TABLE II. CHANGE IN AMINO ACID COMPOSITION OF SURFACTIN AFTER REDUCTION WITH OR WITHOUT PRIOR METHYLATION

	Treatment			Hydrolysis at 105°C for 24 hr with	Relative amount of amino acids in hydrolysate			
	Hydrolysis of lactone with 0.5 N NaOH at room temp. for 1 hr	Methylation with diazomethane for 30 min	Reduction with $LiBH_4$ at and for		Val	Glu	Asp	Leu
Control	−	−	−	6 N HCl	1	0.98	1.0	4.0
(1)	−	−	25°, 1 hr	6 N HCl	1	0.83	1.0	2.7
			75°, 1 hr	6 N HCl	1	0.50	0.76	2.9
			75°, 3 hr	6 N HCl	1	0.78	0.89	2.9
			75°, 3 hr	6 N NaOH	1	0.82	0.75	2.5
(2)	−	+	25°, 1 hr	6 N HCl	1	0	0	2.8
			25°, 3 hr	6 N HCl	1	0	0	2.5
			75°, 1 hr	6 N HCl	1	0	0	2.5
			75°, 1 hr	6 N NaOH	1	0	0	2.9
(3)	+	−	75°, 3 hr	6 N HCl	1	0.75	0.41	3.9
			75°, 3 hr	6 N NaOH	1	1.1	1.0	3.9

Thus the total structure of surfactin has finally been established as shown below.

The possibility exists that the structure of surfactin might be a little different from that of the active entity occurring in culture fluids of *B. subtilis*, since opening and rearrangement of the lactone ring will easily take place within a molecule in the course of purification of surfactin from culture fluids.

Ethyl 3-hydroxy-13-methyl tetradecanoate prepared from surfactin by ethanolysis was proved optically active with $[\alpha]_D^{27} -12.2°$ (c=2, chloroform), +2.7° (c=2, ethanol).

We thank Drs. S. Tatsuoka, R. Takeda and E. Ohmura for their discussions.

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Received August 14, 1969