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New Types of Liposidomycins Produced by *Streptomyces* that Inhibit Bacterial Peptidoglycan Synthesis

Structure Elucidation of Fatty Acid Components by Tandem Mass Spectrometry

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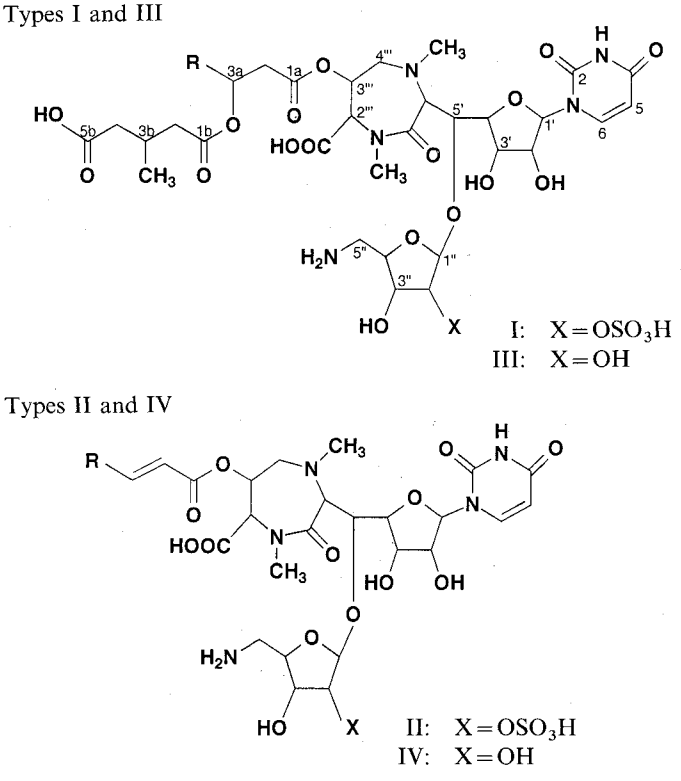
The structures of the fatty acid components of various new liposidomycins were determined using tandem mass spectrometry; the negative FAB/MS/MS/MS technique for liposidomycins with a 3-methylglutaric acid moiety and the negative FAB/MS/MS technique for liposidomycins without a 3-methylglutaric acid moiey. This structural information was obtained by analysis of peaks due to charge-remote fragmentations for 3-hydroxycarboxylate anions or carboxylate anions which were generated from liposidomycin molecules in a mass spectrometer. The MS/MS/MS technique that can exclude the matrix-derived and/or interfering ions showed higher sensitivity than the MS/MS technique.

Liposidomycins, inhibitors of bacterial peptidoglycan synthesis, are produced by *Streptomyces* sp. RK-1061 and consist of at least twelve components (A ~ L) on the basis of their HPLC profiles[1]. The main components, liposidomycins A, B and C, have already been isolated and their structures determined[2,3]. Each of them possesses 5′-substituted uridine, 5-amino-5-deoxyribose-2-sulfate, and perhydro-1, 4-diazepine moieties, and only differs in the structures of their fatty acid side chains. Recently KIMURA *et al.*[4,5] reported the isolation of twenty-four liposidomycins, including the known liposidomycins A, B, C, G and H, from the mutant strain SN-1061M, and classified them into four types, I to IV, based on the presence or absence of sulfate and/or 3-methylglutaric acid moieties, as shown in Fig. 1. Type I represnts the original liposidomycin and contains both moieties. Type II lacks the 3-methylglutaric acid moiety, while type III lacks the sulfate moiety. Type IV lacks both these moieties. A charge-remote fragmentation[6] spectra of fatty acids which are produced by high-energy collision-induced dissociation (CID) give detailed structural information on the alkyl chains. The structures of the fatty acid components in liposidomycins A, B and C have previously been determined from MS/MS anal-

Fig. 1. Structures of the four types of liposidomycins.



yses of the fatty acids obtained by chemical degradation of the liposidomycin molecule[2,3].

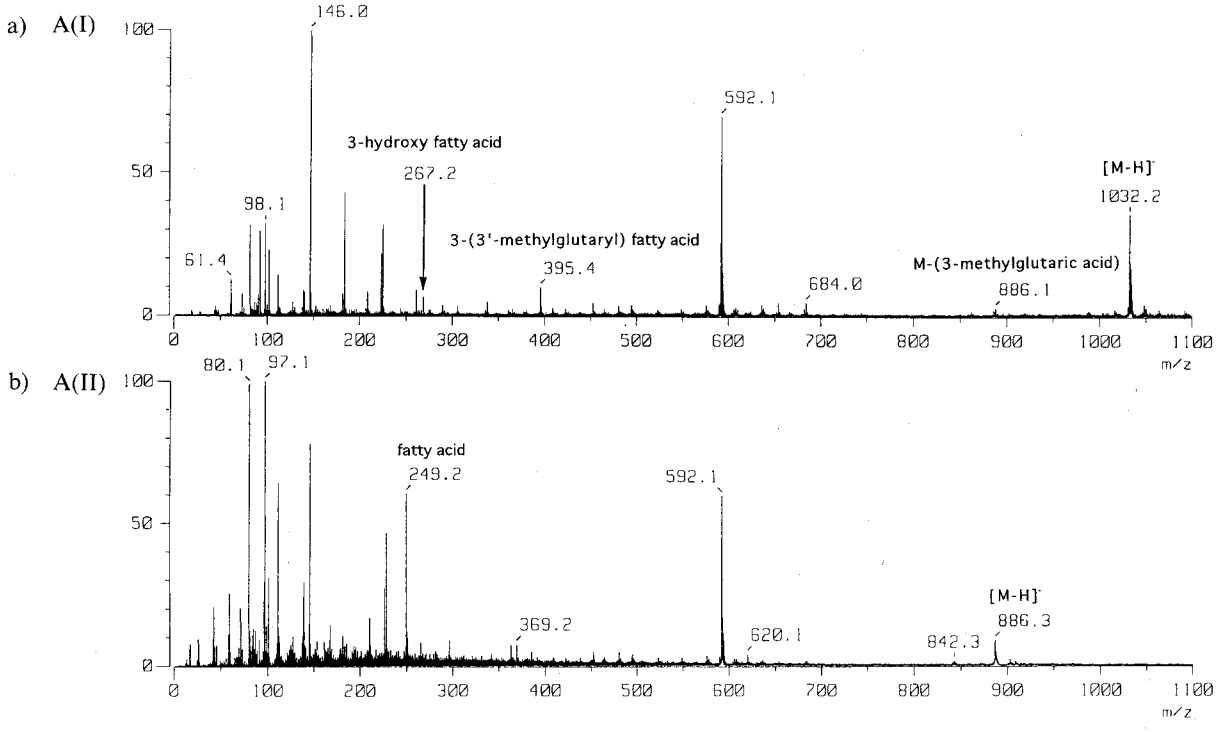
We describe here the elucidation of the structures of the fatty acid components of twenty-seven liposidomycins using MS/MS/MS or MS/MS analyses on the intact liposidomycin molecule without isolation of the fatty acid components. The MS/MS/MS and MS/MS techniques were applied for types I and III, and types II and IV, respectively.

FAB/MS

The negative FAB mass spectrum of liposidomycin A(I) is shown in Fig. 2(a). Four peaks that were assumed to include the fatty acid component were observed at *m/z* 1032 (molecular-related anion), 886 [M-(3-methylglutaric acid) anion], 395 [3-(3′-methylglutaryl)carboxylate anion], and 267 (3-hydroxycarboxylate anion). It is possible to apply the MS/MS method to the peak at *m/z* 267. But in the MS/MS method, the presence of many interfering peaks derived from the peaks overlapping with that of the precursor ion at *m/z* 267 made structural elucidation difficult. It is possible to apply the MS/MS/MS method to the three peaks at *m/z* 1032, 886 and 395. The detection sensitivity of the MS/MS/MS method depends on the magnitude of the energy of the product ion, 3-hydroxycarboxylate anion (*m/z* 267) produced from these three peaks. Therefore the most favorable peak would be the peak at *m/z* 395, which generates the product ion with the largest energy (267/395 × 10 KeV) among the three peaks. Since such 3-(3′-methylglutaryl)carboxylate anion fragments were observed in all liposidomycins belonging to types I and III, the MS/MS/MS method was applied for type I and III liposidomycins.

The negative FAB/MS spectrum of liposidomycin A(II) is shown in Fig. 2(b). Liposidomycin A(II) exhibited two peaks including that of the fatty acid component; *m/z* 886 (molecular-related anion) and 249 (carboxylate anion). When the MS/MS/MS method is applied for the molecular-related anion at *m/z* 886, the detection sensitivity is assumed to be low because of the low energy of the product ion (249/886 × 10 KeV). In addition, the intensity of the carboxylate anion at *m/z* 249 was relatively higher than that of type I and III liposidomycins.

Fig. 2. Negative FAB/MS spectra of liposidomycins A(I) and A(II).



Thus, the MS/MS method was applied for type II and IV liposidomycins.

FAB High-energy CID MS/MS/MS (Types I and III)

(1) Saturated Alkyl Chain

Figure 3 shows the negative FAB MS/MS/MS spectra of liposidomycins M(III) and L(I) with saturated alkyl chains. Spectrum (a) gave rise to a sequence of odd-mass series that was regularly spaced by 14 amu, after an initial loss of methane with the exception of the intense peaks at *m/z* 87 and 59 corresponding to a hydroxyl group on the 3a-carbon. Thus, the saturated alkyl chain of liposidomycin M(III) was shown to be linear. Spectrum (b) exhibited almost the same peak pattern as spectrum (a) except that the peak at *m/z* 241 was no present. This result showed that the saturated alkyl chain of liposidomycin L(I) is the *iso*-type. Both of these characteristic patterns were detected in other liposidomycins with saturated alkyl chains (Table 1).

(2) Unsaturated Alkyl Chain

Figure 4 shows the negative FAB MS/MS/MS spectra of liposidomycins with unsaturated alkyl chains. Spectrum (a) of G(I) with an alkenyl chain shows an obvious "window" of 54 amu (*m/z* 169/115) due to allylic cleavage *via* the 1, 4-elimination of H_2 on either side of the double bond[7~9] and radical-anion fragment (*m/z* 170) due to simple allylic cleavage[9,10]. These results indicate clearly that the double bond in the alkenyl chain of liposidomycin G(I) is located on the 7a-carbon. Spectrum (b) of A(III) with an alkadienyl chain shows two windows of 40 and 54 amu (*m/z* 209/169 and 169/115) and the corresponding two radical anion fragments (*m/z* 210 and 170). These results indicate that the two double bonds in the alkadienyl chain of liposidomycin A(III) are located on the 7a- and 10a-carbons. The locations of the double bonds for other liposidomycins with alkenyl and alkadienyl chains were determined in a similar manner (Table 1). Spectrum (c) of V(III) with an alkatrienyl chain, a fairly noisy spectrum, shows three windows of 40, 40 and 54 amu (*m/z* 249/209, 209/169 and 169/115) and the corresponding three radical anion fragments (*m/z* 250, 210 and 170). These results indicate that the three double bonds in the alkatrienyl chain of liposidomycin V(III) are located on the 7a-, 10a- and 13a-carbons.

FAB High-energy CID MS/MS (Types II and IV)

Figure 5 shows the negative FAB MS/MS spectra of liposidomycins C(II) and A(IV) with saturated alkyl and

Fig. 3. MS/MS/MS spectra of fatty acid components generated from liposidomycins M(III) and L(I) [precursor ion: 3-(3′-methylglutaryl)carboxylate anion].

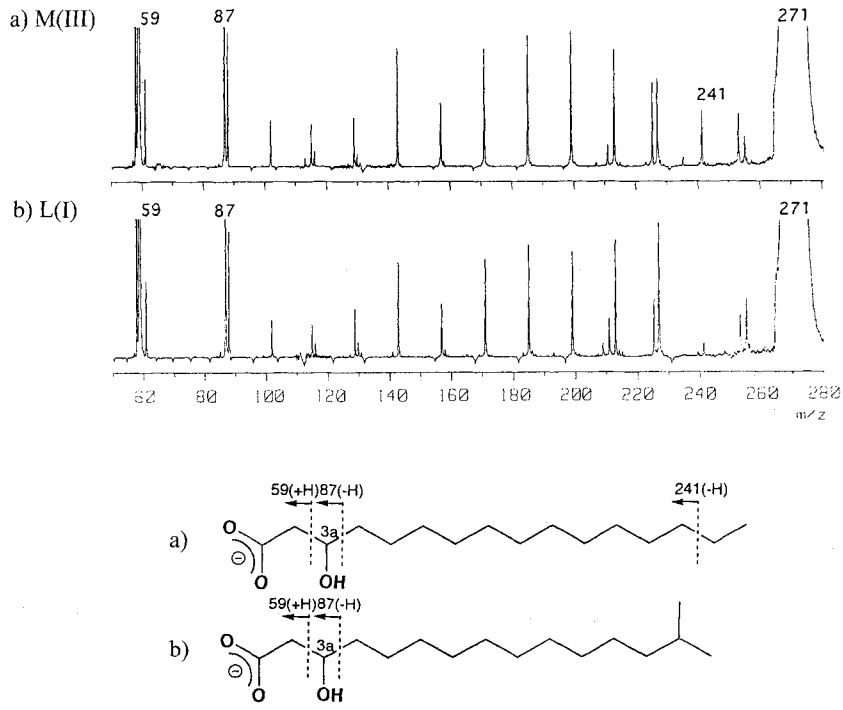
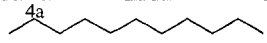
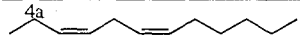
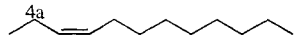
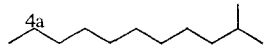
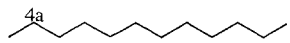
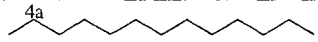
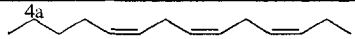
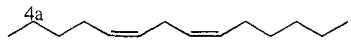
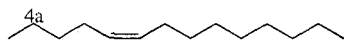
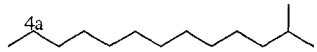
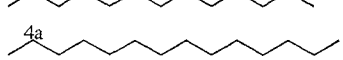
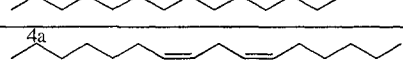
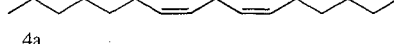
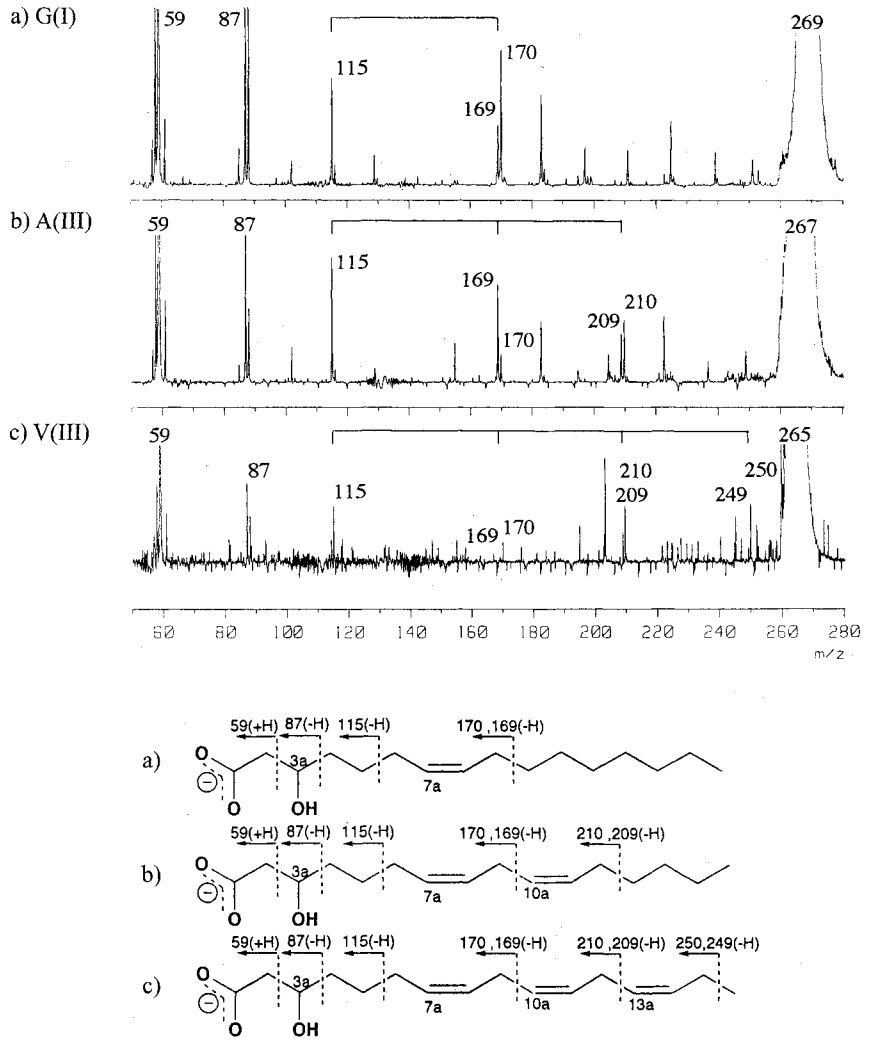


Table 1. Alkyl chain structure and its distribution in type I~IV liposidomycin.

Carbon number of fatty acid	Symbol	Structure of R	Type I	II	III	IV
13	X				#	
14	Y				#	
	Z		#		#	
	B		#*		#**	
	C		#*	#**	#	#**
15	H		#		#**	
16	V				#	
	A		#*	#	#	#
	G		#		#	
	L		#		#**	
	M		#		#	
18	K		#		#	
	N		#		#	

* Alkyl-chain structres reported[2,3].
** Detection only by FAB/MS.

Fig. 4. MS/MS/MS spectra of fatty acid components generated from liposidomycins G(I), A(III) and V(III) [precursor ion: 3-(3′-methylglutaryl)carboxylate anion].

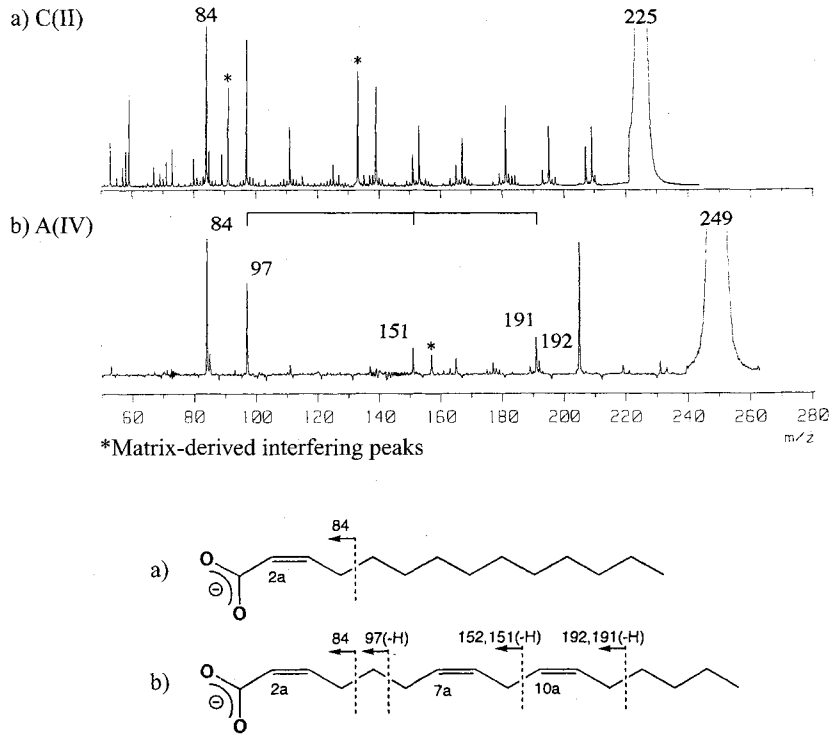


alkadienyl chains, respectively. Both spectra are complex owing to the presence of interfering peaks generated from peaks overlapping that of the precursor ion (mainly derived from the matrix). However, careful analyses of these spectra, similar to those of the MS/MS/MS spectra, except the presence of the fragment peak at *m/z* 84 due to the double bond on the 2a-carbon instead of a 3′-methylglutaryl moiety on the 3a-carbon, led to the structural determination. Thus, liposidomycin C(II) was shown to have a linear saturated alkyl chain and A(IV) was shown to have a homoconjugated (7a- and 10a-carbon) alkadienyl chain. The structures of the alkyl chains in liposidomycins A(II) and C(IV) were also determined in a similar manner (Table 1).

Table 1 lists all the alkyl chain structures of liposidomycins determined in this study. The geometry of the double bonds and the absolute configurations of the 3-methylglutaryl moiety were not determined.

The productivity of these alkyl components varied with the fermentation conditions. For example, liposidomycins C(III) and M(III) with linear-type saturated alkyl chains were produced predominantly, and liposidomycins B(III) and L(III) with *iso*-type saturated alkyl chains and H(III) with odd-numbered carbon chains were detected by FAB/MS only as minor contaminants in 30-liter jar fermentation. However in 200-liter tank fermentation, the production of B(III) and L(III) increased to the same extent as that of C(III) and M(III).

Fig. 5. MS/MS spectra of fatty acid components generated from liposidomycins C(II) and A(IV) [precursor ion: carboxylate anion].



In this report, we analyzed liposidomycins produced by the 30-liter jar fermentation.

As liposidomycins consist of many minor components, the MS/MS/MS technique for types I and III, and the MS/MS technique for types II and IV, were applied to elucidate the structures of the fatty acid components. Remarkable differences in the required sample quantity and fragmentation pattern were observed between the MS/MS/MS and MS/MS techniques. Samples of about 10 pmol for the MS/MS/MS technique and 100 pmol for the MS/MS technique were required. This can be speculated as being due to the following reason; the matrix-derived and/or overlapping ions on the precursor ion can be excluded by operation of the B/E constant-linked scan of the first mass spectrmeter in the MS/MS/MS technique, while this is not possible in the MS/MS technique[11]. The second difference is that, in the MS/MS/MS technique, the relative intensity of the radical fragments due to simple allylic cleavage increased relative to that of the corresponding rearrangement peaks *via* a 1,4-elimination of H_2. The potential of collision cell between the first and the second mass spectrometer was set at 80% of the ion accelerating voltage, so that the collision energy in the MS/MS and MS/MS/MS experiments were estimated to be 2 KeV and 1.3~1.4 KeV, respectively. That is, slight reduction of the collision energy in the MS/MS/MS technique brought a relative increase of the radical-fragment intensity [Fig. 4 and Fig. 5(b)]. This could be attributed to decrease in the rearrangement-fragment intensity, but not increase in the radical-fragment intensity. This finding suggests that the MS/MS technique under such "middle" collision energy may also bring out a relative increase in the radical fragment intensity which is especially useful for the determination of the double-bond locations in a long carbon chain. The MS/MS experiments under such "middle" collision energy are in progress.

Experimental

Fermentation and Isolation

The mutant strain SN-1061M was cultivated under fermentation conditions and liposidomycins were iso-

lated as described previously[4,5].

Mass Spectrometry

FAB/MS, FAB/MS/MS and FAB/MS/MS/MS measurements were performed using a JEOL JMS-HX/HX110A four sector (EBEB) tandem mass spectrometer equipped with an array detector. In the MS/MS/MS experiments, the precursor ion formed in the ion source dissociates in the first field-free region between the ion source and the first electrostatic analyzer to generate the product ion. Then the product ion was scanned manually with B/E constant (a manual B/E constant-linked scan unit was equipped with the first mass spectrometer) and passed into the collision cell located in the third field-free region between the first magnetic sector and the second electrostatic analyzer. The second-generation product ions formed by CID were measured by the second mass spectrometer. Fast atom bombardment (FAB) was generated using xenon as the primary beam with 6 KeV energy and the ion acceleration voltage was 10 KV. High-energy CID was performed by introducing helium as collision gas until the intensity of the precursor ion was reduced to 1/3 of the initial value. Glycerol was purchased from Tokyo Kasei (Tokyo, Japan), and used as matrix without further purification.

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