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New Family of Monoglucosylglyceride Diacyl Glycerol Lipids Containing Very Long Chain Bifunctional Acyl Chains in *Sarcina ventriculi*

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Abstract Recent research on the fatty acyl chains in the membrane lipids in *Sarcina ventriculi* has shown that unusually long chain bifunctional fatty acyl components are the major components of the total lipid. However, these studies did not yield any information on the complete structures of the lipid species containing these fatty acids. In this study, the structures of a new family of glucolipids containing bifunctional acyl chains are described. These structures were determined using NMR (Nuclear Magnetic Resonance) spectroscopy, GC (Gas Chromatography)/MS (Mass Spectrometry), FTIR (Fourier Transform Infrared) spectroscopy, and FAB (Fast Atom Bombardment) mass spectrometric studies. One of the major bifunctional acyl components of the α-glucolipids was an ω-formylmethyl ester indicating the presence of plasmalogen. The general structure of the lipid components was one in which the two head groups were separated by a membrane-spanning acyl species. One head group component is a glycerol moiety of each head group, and the other is a glyceryl glucoside. Two regular chain fatty acids, one on the glycerol moiety of each head group, are also present and meet in the middle of the membrane, roughly equidistant from each head group.

Key word: Very long chain bifunctional fatty acyl component, transmembrane lipids, *Sarcina ventriculi*

Membrane lipids are known to play various important roles in the physiological function of bacteria. These roles include the communication of external information, compartmentalization between the cell and an external medium, and a matrix for the localization and proper function of membrane proteins [5, 6, 13]. The membrane serves as a control center [14] for the regulation of the motional dynamics of the membrane proteins. From the standpoint of signal transduction [1, 2], structural studies of membrane lipids and the induction of the synthesis of unusual structures have a special importance.

Bacteria in their natural ecosystem may experience many changes in environmental factors, including temperature, pH, solvent concentration, nutrient levels, and oxygen concentration. In this respect, the membrane, which is directly exposed to the environment, is one of the critical and vulnerable components of the cell and must adapt to and survive under these changes. Recent studies on the adaptive process in *Sarcina ventriculi* show that very long chain bifunctional acyl components formed in membranes during environmental stress can function in regulating the membrane dynamics [9, 11]. External shocks on temperature, pH, or organic solvents induced these unusual very long chain bifunctional acyl components in the membrane of *S. ventriculi*. The mode of formation of these unusual lipid species in *S. ventriculi* appears to be by the tail-to-tail condensation of alkyl chains across the two leaflets of the membrane bilayer. Although the structures of the isolated fatty acyl species support this idea, no information on the structures of the intact lipid species is available. The focus of this study, therefore, was to isolate and characterize intact lipid species from the membrane of *S. ventriculi* [3, 4, 10]. Special emphasis was placed on the isolation and characterization of the membrane components which contained the unusually very long chain bifunctional fatty acyl species [12]. Information on the structures of such lipid components is critical for understanding the architecture of the membranes as well as for appreciating the possible modes of synthesis for these unusual yet physiologically important components.

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MATERIALS AND METHODS

Culture of Cells

S. ventriculi was cultured in a liquid medium at pH 3.0 as described previously [11].

Membrane Preparations

The cells were disrupted by passing through a French Pressure cell (American Instruments Co. Inc., Silver Spring, U.S.A.) at 20,000 lb/in^2. The disrupted cells were centrifuged at 20,000 ×g to remove any unbroken cells, and the supernatant was centrifuged at 110,000 ×g to sediment the membranes, which were then washed twice with distilled water by suspending and recentrifugation.

Extraction of Lipids

The lipids were extracted from the isolated membrane or whole cells using procedure (a) or (b), respectively: (a) 30 vol of chloroform/methanol (5:1, v/v) was added to each 5–10 ml membrane suspension. The suspensions were then mixed to produce a single phase. The mixtures were shaken or stirred vigorously at 45°C, with intermittent sonication (for approximately 5 min in every 30 min), over a total of 2 h and then taken to dryness on a rotary evaporator. The residues were partitioned between 10 ml chloroform/methanol (5:1, v/v) and 2.5 ml water. The lower organic phase was taken to dryness and redissolved in 1 ml chloroform/methanol (9:1, v/v). (b) Cells of *S. ventriculi* from 50 liters of the culture medium were harvested by centrifugation at 10,000 ×g for 10 min. Lipids from an approximately 50 g wet weight of cells were extracted at 45°C with 400 ml of a mixture of chloroform/methanol/water (15:3:2, by vol.) for 2 h, followed by 200 ml of chloroform/methanol (5:1, v/v). Extraction was performed with intermittent sonication over 2 h or as described in procedure (a). After centrifugation at 20,000 ×g, the pellet (cell debris) was extracted again with the same solvent system. After centrifugation, the supernatant was taken to dryness on a rotary evaporator, dissolved in 10 ml of chloroform/methanol (5:1, v/v), and then shaken with 2.5 water. The lower phase containing the lipids was taken to dryness and the residue dissolved in 1 ml of chloroform.

Analysis of Lipids

The membrane lipids were separated by two-dimensional TLC using chloroform/methanol/ammonia/water (3.3:1.0:0.1:0.05, by vol) for the first dimension and chloroform/methanol/water (7:1.6:0.2, by vol) for the second dimension. The analyses were performed on silica-gel plates (Merck). Spots were visualized either by spraying with 50% ethanolic-sulfuric acid and heating at 250°C to char the organic components, or by spraying with a 0.1% solution of 2',7,-dichlorofluorescein in aqueous ethanol (1:1, v/v) and viewing under ultraviolet light. Standard phospholipids [15] such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and cardiolipin (CL) and neutral lipids (NL) were used as standards in addition to free fatty acids (FA). The spraying agents for the detection of the components included ninhydrin for PE or PS, the Dragendorff agent for PC, orcinol for glycolipid, and molybdenum blue for phosphate. Each lipid band was scraped from the plate onto a column fitted with a sintered disc and the material was eluted from the silica gel using mixtures of methanol and chloroform. Each fraction was concentrated by evaporation and redissolved in chloroform or a chloroform/methanol (5/1,v/v) mixture for further analysis. One of the orcinol-positve lipid bands containing the bifunctional acyl chains (by GC/MS analysis) was chosen for further study.

Head Group Analysis

The isolated lipids were hydrolyzed with 2 M TFA (trifluoroacetic acid) at 120°C for 3 h. The hydrolysate was concentrated to dryness, then 1 ml of water was added and the solution was concentrated to dryness to remove any trace amounts of TFA. The hydrolysate was further analyzed by proton NMR spectroscopy or by conversion to alditol acetates for a GC/MS analysis. For the GC/MS analysis using alditol acetates, the TFA hydrolysate was extracted with 2 vol of 1 ml chloroform. The chloroform extracts were discarded and the aqueous layer was concentrated to dryness under nitrogen. The aqueous residue was dissolved in methanol and reduced with sodium borohydride for 1 h. Three molar HCl was then added to decompose the excess borohydride. The solution was repeatedly concentrated to dryness several times from methanol. One-tenth ml pyridine and 0.1 ml acetic anhydride were added to the residue. The solution was briefly sonicated and left at room temperature for 16 h. The mixture was concentrated to dryness and then 1 ml chloroform and 1 ml 3 M HCl were added to the residue. The chloroform layer was washed once with 1 ml 0.5 M NaCl, dried over anhydrous sodium sulfate, and subjected to a GC analysis on a DB225 capillary column with an initial temperature of 200°C, hold time 5.00 min, rate of 2.0 deg/min, final temperature of 230°C, final hold time of 55 min, and total run time of 75 min. The retention times were compared with alditol acetate derivatives of a number of alditol acetate standards. GC/MS analyses were then performed with a JEOL JMA DA5000 mass spectrometer using a DB225 capillary column.

Fatty Acids Analysis

The fatty acid analyses were performed on the isolated lipids by treatment with methanolic HCl using one of two procedures. Three milliliter of chloroform was added to 1 ml of the lipid suspension followed by 15 ml of a 5% methanolic-HCl solution. The flask was sealed and heated

in an oven at 72°C for 12 h. Three milliliter of chloroform was added every 6 h followed by mild sonication for 5 min. The mixture was then concentrated on a rotary evaporator to dryness and extracted with chloroform. The combined organic fraction was redissolved in 1 ml of hexane. The fatty acid methyl esters prepared by the above procedure were subjected to GC on a 25 M J&W Scientific DB1 capillary column using helium as the carrier gas and a temperature program of 150°C initial temperature, 0.00 min hold time, and 3.0 deg/min rate, to a temperature of 200°C. A second ramp of 4.0 deg/min was then immediately initiated when a final temperature of 300°C was obtained. This temperature was held for 30 min. The relative proportions of the lipid components were calculated from the integrated peak area. The fatty acid identification and molecular weight were determined by a GC/MS analysis using a JEOL JMS-AX505H spectrophotometer interfaced with a Hewlett-Packard 5890A Gas Chromatograph.

FAB (Fast Atom Bombardment) Mass Spectrometry

FAB-MS was performed using a JEOL HX100 (Peabody, MA) double-focusing mass spectrometer (EB configuration) equipped with a high-field magnet operated in the positive ion mode. The ions were produced by bombardment with a beam of Xe atoms (6 kV). The accelerating voltage was 10 kV, and the resolution was set at 1,000 or 3,000 according to the mass range of interest. The samples were dissolved in a chloroform solution. Generally, 1- 1.5 μl of the sample was mixed with 1 μl of *m*-nitrobenzyl alcohol on the FAB-MS stainless steel probe tip. The calibration was performed using Ultramark (443 or 6121) or $(CsI)_nI^-$ cluster ions, depending on the mass range of interest. A JEOL DA-5000 data system was used for recording the spectra. The spectrum was scanned over 2 min from *m/z* 0- 3,000. The data presented were obtained in a single scan and found to be reproducible.

^{1}H-NMR Spectroscopy

The proton NMR spectra were recorded at 300 MHz and 500 MHz on solutions in $CDCl_3$ or D_2O. The chemical shifts were quoted relative to the solvent resonance taken at δ 7.24 for chloroform and at δ 4.65 for water.

Fourier Transform Infrared Spectroscopy

The spectra were obtained with a Nicolet model 710 FTIR spectrometer on a 10% (w/v) solution of isolated lipids in chloroform.

RESULTS AND DISCUSSION

Structural Characterization of Isolated Glycolipids

One of the orcinol-positive glycolipid fractions on the two-dimensional TLC was isolated by preparative TLC. The

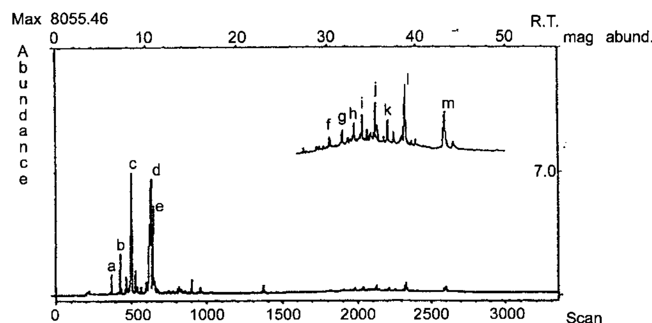

Fig. 1. Total ion chromatogram of the GC/MS analysis for the esterified fatty acyl components of one of the isolated glycolipids.
The later eluting cluster of peaks was due to the membrane spanning α,ω-bifunctional fatty acids. **a** $C_{16:0}$ fatty aldehyde ($OHC(CH_2)_{14}CH_3$), **b** $C_{14:0}$ carboxylic acid methyl ester ($OCH_3CO(CH_2)_{12}CH_3$), **c** $C_{17:0}$ carboxylic acid methyl ester ($OCH_3CO(CH_2)_{15}CH_3$), **d** $C_{18:1}$ carboxylic acid methyl ester ($OCH_3CO(CH_2)_9CH{=}CH(CH_2)_5CH_3$), **e** $C_{18:0}$ carboxylic acid methyl ester ($OCH_3CO(CH_2)_{16}CH_3$), **f** Unknown, **g** $C_{31:2}$ ω-formylmethyl ester (M.W.=490), **h** $C_{33:1}$ ω-formylmethyl ester (M.W.=520), **i** $C_{30:1}$ α,ω-dicarboxylic dimethyl ester (M.W.=508), **j** $C_{32:0}$ α,ω-dicarboxylic dimethyl ester (M.W.=538), **k** $C_{34:1}$ ω-formylmethyl ester (M.W.=534), **l** $C_{34:1}$ ω-dicarboxylic dimethyl ester (M.W.=564), **m** $C_{36:2}$ α,ω-dicarboxylic dimethyl ester (M.W.=590).

total ion chromatogram from the GC/MS analysis of the fatty acid methyl esters obtained from this fraction is shown in Fig. 1. The peaks from **a** to **e** were due to typical membrane fatty acyl components ranging from 14 to 18 carbons. The major regular fatty acid methyl esters were hexadecanoic acid methyl ester (peak **c**, $C_{16:0}$), *cis*-vaccenic acid methyl ester (peak **d**, $C_{18:1(11)}$), and stearic acid methyl ester (peak **e**, $C_{18:0}$). The peaks from **f** to **m**, however, corresponded to very long bifunctional fatty acyl components containing from 28 to 36 carbon atoms. The major bifunctional fatty acyl species were identified as α,ω-15,16-dimethyltricotanedioic acid dimethyl ester (peak **j**), α,ω-(17,18-dimethyl)-*cis*-11-dotriacotaenedioic acid dimethyl ester (peak **i**), and α,ω-(17,18-dimethyl)-*cis*-11-23-hentriacotadienedioic acid methyl ester (peak **m**).

Another unusual, very long chain component (peak **k**) was identified on an ω-formyl methyl ester. Figure 2

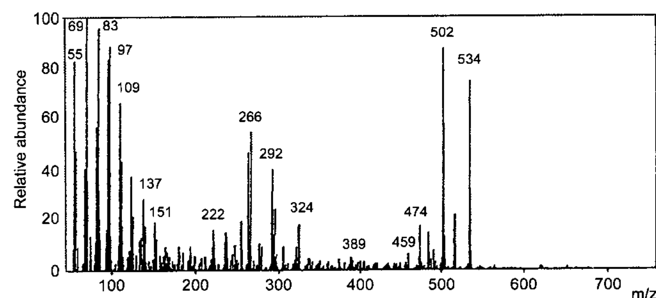

Fig. 2. Electron impact mass spectrum of the peak **k**.
It contained major ions at *m/z* 534, 502, and 474. These correspond to the molecular ion (M^+) with sequential losses of methanol (CH_3OH) and ethylene ($CH_2{=}CH_2$) or carbon dioxide, respectively.

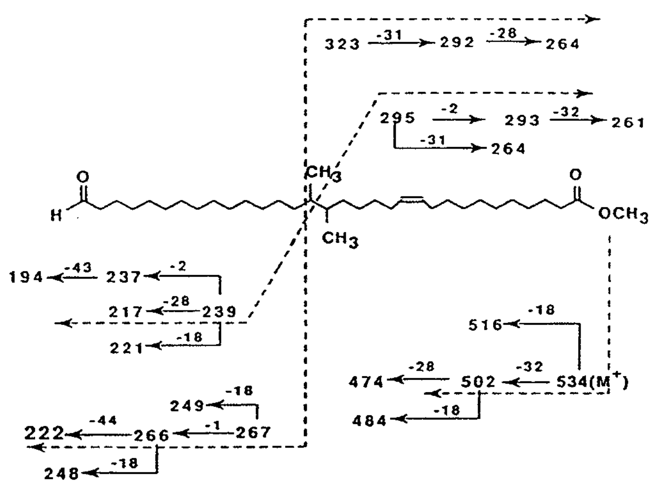

Fig. 3. Mass fragmentation pattern of peak **k** (ω-formyl-(17,18-dimethyl)-*cis*-11-hentriacotanemethyl ester).
1: hydrogen; 2: hydrogen molecule (H_2); 18: water (H_2O); 28: ethylene (CH_2=CH_2); 31: methoxy (CH_3O); 32: methanol (CH_3OH); 43: CH_2=CH-O; 44: CH_2=CH-OH.

shows the EI (electron impact) mass spectrum (70 eV) of this molecule. Major ions appeared at *m/z* 534, 502, and 474. These corresponded to a molecular ion (M^+) with sequential losses of methanol (CH_3OH) and ethylene (CH_2=CH_2) or carbon dioxide, respectively. The ions at *m/z* 516 and 484 were due to the loss of water from the ions at *m/z* 534 and 502, respectively. The primary fragmentation of the alkyl chains between the vicinal methyl groups (Fig. 3) gave rise to the fragments at *m/z* 295 and 239, the ions at *m/z* 295, and to the loss of water from the ion at *m/z* 239, respectively. The characteristic McLafferty fragment of aliphatic methyl ester appeared at *m/z* 74. The other major fragment of peak **k** was, therefore, determined to be ω-formyl-(17,18-dimethyl)-*cis*-11-hentriacotanemethyl ester (Fig. 3). The existence of ω-formylmethyl ester after methanolysis suggested the presence of vinyl ether (-CH=CH-OC-) functional groups in the parent lipid. This functionality defines the lipid class as a plasmalogen. The molar ratio of the regular length (C_{12}-C_{20}) fatty acids to the very long chain bifunctional acyl chains was approximately 2:1. The variety of fatty acid species indicates that, although a single discrete spot was obtained on the analysis of this lipid fraction, there was still considerable heterogeneity with respect to the alkyl chain length and even type. This heterogeneity is quite normal and an important aspect of membrane structure.

NMR Analysis of Glycolipids

The proton NMR spectrum of the glycolipid fraction is shown in Fig. 4. The intense resonance at δ 1.27 was assigned to the methylene group of the lipid chain. The multiplet at δ 1.58 was assigned to the methylene protons β related to the carbonyl group. The resonances at δ 2.28 (*J*=7.69 Hz) were assigned to the methylene groups α related to the carbonyl function. The characteristic peaks at δ 0.74 (*J*=7.20 Hz) confirmed the presence of the vicinal methyl groups of the ω-1-linked bifunctional acyl chains [9, 11]. The peaks at δ 5.36 (*J*=6.92 Hz) were assigned to the vinyl protons of the methylene groups α related to the vinyl carbons. The multiple peaks within the range of δ 3.00 to δ 5.00 strongly indicated the presence of glycerol and carbohydrate functions. This was confirmed by further structural studies.

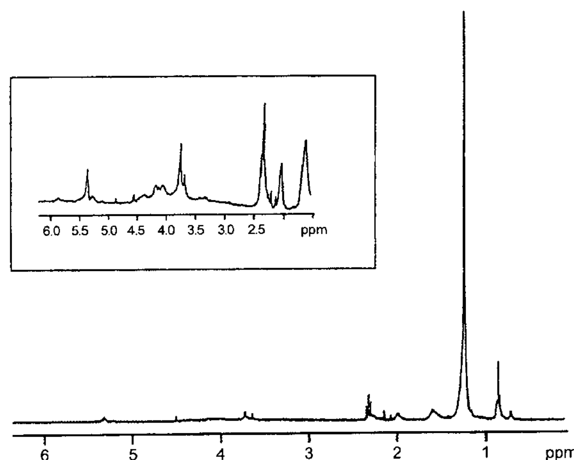

Fig. 4. ^{1}H-NMR spectrum of glycolipids containing bifunctional acyl chains.

Head Group Analyses of Glycolipids

Figure 5 shows the gas chromatographic profile of the alditol acetates obtained from the hydrolysate of the lipid fraction by treatment with 2 M TFA. There were two major components present. Peak B (the later peak) had the same retention time as standard glucitol hexaacetate. The presence

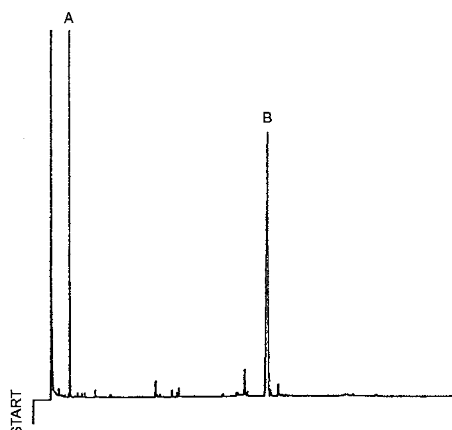

Fig. 5. Gas chromatographic profile of alditol acetates of hydrolysates obtained from 2 M TFA (Trifluoroacetic acid) hydrolysis of lipids.
A, glycerol triacetates; B, glucitol hexaacetates.

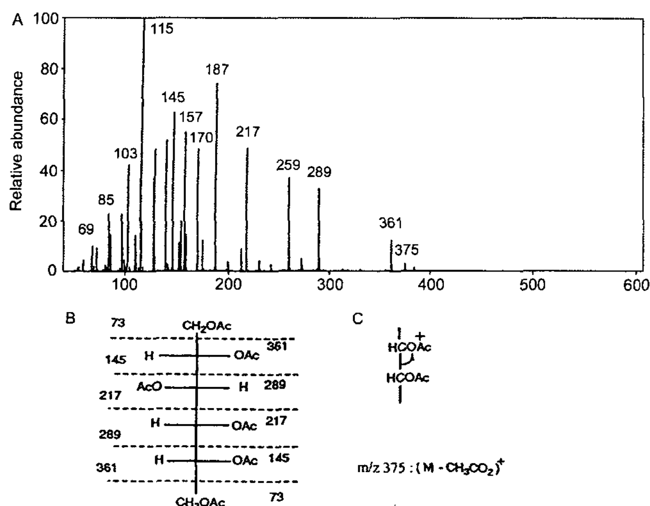

Fig. 6. Mass spectral analysis of peak B.
(A) Electron impact mass spectrum (70 eV) of peak B. (B) Ionic fragmentation pattern of the glucitol hexaacetates. Note the presence of ions at *m/z* 375 $(M\text{-}CH_3CO_2)^+$ and masses corresponding to five other primary fragments. (C) $(M\text{-}CH_3CO_2)^+$ was produced by the elimination of an acetoxyl group, or by cleavage of the alditol chain.

of this alditol acetate was confirmed by mass spectroscopy. The electron impact mass spectrum (70 eV) of peak B is shown in Fig. 6A. Typically, alditol acetate did not produce molecular ions, however, $(M\text{-}CH_3CO_2)^+$ was found in low abundance by the elimination of an acetoxyl group, or by the cleavage of the alditol chains, as shown in Fig. 6C. In this way, *m/z* 375 $(M\text{-}CH_3CO_2)^+$ and five other primary fragments were formed from glucitol hexaacetates (Fig. 6B). Figure 7 shows the EI mass spectrum of peak A. It contained the major ions at *m/z* 159, 145, and 103. They corresponded to $(M\text{-}CH_3CO_2)^+$, $(M\text{-}CH_2OCOCH_3)^+$, and the loss of ketene $(\text{-}CH_2CO)$ from *m/z* 145, respectively. The presence of these ions was indicative of the presence of glycerol triacetate. These data indicated that glucose and glycerol are the components of the head group. This fact was further confirmed by the analysis of the proton NMR spectrum of the hydrolysates obtained from TFA hydrolysis

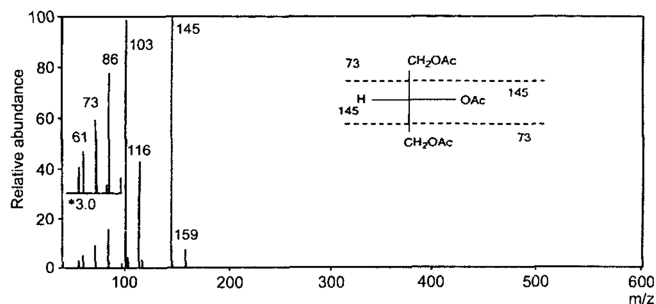

Fig. 7. EI mass spectrum of peak A.
It contained major ions at *m/z* 159, 145, and 103. These corresponded to $(M\text{-}CH_3CO_2)^+$, $(M\text{-}CH_2OCOCH_3)^+$, and the loss of ketene $(\text{-}CH_2CO)$ from *m/z* 145, respectively.

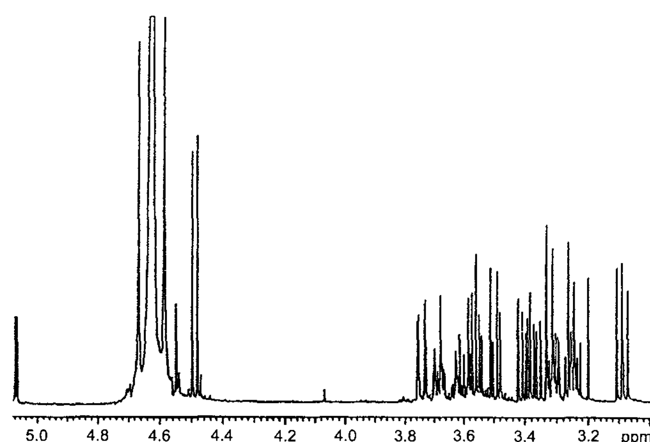

Fig. 8. ^{1}H-NMR spectrum of the hydrolysates obtained from the TFA hydrolysis of glycolipids.

(Fig. 8). The anomeric mixtures of glucose were confirmed by the characteristic H-1 protons at δ 4.45 and δ 5.08. Characteristic signals of glycerol appeared at δ 3.50 (*J*= 4.2 Hz) and δ 3.41 (*J*=4.2 Hz). These data for the glycolipids strongly indicated that they are glucosyl diglycerides that contain the bifunctional acyl chain. Further evidence on the ether linkage between glucose and glycerol was obtained by a Fourier Transform Infrared spectroscopic analysis.

FTIR Analysis of Glycolipids

The Infrared spectrum (Fig. 9) showed a strong aliphatic C-H asymmetric stretching absorption at 2,918 cm^{-1} and symmetric stretching at 2,856 cm^{-1}. Characteristic alkene stretching (=C-H) was observed at 3,038 cm^{-1}. A broad -OH stretching band (3,600-3,000 cm^{-1}) and hydroxyl stretching vibration at 3,411 cm^{-1} were also present. The C-O stretching vibration in the alcohol produced a strong band at 1,166 cm^{-1}. A band due to the bending vibration of the aliphatic C-H bonds in the methylene groups appeared at 1,465 cm^{-1} (scissoring) and one due to the twisting and

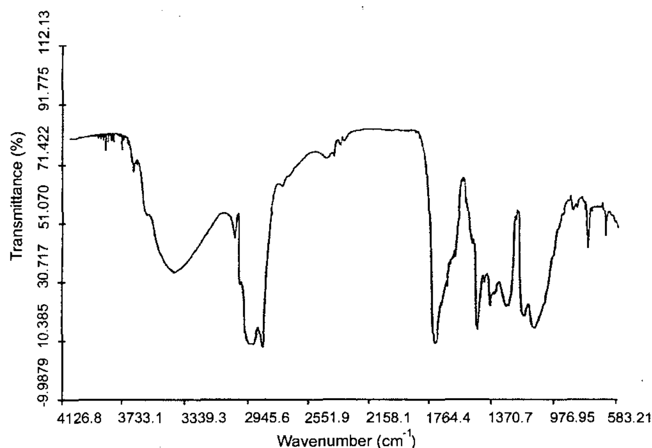

Fig. 9. Fourier Transform Infrared spectrum of glycolipids.

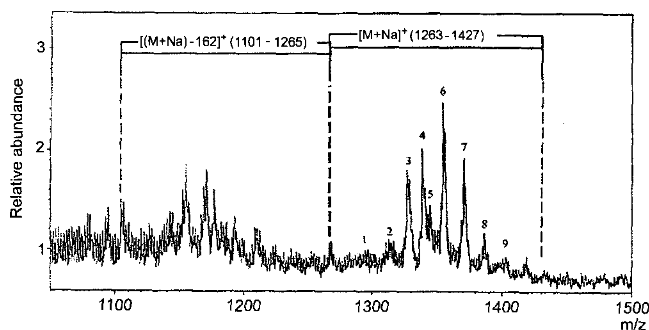

Fig. 10. Positive FAB-Mass Spectrum of two ion clusters ($[M+Na]^+$, $[M+Na-162]^+$) separated by 162 mass units.

This confirmed the presence of one glucose molecule. Each numbered ionic cluster was predicted by the calculated *m/z* values based on the compositional analyses. Table 1 shows the calculated ionic clusters from the basic frame in Fig. 11.

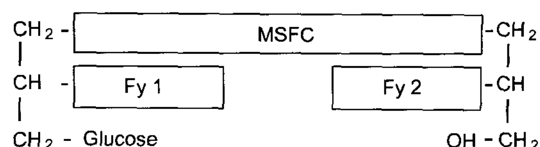

Fig. 11. Basic model structure of monoglucosyldiacylglyceride diacyl glycerol in *S. ventriculi.*

This frame was constructed based on the compositional analyses of the glucolipids. The letters point out the peaks in Fig. 1. The numbers in parenthesis indicate the molecular weights of the fatty acyl components obtained from the methanolysis of the glucolipids. MSFC, Membrane Spanning Fatty Acyl Components; Fy 1, a regular fatty acyl component; Fy 2, another regular fatty acyl component. MSFC: **j** (538) $OCO\text{-}(CH_2)_{13}\text{-}(CH_3)CH\text{-}CH(CH_3)\text{-}(CH_2)_{13}\text{-}OCO$, **l** (564) $OCO\text{-}(CH_2)_{13}\text{-}(CH_3)CH\text{-}CH(CH_3)\text{-}(CH_2)_4\text{-}CH{=}CH(CH_2)_9OCO$, **m** (590) $OCO\text{-}(CH_2)_9CH{=}CH(CH_2)_4\text{-}(CH_3)CH\text{-}CH(CH_3)\text{-}(CH_2)_4\text{-}CH{=}CH(CH_2)_9OCO$, **k** (534) $OCO\text{-}(CH_2)_9CH{=}CH(CH_2)_4\text{-}(CH_3)CH\text{-}CH(CH_3)\text{-}(CH_2)_{12}CH_2CHO$ FY 1; FY 2, **b** (242) $OCO\text{-}(CH_2)_{12}\text{-}CH_3$, **c** (270) $OCO\text{-}(CH_2)_{14}\text{-}CH_3$, **d** (296) $OCO\text{-}(CH_2)_9CH{=}CH(CH_2)_5\text{-}CH_3$, **e** (298) $OCO\text{-}(CH_2)_{16}\text{-}CH_3$.

wagging deformation at 1,340 cm^{-1}. The characteristic C=O absorption band of the aliphatic ester group appeared at 1,733 cm^{-1}, and another ester alkoxy stretch at 1,263 cm^{-1}. The stereochemistry (*cis* or *trans*) of the unsaturated carbons was determined by the clear presence of a strong peak at 660 cm^{-1} due to the C=C-H bending deformation of the *cis*-alkenes [14]. The characteristic response of the ethers in the IR was associated with the stretching vibrations of the C-O-C system. A strong asymmetric C-O-C stretching band appeared at 1,099 cm^{-1}. The C=C stretching band of the vinyl ethers appeared at 1,623 cm^{-1} and 1,636 cm^{-1} as a doublet because of the presence of rotational isomers.

FAB (Fast Atom Bombardment) Mass Spectrometry Analysis of Glycolipids

The positive FAB mass spectra of the glucolipids were obtained to determine the range of the molecular weights of the lipids and the number of attached glucose groups (Fig. 10). Due to the variety of fatty acyl components a

Table 1. Calculated *m/z* values of ionic clusters based on compositional analyses.

MSFC (Fy1, Fy2)	$[(M+Na)-162]^+$				$[M+Na]^+$			
	J(538)	l(564)	m(590)	k(534)	*J*(538)	l(564)	m(590)	k(534)
(b, b) (242, 242)	(*J*, b, b) (1101)	(l, b, b) (1127)	(m, b, b) (1153)	(k, b, b) (1111)	(*J*, b, b) (1263)	(l, b, b) (1279)	[*2](m, b, b) (1315)	(k, b, b) (1273)
(b, c) (242, 270)	(*J*, b, c) (1129)	(l, b, c) (1155)	(m, b, c) (1181)	(k, b, c) (1139)	[*1](*J*, b, c) (1291)	[*2](l, b, c) (1317)	[*4](m, b, c) (1343)	[*1](k, b, c) (1301)
(b, d) (242, 296)	(*J*, b, d) (1155)	(l, b, d) (1181)	(m, b, d) (1207)	(k, b, d) (1165)	[*2](*J*, b, d) (1317)	[*4](l, b, d) (1343)	[*7](m, b, d) (1369)	[*3](k, b, d) (1327)
(b, e) (242, 298)	(*J*, b, e) (1157)	(l, b, e) (1183)	(m, b, e) (1209)	(k, b, e) (1167)	(*J*, b, e) (1319)	[*5](l, b, e) (1345)	[*7](m, b, e) (1371)	[*3](k, b, e) (1329)
(c, c) (270, 270)	(*J*, c, c) (1157)	(l, c, c) (1183)	(m, c, c) (1209)	(k, c, c) (1176)	(*J*, c, c) (1319)	[*5](l, c, c) (1345)	[*7](m, c, c) (1371)	[*3](k, c, c) (1329)
(c, d) (270, 296)	(*J*, c, d) (1183)	(l, c, d) (1209)	(m, c, d) (1235)	(k, c, d) (1193)	[*5](*J*, c, d) (1345)	[*7](l, c, d) (1371)	[*9](m, c, d) (1397)	[*6](k, c, d) (1355)
(c, e) (270, 298)	(*J*, c, e) (1185)	(l, c, e) (1211)	(m, c, e) (1237)	(k, c, e) (1195)	[*5](*J*, c, e) (1347)	[*7](l, c, e) (1373)	[*9](m, c, e) (1399)	[*6](k, c, e) (1357)
(d, d) (296, 296)	(*J*, d, d) (1209)	(l, d, d) (1235)	(m, d, d) (1261)	(k, d, d) (1219)	[*7](*J*, d, d) (1371)	[*9](l, d, d) (1397)	(m, d, d) (1423)	[*8](k, d, d) (1381)
(d, e) (296, 298)	(*J*, d, e) (1211)	(l, d, e) (1237)	(m, d, e) (1263)	(k, d, e) (1221)	[*7](*J*, d, e) (1373)	[*9](l, d, e) (1399)	(m, d, e) (1425)	[*8](k, d, e) (1383)
(e, e) (298, 298)	(*J*, e, e) (1213)	(l, e, e) (1239)	(m, e, e) (1265)	(k, e, e) (1223)	[*7](*J*, e, e) (1375)	[*9](l, e, e) (1401)	(m, e, e) (1427)	[*8](k, e, e) (1385)

MSFC, Membrane spanning Fatty Acyl Components; Fy 1, Fatty acyl component 1; Fy 2, Fatty acyl component 2. Small alphabets (**a**, **b**, ..., **l**, **m**) indicate the peak number in each column and point out of the peak number (1, 2, ..., 9) of the ion fragments observed in FAB-MS. Almost all the observed ionic clusters were predicted with the proposed lipid structures based on the structural model shown in Fig. 11.

cluster of molecular ions ($[M+Na]^+$ or $[M+H]^+$) was observed. The spectra contained two clusters separated by 162 amu. The lower mass cluster was derived from the higher mass cluster at the high mass end of the spectrum. The numbers of the ionic clusters numbered in Fig. 10 could be predicted from the general structure proposed in Fig. 11. This structural formula was based on the compositional data of the lipids, in which the fatty acyl chains and head group components were separately quantitated and analyzed by various spectroscopic methods. There was an approximately 2:1 molar ratio between the regular length acyl chain and membrane spanning the bifunctional acyl chains. Based on the fatty acyl components present, the distribution and molecular weights of the peaks in the clusters were predicted using an unbiased, statistically weighted combination algorithm. Table 1 shows the calculated *m/z* values of the two ionic clusters.

Possible Role of Very Long Chain Bifunctional Fatty Acyl Components

Figure 12 shows a snapshot from a molecular dynamics simulation of the cluster of a phospholipid bilayer in which a very long chain bifunctional fatty acyl component was inserted. By connecting the two leaflets of the lipid bilayer, the very long chain bifunctional fatty acyl component may restrict the motions of neighboring fatty acids, and thus raise the molecular order of the bilayer membrane. A recent theoretical study suggested that the very long chain bifunctional fatty acyl component decreased the diffusion of fatty acids, increased long-axis order parameters, and slowed down the reorientational motions of methylene groups [7]. Accordingly, these results suggest that the increase of membrane rigidity may be caused by the presence of the very long chain bifunctional fatty acyl components. The incorporation of the very long chain bifunctional fatty acyl components in the phospholipid membrane may help the microorganism to survive in severe environmental conditions such as high temperature. It would appear that the very long chain bifunctional fatty acyl components present in *Sarcina ventriculi* contribute to maintenance of appropriate fluidity and integrity of the cellular membrane under severe environmental conditions. Further research is needed in this regard.

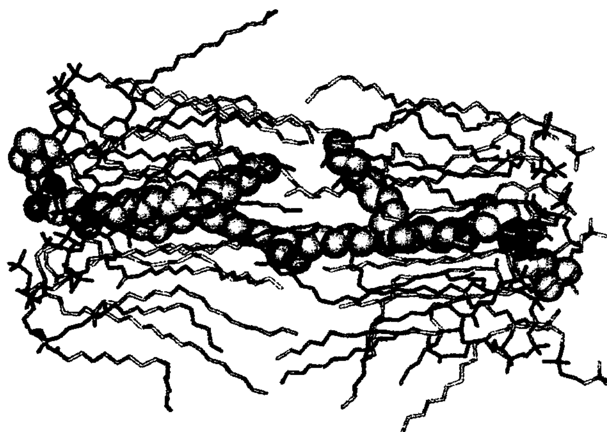

Fig. 12. Molecular graphics illustration of a lipid bilayer membrane with a very long chain bifunctional fatty acyl component.
This snapshot was taken at the end of a 10 picosecond molecular dynamics (MD) simulation. The MD simulation and molecular graphics representation were performed using the InsightII/Discover program (Version 97.0, Molecular Simulations Inc., San Diego, U.S.A.). The molecular model of phospholipid bilayer was made of twenty 1-stearoyl-2-palitoleoyl-3-phosphatidylcholine (PC) and one very long chain bifunctional fatty acyl component, which was constructed by the tail-to-tail coupling of the two stearoyl components of a normal PC at the C17 position. The normal fatty acids were positioned to surround the very long chain bifunctional fatty acyl component with hexagonal packing. The overall molecular model was energy minimized for 100 iterations by a steepest descent algorithm and then another 100 iterations by a conjugate gradient algorithm. The MD simulation was performed for 10 picoseconds at 300 K with a consistent valence force field (CVFF) with 1 femtosecond time step and velocity Verlet integrator. The nonbonded interactions were calculated using the fast multipole method and temperature was controlled by direct velocity scaling. The very long chain bifunctional transmembrane fatty acid was represented with a space-filling model and the other normal fatty acids were represented with a stick model. The hydrogen atoms are not displayed for viewing clarity.

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