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A novel aminoglycosphingolipid found in *Chlorobium limicola* f. *thiosulfatophilum* 6230

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Abstract. An aminolipid from *Chlorobium limicola* f. *thiosulfatophilum* has been purified and characterized by thin-layer chromatography, infrared specroscopy, ^{1}H-NMR, ^{13}C-NMR, plasma desorption mass spectrometry, and fast atom bombardment mass spectrometry. The structure is that of an aminosugar (neuraminic acid) attached to a sphingosine backbone with one myristic acid linked to the sphingosine by an amide bond. Related glycosphingolipids and capnoids are found in the Bacterioides/Flavobacteria which are related to the green sulfur bacteria by the criterion of 16S rRNA structure. No aminoglycosphingolipid was found in *Chloroflexus aurantiacus*.

Key words: Aminoglycosphingolipid – Chlorobiaceae – *Chlorobium limicola* f. *thiosulfatophilum* – *Chloroflexus aurantiacus* – Green sulfur bacteria – Myristic acid – Neuraminic acid – Sphingosine

In green sulfur bacteria (Chlorobiaceae) about half the inner surface of the plasma membrane is covered with chlorosomes which are separated from the membrane by a monolayer of water-soluble bacteriochlorophyll (BChl) *a*-protein trimers (Staehelin et al. 1980; Olson 1980). Reaction centers in the membrane are presumably concentrated under the chlorosomes and the BChl *a*-protein layer. The proteins are thought to be bound to the membrane by hydrogen bonds, because they can be removed from the membrane by guanidine hydrochloride (Olson and Thornber 1979) or arginine hydrochloride (Whitten et al. 1979) but not by the use of high salt or nonionic detergent. However the linking groups on the membrane have not been identified.

In 1974 Kenyon and Gray reported one or more unidentified aminolipids in five strains of Chlorobiaceae. In 1982 E. Knudsen et al. found an amino(glyco)lipid in *Chlorobium limicola* f. *thiosulfatophilum*, and subsequently Olson et al. (1983; 1984) showed that this aminolipid was localized in the plasma membrane and was a secondary or tertiary amide containing one mole of myristic acid. The aminolipid contained no phosphorus or amino acids, but was erroneously thought to be fluorescent. It was suggested that the aminolipid might be required for the unique structure of the photosynthetic apparatus in green sulfur bacteria, since no aminolipid had ever been found in green filamentous bacteria (Chloroflexaceae) Kenyon and Gray 1974; Knudsen et al. 1982).

In this paper we show that the aminolipid from *C. limicola* f. *thiosulfatophilum* (Chlorobium aminolipid) is an aminoglycosphingolipid which does not fluorescence in the pure state. We further suggest that this novel lipid may bind the water-soluble BChl *a*-protein to the inner surface of the plasma membrane.

Materials and methods

Organism and culture conditions. Chlorobium limicola f. *thiosulfatophilum* 6230 was grown in the following medium modified from Pfennig and Trüper (1981). Stock solution A supplied the following ingredients for 1 l of complete medium: Dist. H_2O (900 ml), trace element solution E_7 (Olson et al. 1973) (1 ml), KH_2PO_4 (1 g), NH_4 acetate (0.7 g), $MnSO_4 \cdot 7\ H_2O$ (0.4 g), $CaCl_2 \cdot 2\ H_2O$ (50 mg), Versen-ol Fe solution (0.5 ml) and 2 M H_2SO_4 (1.5 ml). Stock solution B supplied most of the remaining ingredients: Dist. H_2O (100 ml), $NaHCO_3$ (2 g), $Na_2S_2O_3 \cdot 5\ H_2O$ (1.6 g), and $Na_2S \cdot 9\ H_2O$ (0.6 g). Stock solution C (1.5 ml) contained only vitamin B_{12} (20 mg/l) and water. Versen-ol Fe solution (1 liter) consisted of distilled water (500 ml), versen-ol (N-hydroxyethyl ethylene diamine triacetic acid, trisodium, 59 g), $FeSO_4 \cdot 7\ H_2O$ (24.9 g), and enough distilled water to make a final volume of 1 liter. The solution was aerated overnight at pH 9.9

The stock solutions were autoclaved separately, cooled and mixed. To the final medium 3 ml sterile 2 M sulfuric acid was added. The medium was inoculated with bacteria which then remained in the dark overnight. The next day the culture was illuminated in dim

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light from a single 40 or 60 W bulb at a distance of ca. 15–30 cm, and the light intensity was increased as the density of the culture increased. One liter cultures were used to inoculate 23-liter carboys.

Lyophilized cells of *Chloroflexus aurantiacus* J-10-fl were a gift from Prof. R. C. Fuller (University of Massachusetts, Amherst, USA). Frozen cells of the same strain were a gift from Dr. Karin Schmidt (Göttingen).

Purification of the aminolipid. Lipids were extracted by a modification of the Bligh-Dyer procedure (Olson et al. 1983). Acetone precipitation (Kates 1972) of the crude lipid extract was carried out three times to remove the major part of the pigment and some of the MGDG. In the last precipitation some of the phospholipids like DPG and PE were also removed. The precipitate containing the aminolipid was applied to an HPLC column (6 × 250 mm) of Merck Lichrosorp 60 (5-μm particle size). The lipid was eluted isocratically with hexane/isopropanol/water (60/80/12, v/v) at a flow rate of 1.5 ml/min. The fraction containing the aminolipid eluted between 10 and 20 min. The pooled fractions containing the aminolipid were then further purified on the same HPLC column eluted with a gradient of water in hexane/isopropanol (60/80) running from 60/80/5 (solvent B) to 60/80/16 (solvent A). The gradient was as follows: from $t = 0-40$ min the water content increased linearly from 5–16 (v/v); from $t = 40-55$ min the water content decreased linearly from 16–5, and from $t = 55-70$ min the water content remained constant at 5. In this system the aminolipid eluted at about 29 min. When the fractions containing the aminolipid were pooled, the aminolipid precipitated. Contaminating PE, PG and pigments could be removed by washing with the following solvents: methanol, chloroform/methanol/water (65/25/4, v/v) (solvent 3), hexane/isopropanol/water (60/80/11, v/v) (solvent 5), and chloroform/methanol (1/1, v/v). This washing procedure removed all the contaminants and left the aminolipid 99% pure. The yield of aminolipid from 350 g wet cells was ca. 34 mg.

Thin layer chromatography. Plates coated with 0.5 mm kieselgel 60 HR (reinst) from Merck, and the following solvent systems (Olson et al. 1983) were used: Chloroform/methanol/ammonia 25% (13/5/1, v/v) (solvent 1), chloroform/methanol/acetone/acetic acid 100% (10/2/4/1, v/v) (solvent 2), solvent 3, chloroform/methanol/acetic acid/water (6/8/2/2/1, v/v) (solvent 4), and solvent 5.

The lipids were visualized by the following methods: Iodine stain for double bounds, ninhydrin stain for lipids containing amino groups, and molybdate stain for phospholipids (Kates 1972). Glycolipids were visualized with α-naphthol (Kates 1972; Siakotos and Rouser 1965) and orcinol (Christie 1982). Schiff reagent was used for vicinal functional groups like hydroxyl groups (Kates 1972; Christie 1982; Shaw 1968; Skipski and Barclay 1969). For organic carbon charring was used (Christie 1982).

Hydrolysis of the aminolipid for NMR studies. The aminolipid (16 mg) was hydrolyzed in deuterated chloroform/methanol (1/1) (v/v) containing 0.12 M DCl at 80° C for 4 h or 65° C for 16 h. The hydrolysis was followed by TLC in solvent 3, and the products were purified by preparative TLC in the same solvent system.

Analysis of the benzylated sugar derivatives. Dry methanol containing 1 M HCl and 1 M methylacetate was added to the dried aminolipid, and the mixture was heated to 80° C for 4 h or 65° C for 16 h in a closed reaction vessel. The hydrolysis leads to a methylation of the anomeric hydroxyl groups and in the case of sialic acid a methyl esterification of the carboxylic acid group. The method can in the case of sialic acid also lead to a partial hydrolysis of the amide-bound acetyl group. For that reason a reacetylation step was introduced into the method. In order to get rid of an excess of acetyl groups, which combine with the hydroxyl groups upon acetylation, a mild hydrolysis was carried out in the original mixture diluted 10 times. To obtain a derivative which can be detected at 230 nm, the unmethylated hydroxyl groups were benzylated. After benzylation the byproducts were removed by a C-18 SEP-Pack column (H. Rahbek-Nielsen, unpublished). The sugar derivatives were then ready for identification by HPLC (Jentoft 1985).

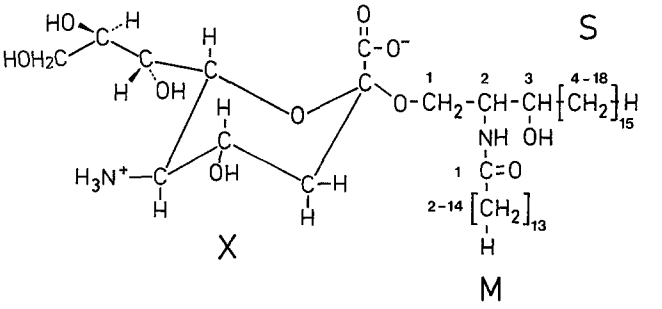


Fig. 1. Proposed structure of the aminoglycosphingolipid

Infrared (IR) spectroscopy. Spectra were recorded on a Perkin-Elmer 1720 FT-IR spectrometer at a resolution of 4 cm^{-1} from 451 to 4000 cm^{-1}. Each sample (1 mg) was mixed with 350 mg KBr and compressed to a 1-mm thick pellet before the spectrum was recorded.

Nuclear magnetic resonance spectroscopy. NMR spectra were recorded with two Bruker instruments, a 250-MHz spectrometer at Odense University and a 500-MHz spectrometer at the Carlsberg Laboratory. One-dimensional ^{1}H-NMR spectra were recorded of the aminolipid dissolved in dimethylsulfoxide (DMSO) at 500 MHz and in deuterated chloroform/methanol (1/1) (v/v) containing 0.12 M DCl at 250 MHz. A two-dimensional COSY spectrum at 250 MHz was also recorded in the latter solvent. A ^{13}C-NMR spectrum of the aminolipid in DMSO was recorded on the 500-MHz instrument. One- and two-dimensional spectra at 250 MHz were recorded of the TLC-purified sugar dissolved in deuterated water. ^{1}H-NMR spectra at 250 MHz were also recorded for the purified sphingosines A, B and C in chloroform/methanol, and for the fatty acid in chloroform. All solvents were deuterated.

Mass spectrometry. Plasma desorption mass spectroscopy (PDMS) (Sundquist et al. 1984) of the purified benzylated sugar derivatives were carried out on a Bio-Ion Bin 10K instrument. Fast atom bombardment mass spectroscopy (FABMS) (Barber et al. 1981) of the intact aminolipid and the benzylated carbohydrate part was carried out on a Kratos MS50TC mass spectrometer in the positive ion mode. Masses were determined by peak matching; the limit of error was ± 0.002.

Results and discussion

Extracts of *C. limicola* and *Cf. aurantiacus* were analyzed by TLC. No lipid corresponding to Chlorobium aminolipid could be found in the extracts of *Cf. aurantiacus* (cf. Kenyon and Gray 1974; Knudsen et al. 1982). The structure of Chlorobium aminolipid as an aminoglycosphingolipid (Fig. 1) was deduced from the following evidence.

The aminolipid reacted positively with the following TLC stains: Iodine, ninhydrin, α-naphthol, Schiff reagent and orcinol. The lipid was molybdate negative. These reactions showed that the aminolipid contains no phosphate but does contain a carbohydrate residue and at least one amino group.

Hydrolysis of 16 mg aminolipid in the NMR-solvent gave 5 components. When the hydrolysate was run on TLC plates with solvent 3, the 5 components were revealed by various staining solutions as shown in Table 1. On the same plates the intact aminolipid stained olive-green with α-naphthol. These results indicated that the aminolipid contained more than one anino group.

Table 1. Staining reactions of the hydrolysis products of the aminolipid after separation on kieselgel 60 TLC plates developed in solvent 3

Component	Iodine	Ninhydrin	α-Naphthol	Identification
(1)	(+)	+	Purple	Carbohydrate
(2)	+	+	Yellow	"Sphingosine A"
(3)	(+)	+	Yellow	"Sphingosine B"
(4)	(+)	+	Yellow	"Sphingosine C"
(5)	−	−	Yellow	Fatty acid

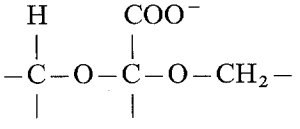
(+) = weakly iodine positive

Table 2. Infrared spectrum of the intact lipid

ν cm^{-1}	Assignment
3851	Noise
3402	N-H and O-H stretch
2922, 2853, 2029	Aliphatic C-H stretch
1610	C-O stretch, amide 1 band
1524	Monosubstituted amide or secondary amine
1468	CH_2 scissoring
1398	
1294	
1207	O-H bend
1068, 1034	C-O and C-N stretch in amines dependent on hydrogen bonds
888	
836	
722	CH_2 rock, straight chained alkanes with 7 or more carbon atoms
672	

Infrared spectroscopy. The IR-spectrum of the aminolipid (Table 2) is identical to the IR-spectrum of the α-form of the aminolipid published by Olson et al. (1983). This spectrum shows that the lipid contains methylene groups (presumably in long chains), amide bonds, and some hydroxy groups. The C-O stretch lines in the IR spectrum confirm the existence of the carbohydrate part of the aminolipid. Knudsen et al. (1982) and Bias (1985) have also detected the glycolipid structure in *Chlorobium*.

The IR-spectrum also showed that the lipid does not contain double bonds, ketones, protonized carboxylic acids, aldehydes or esters. Amines and ethers cannot be excluded however. Carboxylic acids in the carboxylate form and/or with a hydrogen bond to the carbonyl oxygen atom also cannot be excluded (Silverstein et al. 1981). Figure 1 contains an ether-like configuration



but strictly speaking, there are no true ether bonds in the proposed structure.

Proton NMR. The proton-NMR spectrum from 1.9 to 4.2 PPM of the aminolipid dissolved in deuterated chloroform/methanol 1/1 made 0.12 M with DCl is shown in Fig. 2; the entire spectrum is summarized in Table 3. The spectrum in DMSO (data not shown) shows 6 additional lines not present in the spectrum of aminolipid in deuterated chloroform/methanol. These additional lines show the presence of at least 3 hydroxy protons (4.7−4.5 PPM), a proton from a secondary amide (7.55 PPM) and two broad peaks (7.6 and 7.1 PPM) from the 3 protons in a protonized primary amine (Silverstein et al. 1981).

Only about 10% of the lipid molecules contain a double bound. The integral of the double-bound protons at 5.35 PPM is 0.19, while the integral of a single proton in the carbohydrate part (the quartet at 2.65 PPM) is 1.485. Further discussion will focus on the major aminolipid species which does not contain a double bond. The triplet in the ^{1}H-NMR spectrum at 2.35 PPM originates from the methylene group vicinal to the carbonyl group in the fatty acid; the area of this triplet is 2.853. The triplet at 0.88 PPM from methyl groups has an area of 9.07. The number of protons in the methyl groups peak is then 9.07/(2.853/2) = 6.4. The number of aliphatic chains in the molecule must therefore be two, one of which belongs to the fatty acid and the other to sphingosine. The area of the triplet at 2.35 PPM also confirmed the presence of only one fatty acid per mole of aminolipid, as shown by gas chromatography.

^{13}C-NMR. The ^{13}C-NMR spectrum of the entire aminolipid is summarized in Table 4. The assignments for the lines 42.5059 and 41.5620 PPM can be interchanged, because it is not possible to assign lines unambigously when they are so close together. The same is true for the lines at 171.5 ± 0.1 and 171.9592 PPM. From the ^{13}C-NMR spectrum it is concluded that the aminolipid contains a carbohydrate part (possibly a deoxysugar). Other carbon atoms are bonded to hydroxygroups; two carbon atoms are double-bonded to oxygen; and three carbon atoms are single-bonded to nitrogen atoms.

Comparison of the ^{13}C-NMR spectrum of the aminolipid in DMSO (Table 4) with data from a known glycosphingolipid (Dabrowski et al. 1980) indicates good agreement between the two spectra for those lines arising from the alphatic parts of the two molecules.

Fatty acid. Gas chromatography of the methyl esters and the free fatty acids gave myristic acid as the main component as found by Olson et al. (1983); the presence of other fatty acids like C-15 and C-16 could not be absolutely excluded, but the peaks were small and they also were present in the control. By comparison with the internal standard it was calculated that only one mole of myristic acid is present in each mole of the aminolipid. The myristic acid must be bound to the rest of the molecule by an amide bond, because the IR-spectrum (Table 2) indicates the existence of amide bonds but no ester bonds (Olson et al. 1983).

Carbohydrate. HPLC of the benzylated carbohydrate showed two peaks, a main peak and a later eluting small

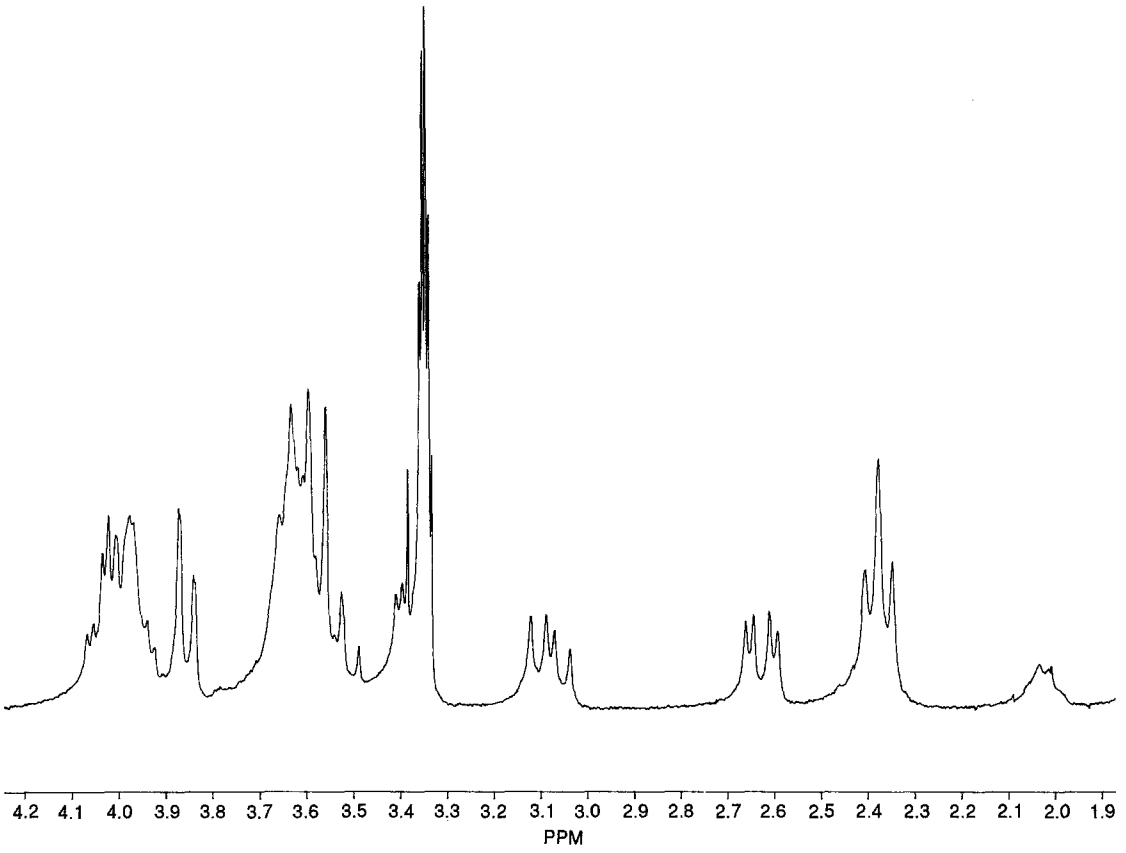


Fig. 2. Partial ^{1}H-NMR spectrum recorded at 250 MHz of intact aminolipid in chloroform/methanol and hydrochloric acid. Table 3 shows the complete spectrum

Table 3. Proton NMR spectrum of the intact aminolipid dissolved in deuterated chloroform/methanol (1:1) and 0.12 M DCL

PPM	Area	Assignment
0.88	9.070	Triplet, methyl groups
1.28	95.333	Peak complex, methylene groups
2.0	0.726	Imputity
2.35	2.853	Triplet, methylene group vicinal to carbonyl
2.6	1.485	Quartet, methine proton
3.1	1.643	Quartet, methine proton
3.3–4.1	18.595	Peak complex, carbohydrate skeleton protons
5.35	0.190	Triplet, double bond
5.7	23.030	Singlet, HOD in acid solvent
7.7		Singlet, chloroform solvent

peak with same shape as the main peak, indicating an α-β anomerization of the sugar during the hydrolysis. FABMS of the benzylated carbohydrate collected from HPLC gave a molecular weight of 753.24 which indicates an odd number of nitrogen atoms (Brittain et al. 1970). As with the intact aminolipid several peaks from glycerol appeared in the spectrum. The peak at 599.9 can be assigned to a fragment. PDMS gave a molecular ion at 754.7 which corresponds to a mass of 753.7 for the uncharged molecule. A custer ion at 1505.9 corresponds to a mass of 1504.9 which is very close to double that of the uncharged molecule ($2 \times 753.7 = 1507.4$). A fragment of 601.1 corresponds to the peak at 599.9 in the FABMS spectrum. PDMS of the later eluting small peak from HPLC of the benzylated carbohydrate, gave essentially the same resuls, confirming the α-β anomerization during hydrolysis.

The PDMS spectra of the main HPLC peak of the benzylated carbohydrate and benzylated sialic acid (data not shown) (Rahbek-Nielsen, unpublished) showed that the two derivatives are identical.

The similarity of the carbohydrate part of the aminolipid and sialic acid was further confirmed by comparison with lines in the ^{13}C-NMR spectrum with the literature (Vliegenthart et al. 1982). The ^{1}H-NMR spectrum of the aminolipid shows the absence of methyl protons associated with the acetyl group in sialic acid (which should give a strong singlet with area 3 at 2 PPM). The carbohydrate in the aminolipid is therefore not sialic acid. But removal of the N-acetyl group leaving an amino group would give a structure with almost the same ^{13}C-NMR spectrum; furthermore the compound would give a positive reaction with ninhydrin on TLC. The derivatizing of this carbohydrate for HPLC would give exactly the same product as sialic acid. The carbohydrate residue of the molecule is therefore neuraminic acid.

The deoxysugar structure is further supported by the two dimensional ^{1}H-NMR spectrum of the partially purified sugar (data not shown). The triplet at 1.65 PPM couples with the quarted at 2.25 PPM, and the two protons are bound to the same carbon atom. The two deoxy

Table 4. Comparison of ^{13}C-NMR spectra (PPM) for the entire aminolipid and a known glycosphingolipid (Dabrowski et al. 1980). M = myristic acid; S = sphingosine base; X = carbohydrate. See Fig. 1 for number assignments

Aminolipid	Glycosphingolipid	Assignment
0.1677	–	TMS, internal standard
5.9452	–	Impurity
13.9978	13.67	Methyl groups 14M, 18S
22.1547	21.93	13M, 17S
25.3416	25.26	3M
28.6671	28.88	4M, 7S
29.3957	28.88	11M, 15S
31.3632	31.19	12M, 6S, 16S
35.2732	35.67	2M
40	–	DMSO, solvent
41.5620	–	Deoxycarbon in X
42.5059	–	Impurity
51	–	C-N in X
54	53.40	2S
64	–	C-OH in X
68.8814	68.86	1S
69.5839	–	X
70.3586	–	X
70.9836	70.88	3S
73.0962	–	X
101.5	–	Anomeric C in X
129.5	131.19	5S
129.5	131.3	4S
171.5	–	Carboxylic acid
171.9592	172.02	1M

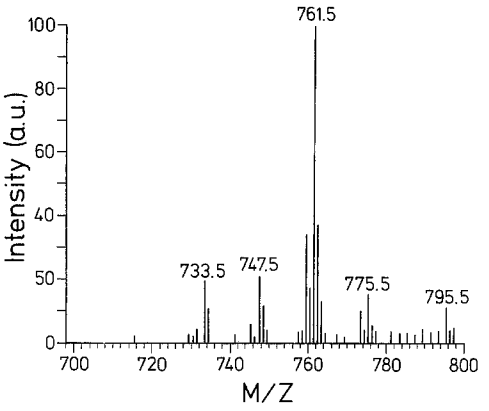


Fig. 3. FABMS spectrum of intact aminolipid. The peak at 761.5 belongs to MH^+

Table 5. Elemental compositions of the aminolipid consistent with the MH^+ mass of 761.58

C	O	N	H	Deviation from the measured mass	
				Millimass units	Parts per million
48	5	2	77	−3.21	− 4.2
43	7	4	77	0.81	1.1
41	10	2	81	−9.08	−11.9

carbons further couple to a single proton at the vicinal carbon. This evidence further confirms the structure of the carbohydrate part. This structure is also supported by Vliegenthart et al. (1982), where the quartet is assigned to the equatorial proton and the triplet is assigned to the axial proton. As neuraminic acid is lacking an anomeric proton, the structure is further supported by the absence of signals from an anomeric proton in the ^{1}H-NMR spectrum of the intact aminolipid (Table 3). The anomeric configuration of the carbohydrate part in the intact aminolipid, can be determined from the ^{1}H-NMR spectrum (Vliegenthart et al. 1982). In the α-anomer the equatorial proton will be between 2.6 and 2.8 PPM and H4 between 3.6 and 3.8 PPM. In the β-form the corresponding values will be 2.1 and 2.5 PPM for the equatorial proton and 3.9 to 4.2 PPM for H4. In Fig. 2 the equatorial quartet in the intact aminolipid is found at 2.65 PPM; the neuraminic acid in the intact aminolipid is therefore in the α-form.

Intact aminolipid. The molecular weight of the aminolipid was determined by FABMS to be 760.58. The even molecular weight indicates an even number of nitrogen atoms (Brittain et al. 1970). The mass spectrum in Fig. 3 shows a cluster of peaks around the molecular ion, with a spacing of 14 mass units; this indicates microheterogeneity in the aliphatic part of the aminolipid (sphingosine or fatty acid). Because of the presence of the prominent glycerol peaks (data not shown) the fragmentation pattern is not clear. The molecular weight makes it possible to calculate possible elemental compositions of the molecule. If elemental compositions with odd numbers of nitrogen atoms are excluded, 17 compositions are possible. Because more than 4 nitrogen atoms and more than 10 oxygen atoms are unlikely from the C-13 NMR spectrum, there are then only 3 possible elemental compositions: $C_{48}O_5N_2H_{77}$, $C_{41}O_{10}N_2H_{81}$ and $C_{43}O_7N_4H_{77}$ (Table 5). The composition of the aminoglycosphingolipid structure in Fig. 1 is $C_{41}O_{10}N_2H_{80}$ in good agreement with $MH^+ = C_{41}O_{10}N_2H_{81} = 761.58$.

Function. Since the aminolipid is found in the plasma membrane and not in the chlorosomes (Olson et al. 1984), a possible function might be to serve as a membrane anchor (Low and Slatid 1988; Ferguson and Williams 1988) for the hydrophilic BChl α-protein found in Chlorobiaceae but not in Chloroflexaceae. In Chlorobiaceae this protein forms a two dimensional crystal between the plasma membrane and the chlorosome (Olson 1980) and is thought to be bound to the membrane by hydrogen bonds, as it can be removed from the membrane by guanidine hydrochloride or argininehydrochloride but not by the use of high salt or nonionic detergent (Olson 1980; Olson and Thornber 1977; Whitten et al. 1979). The binding of galactosylsphingosine by albumin (Igisu et al. 1990) might be a model for the binding of the aminolipid by BChl *a*-protein. Another function of the aminolipid could be to substitute for phospholipids during phosphate limitation (Minnikin et al. 1973), thereby enabling the organism to grow at lower phosphate levels. The two functions do not necessarily exclude each other.

Table 6. Distribution of sphingolipids and capnoids in bacteria

Genus	Type of sphingolipid or capnoid	Reference
Bacteroides	Phosphosphingolipids	Riazza et al. 1970
Bdellovibrio	Phosphonosphingolipids	Steiner et al. 1973
Capnocytophaga	Capnoids	Godchaux III and Leadbetter 1980
Chlorobium	Aminoglycosphingolipid	this study
Cytophaga	Capnoids	Godchaux III and Leadbetter 1983
Flavobacterium	Glucuronic acid sphingolipid	Yamamoto et al. 1978
	Capnoids	Godchaux III and Leadbetter 1983
Flexibacter	Capnoids	Godchaux III and Leadbetter 1983
Mycoplasma	Sphingomyelin	Tourtellotte et al. 1963
Sporocytophaga	Capnoids	Godchaux III and Leadbetter 1983

Sphingolipids and the related capnoids (containing a "backbone" of 2-amino-3-hydroxy-15-methylhexadecan-1-sulfonic acid with the amino group amide-bonded to a fatty acid) are found in only a few types of bacteria (see Table 6). The Flavobacteria contain both types of lipids, while the Bacteroides family contain only sphingolipids. By the criterion of 16S and 23S rRNA structure Chlorobiaceae are related to the Flavobacteria/Bacteroides group, but Chloroflexaceae are not (Woese 1987; Woese et al. 1990). The presence of an aminoglycosphingolipid in *C. limicola* and the absence of any aminoglycosphingolipid in *Cf. aurantiacus* (Kenyon and Gray 1974; Knudsen et al. 1982, this work) will be in harmony with the phylogenetic distance between these two green bacteria on the basis of rRNA structure as long as no sphingolipids are discovered in *Cf. aurantiacus*.

If the aminoglycosphingolipid turns out to be universally present in Chlorobiaceae and universally absent in Chloroflexaceae, newly discovered green bacteria can be classified on the basis of a lipid extraction of whole cells followed by TLC in solvent 1 (Olson et al. 1983). The aminoglycosphingolipid can easily be identified by its distinctive staining with ninhydrin and α-naphthol and its distinctive position near the origin of the plate.

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References

Barber M, Bordoli RS, Sedgwick RD, Tyler AN (1981) Fast atom bombardment of solids (FAB.): a new ion source for mass spectrometry. J Chem Soc Chem Commun: 325–327

Bias U (1985) Zur Freisetzung von Sulfat, Verwertung von Cystein und Vorkommen von Sulfolipiden bei *Chlorobium*. PhD-thesis, Bonn, FRG

Brittain EFH, George WO, Wells CHJ (1970) Introduction to molecular spectroscopy, theory and experiment. Academic Press, London

Christie WW (1982) Lipid analysis, 2nd edn. Pergamon Press, Oxford

Dabrowski J, Egge H, Hanfland P (1980) High resolution molecular magnetic resonance spectroscopy of glycosphingolipids. I: 360 MHz ^{1}H and 90.5 MHz ^{13}C NMR analysis of galactosylceramides. Chem Phys Lipids 26:187–196

Ferguson MAJ, Williams AF (1988) Cell-surface anchoring of proteins via glycosylphosphatidyl inositol structures. Annu Rev Biochem 57:285–320

Godchaux III W, Leadbetter ER (1980) *Capnocytophaga* spp. contain sulfonolipids that are novel in prokaryotes. J Bacteriol 144:592–602

Godchaux III W, Leadbetter ER (1983) Unusual sulfonolipids are characteristic of the *Cytophaga-Flexibacter* group. J Bacteriol 153:1238–1246

Igishu H, Matsuoka M, Hamasaki N (1990) Binding of galactosylsphingosine (psychosine) by albumin. Lipids 25:65–68

Jentoft N (1985) Analysis of sugars in glycoproteins by high-pressure liquid chromatography. Anal Biochem 148:424–433

Kates M (1972) Techniques in lipidology. Elsevier, North-Holland, Amsterdam

Kenyon CN, Gray AM (1974) Preliminary analysis of lipids and fatty acids of green bacteria and *Chloroflexus aurantiacus*. J Bacteriol 120:131–138

Knudsen E, Jantzen E, Bryn K, Ormerod JG, Sirevåg R (1982) Quantitative and structural characteristics of lipids in *Chlorobium* and *Chloroflexus*. Arch Microbiol 132:149–154

Low MG, Saltiel AE (1988) Structural and functional roles of glycosyl phosphatidyl inositol in membranes. Science 239:268–275

Minnikin DE, Abdolrahimzadeh H, Babbiley J (1973) Glycolipids and biological membranes: the replacement of phospholipids by glycolipids in *Pseudomonas diminuta*. Biochem Soc Trans 1:431–432

Olson JM (1980) Chlorophyll organization in green photosynthetic bacteria. Biochim Biophys Acta 594:33–51

Olson JM, Philipson KD, Sauer K (1973) Circular dichroism and absorption spectra of bacteriochlorophyll-protein and reaction center complexes from *Chlorobium thiosulfatophilum*. Biochim Biophys Acta 292:206–217

Olson JM, Shaw EK, Gaffney JS, Scandella CJ (1983) A fluorescent aminolipid from a green photosynthetic bacterium. Biochemistry 22:1819–1827

Olson JM, Shaw EK, Gaffney JS, Scandella CJ (1984) Chlorobium aminolipid. A new membrane lipid from green sulfur bacteria. In: Sybesma C (ed) Advances in photosynthesis research, vol 3. Nijhoff/Junk, The Hague, pp 139–142

Olson JM, Thornber JP (1979) Photosynthetic reaction centers. In: Capaldi RA (ed) Membrane proteins in energy transduction. Marcel Dekker, New York, pp 279–340

Pfennig N, Trüper HG (1981) Isolation of members of the families Chromatiaceae and Chlorobiaceae. In: Starr MP, Stolp H, Trüper HG, Balows, Schlegel HG (eds) The prokaryotes, vol 1. Springer, Berlin Heidelberg New York, pp 279–289

Riazza V, Tucker AN, White DC (1970) Lipids of *Bacteroides melaninogenicus*. J Bacteriol 101:84–91

Shaw N (1968) The detection of lipids on thin-layer chromatograms with the periodate-Schiff reagent. Biochim Biophys Acta 164:435–436

Siakotos AN, Rouser G (1965) Analytical separation of nonlipid water soluble substances and gangliosides from other lipids by dextran gel column chromatography. J Am Oil Chem Soc 42:913–919

Silverstein RM, Bassler GC, Morill TC (1981) Spectrometric identification of organic compounds. Wiley, New York

Skipski VP, Barclay M (1969) Thin-layer chromatography of lipids. Methods Enzymol 14:530–598

Staehelin LA, Golecki JR, Drews G (1980) Supramolecular organization of chlorosomes (Chlorobium vesicles) and of their membrane attachment sites in *Chlorobium limicola*. Biochim Biophys Acta 589:30–45

Steiner S, Conti SF, Lester RL (1973) Occurrence of phosphonosphingolipids in *Bdellovibrio bacteriovorus* strain UKi2. J Bacteriol 16:1199–1211

Sundquist B, Kamensky I, Håkansson P, Kjellberg J, Salehpour M, Widdiyasekera S, Fohlman J, Peterson PA, Roepstorff P (1984) Californium-252 plasma desorption time of flight mass spectroscopy of proteins. BioMed Mass Spec 11:242–257

Tourtellote ME, Jensen RG, Gander GW, Morowitz HJ (1963) Lipid composition and synthesis in the pleuropneumonia-like organism *Mycoplasma gallisepticum*. J Bacteriol 86:370–379

Vliegenthart JFG, Dorland L, van Halbeek H, Haverkamp J (1982) NMR spectroscopy of sialic acids. In: Schauer R (ed) Sialic acids. Springer, Vienna, pp 127–172

Whitten WB, Pearlstein RM, Olson JM (1979) New spectral components in high resolution absorption spectra of green bacterial reaction center complexes at 5K. Photochem Photobiol 29:823–828

Woese CR (1987) Bacterial evolution. Microbiol Rev 51:221–271

Woese CR, Mandelco L, Yang D, Gherna R, Madigan MT (1990) The case for relationship of the flavobacteria and relatives to the green sulfur bacteria. Syst Appl Microbiol 13:258–262

Yamamoto A, Yano I, Masui M, Yabuuchi E (1978) Isolation of a novel sphingoglycolipid containing glucuronic acid and 2-hydroxy fatty acid from *Flavobacterium devorans* ATTC 10829. J Biochem 83:1213–1216