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Cell wall and sheath constituents of the cyanobacterium *Gloeobacter violaceus*

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Abstract. Sheaths isolated from *Gloeobacter violaceus* were found to be composed of a major polysaccharide moiety (glucose, galactose, rhamnose, mannose, arabinose), a protein moiety, and negatively charged components (glucuronic acids, phosphate, sulfate). Outer membrane polypeptide patterns were dominated by two major peptidoglycan-associated proteins (M_r 62,000 and 53,000). Lipopolysaccharide constituents were glucosamine, 3-hydroxy fatty acids (3-OH-14:0, *anteiso*-3-OH-15:0, 3-OH-16:0, 3-OH-18:0), carbohydrates, and phosphate. A1γ-type peptidoglycan and non-peptidoglycan components (mannosamine, glucose, mannose, and glucosamine) indicated the presence of a peptidoglycan-polysaccharide complex in the cell walls of *Gloeobacter violaceus*.

Key words: Cell wall – Cyanobacterium – *Gloeobacter violaceus* – Lipopolysaccharide – Outer membrane protein – Peptidoglycan-polysaccharide complex – Sheath

The unicellular sheathed *Gloeobacter violaceus*, holotype of the genus *Gloeobacter*, is an atypical cyanobacterium, since it lacks an internal thylakoid membrane system (Rippka et al. 1974). The densely packed rod-shaped phycobilisomes of this organism are attached to the plasmic surface of the cytoplasmic membrane and appear as an electron-dense cortical layer (width of 80 nm) when viewed by transmission electron microscopy (Guglielmi et al. 1981). Ultrathin sections of the cell walls of *Gloeobacter violaceus* revealed a complex fine structure, comprising, from interior to exterior, the electron-dense peptidoglycan layer, an intermediate electron-dense cell wall layer, the double track-structured outer membrane, and closely adherant to the latter a sheath layer. Cell division seems to lead to detachment and expansion of the sheath layer, and the resulting thinner and loosely adherant sheath may accomodate several generations, whose nowly formed cells synthesize again their own thicker and tightly adhering sheath layer (Rippka et al. 1974). The intermediate cell wall layer has not been observed on ultrathin sections of other cyanobacterial cell walls. However, two complementary fracture faces with substructures comparable to those of various other cyanobacteria (Golecki and Drews 1974; Golecki 1977, 1979; Jürgens and Weckesser 1985) were revealed for the outer membrane of *Gloeobacter violaceus* by freeze-etching studies (Guglielmi et al. 1981). The peptidoglycan preparation of *Gloeobacter violaceus* obtained by phenol-extraction of cell walls contained A_2pm, GlcN, MurN in approximate equimolar ratios in addition to GalN and amino acids in varying amounts (Aitken and Stanier 1979).

Inspite of these studies the knowledge on the cell envelope of *Gloeobacter violaceus* is still fragmentary. This paper describes the constituents of the cell wall and sheath of *Gloeobacter violaceus* in order to reveal whether this unusual cyanobacterium has a different cell wall composition compared to that of other cyanobacteria.

Materials and methods

Organism and cultivation

Gloeobacter violaceus sp. strain PCC 7421 (ATCC 29082) obtained from the Pasteur Culture Collection (PCC), Paris (France), was grown photoautotrophically in BG-11 medium, pH 7.5, at 25°C (Rippka et al. 1979). Axenic mass cultures, gassed with air and carbon dioxide (flow rates of 100 l/h and 1 l/h respectively), were prepared in a 10 l Biostat E fermentor (Braun, Melsungen, FRG)

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Abbreviations: A_2pm, diaminopimelic acid; ATCC, American Type Culture Collection; CE, cell envelope; CM, cytoplasmic membrane; CW, cell wall; dOclA, 3-deoxy-D-manno-2-octulosonic acid; GalN, galactosamine; GlcN, glucosamine; GlcUA, glucuronic acid; HF, hydrofluoric acid; LPS, lipopolysaccharide; ManN, mannosamine; M_r, relative molecular mass; MurN, muramic acid; MurN-6-P, muramic acid-6-phosphate; *O*Me, *O*-methyl; PAGE, polyacrylamide gel electrophoresis; PCC, Pasteur Culture Collection; SDS, sodium dodecyl sulfate; SH, sheath

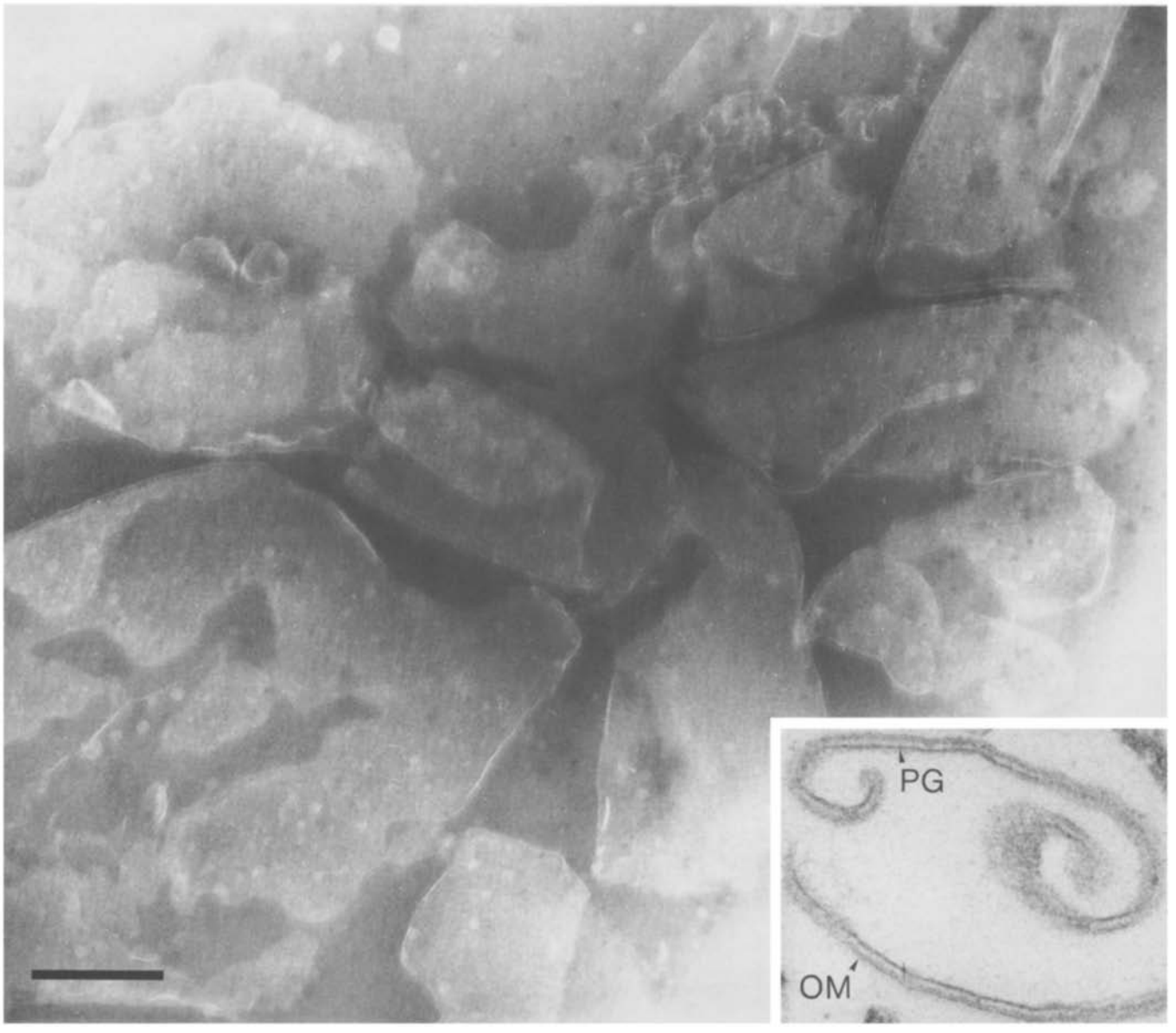

Fig. 1. Negatively stained preparation and ultrathin sections (*inset* in Fig. 1: same magnification) of gradient-purified cell walls (CWII fraction) of *Gloeobacter violaceus*, *OM*, outer membrane; *PG*, peptidoglycan layer. Bar represents 0.2 μm

under continuous irradiation with white fluorescent lamps (6,000 lx) and stirring (250 rpm). Cells were harvested after 28 days of growth and stored as pellet at −20° C until further use.

Sheath and cell wall isolation

A cell homogenate of *Gloeobacter violaceus* was prepared by mechanical disruption of cells as described previously (Jürgens et al. 1985). Differential centrifugation of the cell homogenate was performed by using sequential centrifugation steps (750 × *g*; 3,000 × *g*; 12,000 × *g*; 4° C, 30 min each). The individual pellets (crude cell envelope fractions) from each centrifugation step were resuspended in a small volume (5 to 10 ml) of 20 mM Tris/HCl buffer, pH 8.0 ("Tris buffer", used throughout the isolation procedure), and 5 ml of each suspension was subjected to discontinuous sucrose density gradient centrifugation as described by Jürgens et al. (1985). Sheaths were recovered from the pellet of the gradient at 60% sucrose and cell walls, isolated from the bands at 48% and 53% sucrose, were designated as CWI and CWII fractions, respectively. Sucrose was removed by repeated washings of the particle fractions in Tris buffer and centrifugation (48,000 × *g*, 4° C, 30 min), until no sucrose could be detected in the final supernatant as estimated by measuring the refractive index of an aliquot from the supernatant at room temperature. The sheath and cell wall fractions were finally resuspended in Tris buffer and stored at −20° C. For chemical analyses, aliquots of the isolated fractions were lyophilized after elimination of the Tris buffer by washing in distilled water and centrifugation (13,000 × *g*, 15 min) in an Eppendorf centrifuge. For removal of residual cytoplasmic membranes the cell walls (CWI or CWII fractions, respectively) were extracted (20° C, 20 min) with 2% (w/v) Triton X-100 in 20 mM Tris/HCl buffer, pH 8.0, containing 10 mM $MgCl_2$ (Schnaitman 1971). Triton-insoluble cell walls (CWIII fraction) were recovered by centrifugation (48,000 × *g*, 15° C, 30 min), and washed in Tris buffer containing 10 mM $MgCl_2$ until no detergent could be detected in the final supernatant (Garewal 1973).

Preparation of cell wall constituents

Rigid layers (SDS-insoluble cell wall fraction), the peptidoglycan-polysaccharide complex, and peptidoglycan were prepared from gradient-purified cell walls (CWI plus CWII fractions) of *Gloeobacter violaceus* by applying hot SDS-extraction, Pronase-digestion, and hydrofluoric acid (HF)-treatment in the cold as described previously (Jürgens et al. 1983). The isolated peptidoglycan-polysaccharide complex was digested with lysozyme (EC 3.2.1.17, 50,000 units/mg protein, Sigma Chemical Company, St. Louis, MO, USA) in an enzyme/substrate ratio of 1:25 (w/w) and yielded after degradation and centrifugation (48,000 × *g*, 4°C, 12 h) the glycopeptide-bound polysaccharide and peptidoglycan subunits in the supernatant. Lipopolysaccharide (LPS) was isolated from gradient-purified cell walls (CWI plus CWII fractions) by using hot phenol-water treatment (Westphal and Jann 1965).

Electron microscopy

Sheath and cell wall fractions were fixed with 1% osmiumtetroxide (OsO_4) in cacodylate buffer (Kellenberger et al. 1958) or with 2% glutaraldehyde followed by 1% OsO_4, dehydrated with ethanol (70 to 100%, v/v), and embedded in Epon resin (Luft 1961). Ultrathin sections, stained with uranylacetate (Watson 1958) and lead citrate (Reynolds 1963), were mounted on Formvar-coated copper grids. Negative staining of cell walls was performed by using 1% phosphotungstic acid, pH 7.2 for 1 min as described by (Golecki 1988). The specimens were examined in a transmission electron microscope EM 400 (Philips, Eindhoven, The Netherlands) at 80 kV.

Biochemical-analytical methods

SDS-PAGE was performed on slab gels (Laemmli 1970), consisting of an upper stacking gel (3% acrylamide) and a lower running gel (15% acrylamide), at a constant current of 15 mA. Neutral sugars and fatty acids were analyzed as alditol acetates and methyl ester derivatives, respectively, by combined gas-liquid chromatography/mass spectrometry (Mayer et al. 1985; Weckesser and Jürgens 1988). Amino acids and amino sugars were determined on an automatic amino acid analyzer, model LC 6001 (Biotronik, München, FRG) (Jürgens and Speth 1991). Total hexuronic acids were estimated according to Blumenkrantz and Asboe-Hansen (1973) and identified after separation by high voltage paper electrophoresis (Kickhöfen and Warth 1968). Protein (Lowry et al. 1951) and phosphate (Lowry et al. 1954) were determined colorimetrically. Sulfate was quantified by the turbidimetric method (Tease and Walker 1987).

Results

Isolation and fine structure of cell walls and sheaths

Crude cell envelopes (CE fraction) were enriched as the pellets upon differential centrifugation of 750 × *g* to 12,000 × *g* (for yields see Table 1), whereas cytoplasmic membranes were mainly found in the supernatant of the 48,000 × *g* centrifugation step (data not shown). Isolation of the fractions analyzed, namely sheaths (SH) and cell walls (CWI and CWII) was achieved by sucrose density gradient centrifugation. The sheaths were recovered in the pellet of the gradient at a concentration of 60% sucrose, corresponding to a relatively high buoyant density (more than 1.442 g/cm^3), whereas the cell walls (CWI and CWII fractions) banded at 48% and 53% sucrose, corresponding to buoyant densities of 1.416 g/cm^3 and 1.427 g/cm^3, respectively.

Table 1. Yields of cell envelopes (CE fractions), recovered in the pellets after differential centrifugation of a cell homogenate from *Gloeobacter violaceus* (50 g wet weight) and yields of cell walls (CWI and CWII) and sheaths (SH) after sucrose density gradient centrifugation (for further details see the 'Materials and methods' section)

Centrifugation Speed	Yields (%) of fraction:			
	CE[a]	CWI	CWII	SH[b]
750 × *g*	18.2	0.2	4.6	36.0
3,000 × *g*	24.8	3.2	12.5	17.4
12,000 × *g*	12.8	3.6	4.4	13.8
48,000 × *g*	3.0	1.1	1.9	1.2

[a] Wet weight basis
[b] Dry weight basis

The isolated sheath fragments presented electron-dense layers without uniform thickness on ultrathin sections (data not shown). A network of fine fibrils was seen in cross-sections of some sheaths. Negatively stained preparations of the purified cell wall fraction (CWII) presented flat cell wall fragments with an irregular particle pattern on the surface (Fig. 1), whereas ultrathin sections of this fraction (inset in Fig. 1) revealed the typical C-shaped configuration of cell wall fragments (total width of 23 nm) comprising the electron-dense peptidoglycan layer (width of 6 nm) and the double-track structured outer membrane (width of 8 nm). However, the intermediate electron-dense cell wall layer, described for *Gloeobacter violaceus* cells (Rippka et al. 1974) was not observed on ultrathin sections of isolated cell walls. Instead, an electron-translucent space (width of 9 nm) was seen separating the outer membrane from the peptidoglycan layer.

Cell wall and sheath components

The protein contents (dry weight basis) of gradient-purified CWI and CWII fractions (Table 1) from *Gloeobacter violaceus* were slightly different (28.3% and 30.9%), respectively. The carbohydrates accounted for 17.6% and 17.1% of the dry weights of CWI and CWII fractions, respectively. The presence of A_2pm and MurN indicated peptidoglycan as a characteristic cell wall component. The total peptidoglycan components were found to be enriched in CWII fraction (13.4%) compared with CWI fraction (10.2%). A significant difference between the CWI and CWII fractions was found with respect to the contents of fatty acids (Table 2). The denser CWII fraction had a total fatty acid content of 1.0%, the less dense CWI fraction 8.9% fatty acids (dry weight basis). The fatty acid content of CWI fraction decreased to 0.9% after Triton X-100 extraction, indicating partial removal of membrane lipids and LPS by the detergent treatment. The orange-brownish pigmentation of the cell walls (CWI or CWII fraction) indicated the presence of

Table 2. Chemical composition of gradient-purified cell walls (CWI and CWII fractions), Triton-insoluble cell walls (CWIII fraction), and gradient-purified sheaths (SH fraction) from *Gloeobacter violaceus*

Component	Content per fraction dry weight (nmol/mg):			
	CWI	CWII	CWIII	SH
Amino sugars				
MurN	48	79	63	6
GlcN	192	230	172	30
ManN	27	34	39	–[a]
Unknown[b]	–	–	–	+[c]
Amino acids				
A_2pm	94	126	99	18
Glu[d]	352	402	511	195
Ala[d]	458	506	577	242
Other amino acids[e]	1970	2204	2794	1288
Neutral sugars				
Rha	77	92	44	118
Fuc	67	101	28	–
Ara	79	105	57	87
Xyl	+	+	+	25
Man	87	83	36	114
Gal	171	170	126	398
Glc	508	361	315	1155
2-*O*Me-6-deoxyhexose	+	51	5	ND[f]
2-*O*Me-pentose	+	+	+	ND
2-*O*Me-hexose	8	9	3	ND
GlcUA	+	+	+	+
Fatty acids				traces
16:0	29	14	2	
18:0	3	4	–	
18:1	14	+	–	
20:0	62	2	–	
3-OH-14:0	22	2	21	
anteiso-3-OH-15:0	11	4	3	
3-OH-16:0	+	+	+	
3-OH-18:0	+	+	+	
Phosphate	+	+	+	81
Sulfate	+	233	+	720
Carotenoids	+	+	+	–

[a] –, absent
[b] Unknown amino sugar (R_{GlcN} = 1.24 on high voltage paper electrophoresis)
[c] +, present, but not quantified
[d] Components of peptidoglycan and protein
[e] Amino acids except Pro, Met, Cys, Trp
[f] ND, not determined

carotenoids as constituents of the outer membrane of *Gloeobacter violaceus*.

The sheath (SH) fraction of *Gloeobacter violaceus* revealed highly acidic components such as GlcUA, sulfate, and phosphate (Table 2). In addition, a major carbohydrate moiety (33.7% of the SH dry weight), composed of Glc, Gal, Rha, Man, Ara, and Xyl in molar ratios of 10.1:3.5:1.0:1.0:0.9:0.2, and a protein moiety (18.7%) was found. An unknown amino sugar (R_{GlcN} = 1.24) as a characteristic sheath constituent (being absent from cell wall fractions of *Gloeobacter violaceus*) was detected after separation of an acidic sheath hydrolysate (1 N sulfuric acid, 100° C, 4 h) by high voltage paper electrophoresis. Small amounts of peptidoglycan components (total

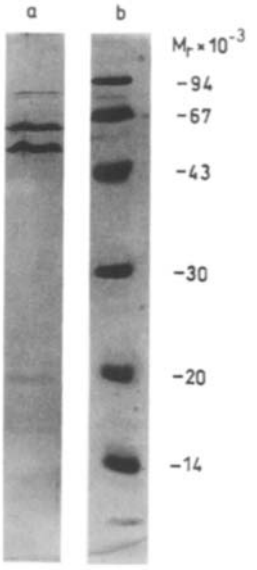

Fig. 2. SDS-PAGE polypeptide patterns of gradient-purified cell walls (CWII fraction) of *Gloeobacter violaceus* (*lane a*) and marker polypeptides (*lane b*)

1.6%) and some fatty acids (0.5%) were detected as contaminants.

Major outer membrane proteins

The SDS-PAGE polypeptide pattern of the gradient-purified cell wall (CWI fraction, band at 48% sucrose) of *Gloeobacter violaceus* was dominated by two major polypeptides (M_r 62,000 and 53,000) (Fig. 2). A release of these proteins from, the outer membrane was only achieved by differential SDS-extraction at temperatures above 70° C, indicating a relatively strong ionic interaction with the peptidoglycan layer. No obvious differences were observed between the polypeptide patterns of the isolated gradient-purified cell walls (CWI and CWII) or the Triton-insoluble cell wall (CWIII) (data not shown).

LPS constituents

LPS fractions from *Gloeobacter violaceus* were obtained from hot phenol-water extracts of gradient-purified cell walls (CWI plus CWII fraction) in yields of 4% (waterphase-LPS) and 1% (phenolphase-LPS), respectively. Both extracts were enriched in GlcN, neutral sugars (including characteristic *O*-methyl sugars), and 3-hydroxy fatty acids (Table 3). The waterphase-LPS contained additional amino sugars (MurN and ManN) indicating some contamination by components of the peptidoglycan-polysaccharide complex of *Gloeobacter violaceus* (see below). The phenolphase-LPS was free of peptidoglycan components, since GlcN was the only amino sugar present. However, significant amounts of lipids were present in the phenolphase-LPS as indicated by the level of 16:0 and 18:0 fatty acids. Neutral sugars of the waterphase-LPS were Glc, Ara, Fuc, Rha, Gal,

Table 3. Chemical composition of LPS fractions (waterphase-LPS and phenolphase-LPS) from *Gloeobacter violaceus*

Component	Content per fraction dry weight (nmol/mg):	
	Waterphase-LPS	Phenol phase-LPS
Amino sugars		
MurN	15	–[a]
GlcN	169	255
ManN	25	–
Amino acids	305	660
Neutral sugars		
Rha	465	–
Fuc	704	430
Ara	738	847
Man	254	216
Gal	270	333
Glc	1318	211
2-*O*Me-6-deoxyhexose	44	75
2-*O*Me-pentose	140	323
2-*O*Me-hexose	28	35
d0clA	ND[b]	ND
Fatty acids		
14:0	6	14
16:0	9	57
18:0	–	17
18:1	–	3
20:0	–	33
3-OH-14:0	75	117
anteiso-3-OH-15:0	26	43
3-OH-16:0	19	40
3-OH-18:0	30	49
Phosphate	67	ND

[a] –, absent
[b] ND, not determined

and Man in molar ratios of 7.8:4.4:4.2:2.8:1.6:1.5 per mol GlcN, whereas the phenolphase-LPS contained Ara, Fuc, Gal, Man, Glc in molar ratios of 3.3:1.7:1.3:0.9:0.8 per mol GlcN. In addition, both LPS fractions contained 3-OH-14:0, *anteiso*-3-OH-15:0 (rarely detected in bacterial LPS), 3-OH-16:0 fatty acids in molar ratios of 2.5:0.9:0.6:1.0 (waterphase-LPS) and 2.4:0.9:0.8:1.0 (phenolphase-LPS), respectively. In addition, significant amounts of phosphate (1 mol per 2.5 mol GlcN) were found in the waterphase-LPS, indicating the presence of phosphorylated LPS.

Peptidoglycan components and degradation products

The peptidoglycan-polysaccharide complex (Table 4) of *Gloeobacter violaceus* was obtained from the SDS-insoluble fraction (rigid layer) of CWI and CWII after digestion with pronase, which removed residual protein (8.7%, dry weight basis) from the fraction. The typical peptidoglycan components, A_2pm, MurN , GlcN, Glu, and Ala were found in molar ratios of 1.0:0.9:1.8:1.0:1.8 indicating the presence of A1γ-type peptidoglycan (Schleifer and Kandler 1972). Non-peptidoglycan components of this complex were ManN, Man, Glc, as well as GlcN (as judged from the relatively high molar

Table 4. Chemical composition of rigid layer, peptidoglycan-polysaccharide complex, HF-insoluble peptidoglycan, and glycopeptide-bound polysaccharide from *Gloeobacter violaceus*

Component	Content per fraction dry weight (nmol/mg):			
	Rigid layer	Peptido-glycan-polysac-charide-complex	HF-insol-uble peptido-glycan	Glyco-peptide-bound poly-saccharide
Amino sugars				
MurN	273	272	410	195
GlcN	471	528	941	479
ManN	131	139	233	113
Amino acids				
A_2pm	261	294	567	329
Ala[a]	550	534	860	754
Glu[a]	339	286	495	457
Compound X[b]	45	50	76	19
Other amino acids[c]	617	35	69	946[d]
Neutral sugars				
Rha	268	109	–[e]	3
Ara	13	5	+[f]	8
Xyl	21	34	41	4
Man	161	109	186	223
Gal	325	157	31	–
Glc	932	508	145	696
2-*O*Me-6-deoxyhexose	24	26	16	ND[g]
GlcUA	+	+	ND	ND
Phosphate	7	11	–	8

[a] Components of peptidoglycan and protein
[b] Co-migrating with His on the amino acid analyzer
[c] Amino acids except Pro, Met, Trp, Cys
[d] Amino acid enrichment due to lysozyme contamination
[e] –, absent
[f] +, present, but not quantified
[g] ND, not determined

ratio of GlcN to A_2pm or MurN, respectively) (Table 4). The phosphate content was negligible (0.07% of fraction dry weight). Additional non-peptidoglycan components were GlcUA, which was detected by high voltage paper electrophoresis of an acidic hydrolysate of the peptidoglycan-polysaccharide complex (data not shown) and a ninhydrin-positively reacting compound X, which co-migrated with histidine on the amino acid analyzer (Table 4).

The incomplete cleavage of peptidoglycan and polysaccharide by treatment of the peptidoglycan-polysaccharide complex with hydrofluoric acid in the cold (48% HF, 0°C, 48 h), together with the almost complete absence of phosphate (Table 4), suggested that phosphodiester bonds are not involved in the linkage of peptidoglycan and polysaccharide. Digestion of the peptidoglycan-polysaccharide complex from *Gloeobacter violaceus* with lysozyme yielded, after centrifugation (48,000 × *g*, 4°C, 30 min), a soluble glycopeptide-bound polysaccharide fraction in the supernatant of the digest. In this fraction GlcN, ManN, Glc, and Man were present in molar ratios of 1.5:0.3:2.1:0.7 per mol A_2pm (Table 4). The low amounts or lack of Rha, Gal, Xyl, and

Ara as components of rigid layer degradation products (Table 4) indicated that these neutral sugars are attributable to residual cell envelope polysaccharide.

Discussion

LPS was successfully extracted by hot phenol-water treatment of cell wall fractions from *Gloeobacter violaceus*. This is in contrast to previous attempts, performed with whole cells, which had failed to prove the presence of LPS in other sheated cyanobacteria (Schrader et al. 1982). Characteristic LPS constituents, such as GlcN, *O*-methyl sugars, and 3-hydroxy fatty acids (3-OH-14:0, *anteiso*-3-OH-15:0, 3-OH-16:0, and 3-OH-18:0 fatty acids) were identified and indicated the presence of lipid A in *Gloeobacter violaceus*. Thus, it seems likely that the lipid A from *Gloeobacter violaceus* contains as that from other cyanobacteria (Weckesser et al. 1979) GlcN as the backbone amino sugar, and which may be linked to 3-hydroxy fatty acids by amide and/or ester bonds. Phosphate was found in nonequimolar amounts relative to GlcN in the LPS. This indicated the presence of phosphorylated lipid A, which was shown to be important for endotoxic activity of LPS from gram-negative bacteria (Rietschel et al. 1987). However its role in *Gloeobacter violaceus* remains to be examined. In accordance with data on LPS fractions from the unicellular cyanobacterium *Microcystis aeruginosa* (Martin et al. 1989), L-*glycero*-D-mannoheptose, a characteristic core-constituent of enterobacterial LPS, was not detectable in *Gloeobacter violaceus* LPS.

The two dominant outer membrane polypeptides (M_r 62,000 and 53,000) of *Gloeobacter violaceus* revealed by SDS-PAGE, were of different molecular mass than those from porins of gram-negative bacteria (Nikaido and Vaara 1985). This is in agreement with data obtained for the dominant outer membrane proteins of other cyanobacteria (Murata et al. 1981; Resch and Gibson 1983; Omata and Murata 1984; Jürgens et al. 1985; Woitzik et al. 1988; Jürgens et al. 1989; Pritzer et al. 1989). These proteins are associated with the peptidoglycan layer of *Gloeobacter violaceus* by ionic interactions, which seem to be stronger than those observed between porins and the peptidoglycan of gram-negative bacteria (Rosenbusch 1974). In addition, the resistance to desintegration of the cell walls of *Gloeobacter violaceus* by detergent treatment (SDS or Triton X-100) indicated a relatively high stability of its outer membrane. Similar observations have been made for a number of other cyanobacterial cell walls (Resch and Gibson 1983; Jürgens et al. 1985; Woitzik et al. 1988; Jürgens and Benz 1989; Jürgens et al. 1989).

The rigid layer fraction of *Gloeobacter violaceus* contained GlcN in a higher molar ratio compared to other peptidoglycan components. This is due to the presence of a peptidoglycan-bound polysaccharide containing GlcN, ManN, Man, and Glc as major constituents. These additional carbohydrates have also been found as characteristic components in the peptidoglycan-bound polysaccharides of several cyanobacteria such as *Synechocystis* PCC 6714 (Jürgens and Weckesser 1986), *Synechococcus* PCC 6307 (Woitzik et al. 1988), *Microcystis* PCC 7806 (Jürgens et al. 1989), and in that of the prochlorophyte, *Prochlorothrix hollandica* (Jürgens and Burger-Wiersma 1989). In all cases examined, this polysaccharide seems to be covalently bound via MurN-6-P to the peptidoglycan.

The gradient-purified sheath of *Gloeobacter* PCC 7421 presented a relatively high acidic character due to the presence of considerable amounts of GlcUA, sulfate, and phosphate. Sulfate as a constituent of sheath fractions from cyanobacteria has also been found in *Gloeothece* ATCC 27152 (Tease and Walker 1987) and *Fischerella* PCC 7414 (Pritzer et al. 1989).

It is concluded that the sheathed cyanobacterium *Gloeobacter violaceus*, as cyanobacteria that lack external cell envelope layers, contains LPS, two major outer membrane proteins and an A1γ-type peptidoglycan, with presumably covalently bound polysaccharide, as constituents of its cell walls. However, in *Gloeobacter violaceus* phosphodiester bonds do not seem to be involved in the linkage of peptidoglycan and polysaccharide as indicated by the almost complete lack of phosphate in the peptidoglycan-polysaccharide complex. A further difference of the cell walls of *Gloeobacter violaceus* compared to those of other unicellular cyanobacteria is the presence of an intermediate electron-dense cell wall layer, localized between the outer membrane and the peptidoglycan layer, which was observed on ultrathin sections of whole cells (Rippka et al. 1974). However, this layer was not observed on electron micrographs of isolated cell walls described here and therefore an assignment of its constituents is presently impossible. On the other hand, it cannot be excluded that the presence of this layer on ultrathin sections is an artefact due to incomplete extraction during embedding procedures of whole sheathed cells. Further studies are required to clarify this point.

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References

Aitken A, Stanier RY (1979) Characterization of peptidoglycan from the cyanelles of *Cyanophora paradoxa*. J Gen Microbiol 112:219–223

Blumenkrantz N, Asboe-Hansen G (1973) New method for quantitative determination of uronic acids. Anal Biochem 54:484–489

Garewal HS (1973) A procedure for the estimation of microgram quantities of Triton X-100. Anal Biochem 54:319–324

Golecki JR (1977) Studies on ultrastructure and composition of cell walls of the cyanobacterium *Anacystis nidulans*. Arch Microbiol 114:35–41

Golecki JR (1979) Ultrastructure of cell wall and thylakoid membranes of the thermophilic cyanobacterium *Synechococcus lividus* under the influence of temperature shifts. Arch Microbiol 120:125–133

Golecki JR (1988) Analysis of structure and development of bacterial membranes (outer, cytoplasmic, and intracytoplasmic membranes). In: Mayer F (ed) Methods in microbiology, vol 20. Academic Press, London, pp 61–77

Golecki JR, Drews G (1974) Zur Struktur der Blaualgen-Zellwand. Gefrierätzuntersuchungen an normalen und extrahierten Zellwänden von *Anabaena variabilis*. Cytobiologie 8:213–227

Guglielmi G, Cohen-Bazire G, Bryant DA (1981) The structure of *Gloeobacter violaceus* and its phycobilisomes. Arch Microbiol 129:181–189

Jürgens UJ, Weckesser J (1985) Carotenoid-containing outer membrane of *Synechocystis* sp. strain PCC 6714. J Bacteriol 154:471–478

Jürgens UJ, Weckesser J (1986) Polysaccharide covalently linked to the peptidoglycan of the cyanobacterium *Synechocystis* sp. PCC 6714. J Bacteriol 168:568–573

Jürgens UJ, Benz R (1989) Pore-forming activity of outer membrane extracts from the unicellular cyanobacterium *Synechocystis* sp. PCC 6714. Z Naturforsch 44c:165–169

Jürgens UJ, Burger-Wiersma T (1989) Peptidoglycan-polysaccharide complex in the cell wall of the filamentous prochlorophyte *Prochlorothrix hollandica*. J Bacteriol 171:498–502

Jürgens UJ, Speth V (1991) Ultrastructure and biochemistry of the cell wall of *Prochlorothrix hollandica* (Prochloraceae). Arch Hydrobiol (in press)

Jürgens UJ, Drews G, Weckesser J (1983) Primary structure of the peptidoglycan of the cyanobacterium *Synechocystis* sp. strain PCC 6714. J Bacteriol 154:471–478

Jürgens UJ, Golecki JR, Weckesser J (1985) Characterization of the cell wall of the unicellular cyanobacterium *Synechocystis* PCC 6714. Arch Microbiol 142:168–174

Jürgens UJ, Martin C, Weckesser J (1989) Cell wall constituents of *Microcystis* sp. PCC 7806. FEMS Microbiol Lett 65:47–52

Kellenberger E, Ryter A, Sechaud J (1958) Electronmicroscope study of DNA containing plasm. J Biophys Biochem Cytol 4:671–683

Kickhöfen B, Warth R (1968) Eine Trennkammer für die Hochspannungselektrophorese nach dem Michl'schen Prinzip. J Chromatography 33:558–560

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Lowry OH, Roberts NR, Leiner KY, Wu ML, Farr AL (1954) The quantitative histochemistry of brain. J Biol Chem 207:1–17

Martin C, Codd GA, Siegelman HW, Weckesser J (1989) Lipopolysaccharides and polysaccharides of the cell envelope of toxic *Microcystis aeruginosa* strains. Arch Microbiol 152:90–94

Mayer H, Tharanathan RN, Weckesser J (1985) Analysis of lipopolysaccharides of Gram-negative bacteria. In: Gottschalk G (ed) Methods in microbiology, vol 18. Academic Press, New York, pp 157–207

Murata N, Sato N, Omata T, Kuwabara T (1981) Separation and characterization of thylakoids and cell envelope of the blue-green alga (cyanobacterium) *Anacystis nidulans*. Plant Cell Physiol 22:855–866

Nikaido H, Vaara M (1985) Molecular basis of bacterial outer membrane permeability. Microbiol Rev 49:1–32

Omata T, Murata N (1984) Isolation and characterization of three types of membranes from the cyanobacterium (blue-green alga) *Synechocystis* PCC 6714. Arch Microbiol 139:113–116

Pritzer M, Weckesser J, Jürgens UJ (1989) Sheath and outer membrane components from the cyanobacterium *Fischerella* sp. PCC 7414. Arch Microbiol 153:7–11

Resch CM, Gibson J (1983) Isolation of the carotenoid-containing cell wall of three unicellular cyanobacteria. J Bacteriol 155:345–350

Reynolds ES (1963) The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J Cell Biol 17:208–212

Rietschel ET, Brade L, Schade U, Seydel U, Zähringer U, Kusumoto S, Brade H (1987) Bacterial endotoxin: properties and structure of biologically active domains. In: Schrinner E, Richmond MH, Seibert G, Schwarz U (eds) Surface structures of microorganisms and their interactions with the mammalian host. Verlag Chemie, Weinheim, pp 1–41

Rippka R Waterbury J, Cohen-Bazire G (1974) A cyanobacterium which lacks thylakoids. Arch Microbiol 100:419–436

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Rosenbusch J (1974) Characterization of the major envelope protein from *Escherichia coli*. Regular arrangement on the peptidoglycan and unusual dodecyl sulfate binding. J Biol Chem 249:8019–8029

Schleifer KH, Kandler O (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol Rev 36:407–477

Schnaitman CA (1971) Solubilization of the cytoplasmic membrane of *Escherichia coli* by Triton X-100. J Bacteriol 108:545–552

Schrader M, Drews G, Golecki JR, Weckesser J (1982) Isolation and characterization of the sheath from the unicellular cyanobacterium *Chlorogloeopsis* PCC 6912. J Gen Microbiol 128:267–272

Tease B, Walker RW (1987) Comparative composition of the sheath of the cyanobacterium *Gloeothece* ATCC 27152 cultured with and without combined nitrogen. J Gen Microbiol 133:3331–3339

Watson ML (1977) Staining of tissue sections for electron microscopy with heavy metals. Arch Microbiol 114:35–41

Weckesser J, Drews G, Mayer H (1979) Lipopolysaccharides of photosynthetic procaryotes. Annu Rev Microbiol 33:215–239

Weckesser J, Jürgens UJ (1988) Cell walls and external layers. In: Packer L, Glazer, AN (eds) Cyanobacteria. Methods Enzymol 167, pp 173–188

Westphal O, Jann K (1965) Bacterial lipopolysaccharides. Extraction with phenol-water and further application of the procedure. Methods Carbohydr Chem 5:83–91

Woitzik D, Weckesser J, Jürgens UJ (1988) Isolation and characterization of cell wall components of the unicellular cyanobacterium *Synechococcus* sp. PCC 6307. J Gen Microbiol 134:519–627