Arch Microbiol (1987) 147:300–303

Archives of
Microbiology

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2-O-Methyl-D-xylose containing sheath in the cyanobacterium *Gloeothece* sp. PCC 6501

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Abstract. The sheath of the unicellular cyanobacterium *Gloeothece* sp. PCC 6501 was isolated from cell homogenates by differential and sucrose gradient centrifugation, followed by lysozyme treatment and hot sodium dodecyl sulfate extraction. The sheath contains a major fraction of carbohydrate consisting of galactose, glucose, mannose, rhamnose, 2-O-methyl-D-xylose, xylose, glucuronic and galacturonic acids, but only traces of fatty acids and phosphate. A protein content of about 2% (of fraction dry weight) could not be removed by the detergent treatment.

Key words: Cyanobacteria – *Gloeothece* – 2-O-Methyl-D-xylose – Polysaccharide – Sheath

Sheaths from cyanobacteria can be defined as distinct and fine-structured layers which loosely surround cells or cell groups (Drews and Weckesser 1982; Vaara 1982). They are often directly visible in the light-microscope. Presence or lack of sheath is used for taxonomical purposes among unicellular cyanobacteria (Rippka et al. 1979): strains having a typical sheath belong to either *Gloeobacter*, *Gloeothece*, or *Gloeocapsa*. The enclosed cells produce new layers, forming a multilayered sheath surrounding finally single cells and cell groups as well. The sheath of *Gloeobacter* seems to be formed by successive excretion from the outer membrane (Rippka et al. 1974).

Little is known about the chemistry of the cyanobacterial sheath. Positive staining with ruthenium-red indicated acidic polysaccharides (Vaara 1982). Polysaccharide compounds were confirmed by analyses on isolated sheath fractions, such as of *Chlorogloeopsis* PCC 6912 (Schrader et al. 1982) or *Chroococcus minutus* (Adhikary et al. 1986). The present paper describes the isolation and gives chemical data on the sheath of *Gloeothece* sp. PCC 6501, a unicellular cyanobacterium with cell division in one plane (Rippka et al. 1979).

Materials and methods

Strain and cultivation

Axenic *Gloeothece* sp. PCC 6501 was kindly provided by the Pasteur Culture Collection (PCC), Institut Pasteur, Paris, France. Mass cultures were grown photoautotrophically in BG-11 medium (Stanier et al. 1971), pH 7.5 at 25° C in a 12 l fermentor (Jungkeit, Göttingen, FRG). Cultures were irradiated with white fluorescent lamps (1–4 klx) and continuously gassed by a stream of air/CO_2 (99:1, v/v, 250 l h^{-1}). Cells were harvested after 15 days cultivation and washed once with 20 mM Tris-HCl buffer, pH 8.0 (used throughout sheath isolation).

Isolation of sheath

For cell homogenization, freshly harvested cells were suspended in buffer (see above), mixed with glass beads (0.25 mm in diameter; cell to glass bead ratio 1:2, v/v; with some DNase added) and broken in a Vibrogen shaker (type Vi2, E. Bühler, Tübingen, FRG) at 4° C at full speed for 2 h. Lysozyme treatment (EC 3.2.1.17; 5 mg in 25 ml ammonium acetate buffer, pH 6.8) was at 37° C for 12 h, sodium dodecyl sulfate (SDS, 2%, w/v) – extraction in Tris-HCl buffer (as above) at 100° C for 5 min (for further details see Adhikary et al. 1986).

Microscopy and analytical procedures

For microscopy, cells were fixed with OsO_4 (see Golecki 1977), dehydrated by ethanol (70 to 100%), and embedded according to the procedure of Spurr (1969). For light-micrographs semi-thin sections (0.5–1 µm) were stained with methylene-blue Azur II (Richardson 1960), for electron microscopy ultrathin sections were contrasted with uranyl acetate and lead citrate before being examined in a Philips EM 400 at 80 kV (Golecki 1977).

Hydrolysis conditions for liberation of monomeric compounds and experimental details of gas-chromatographic determination and mass-spectrometric fragmentation of neutral sugars (as alditol acetates) were as described elsewhere (Adhikary et al. 1986). 2-O-Methyl-D-xylose was isolated in preparative amounts (1 mg) by preparative paper chromatography (solvent system: ethylacetate-pyridine-water = 12:5:4, v/v/v). Optical rotation was measured in a Perkin-Elmer polarimeter, type 141, using a 1 ml quartz cuvette and a light pathway of 10 cm (wavelength: 589 nm). Demethylation of O-methyl sugar (1–2 mg, dissolved in 0.5 ml dichloro-methane) was carried out with BBr_3 (3 ml) in an ice-bath during 18 h (McOmie et al. 1968). Solvent and excess of BBr_3 were removed by evaporation in vacuo (30° C, repeated 5 times with the addition of methanol after the first evaporation). Uronic acids were detected by high-voltage paper-electrophoresis and quantitatively determined by

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Abbreviation. SDS, sodium dodecyl sulfate

Fig. 1. Interference-contrast picture of *Gloeothece* sp. PCC 6501 cells. Note the distinctly visible layers in the sheath surrounding single cells and cell groups as well. Bar represents 20 μm

Fig. 3. Light-microscopy of sheath isolated from cell homogenates, purified by sucrose gradient centrifugation, lysozyme digestion and hot SDS treatment (for experimental details see text). Staining with methylene-blue Azur II. Bar represents 200 μm

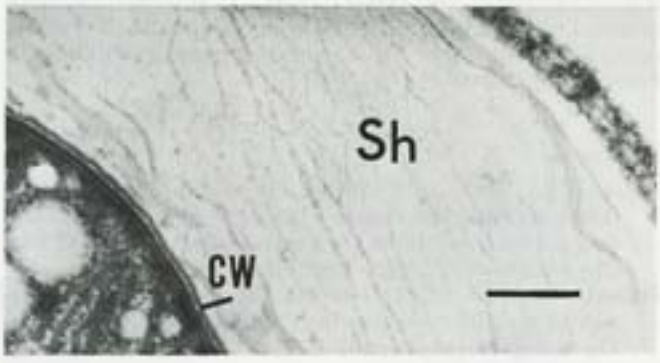

Fig. 2. Ultrathin section of *Gloeothece* sp. PCC 6501 cells (OsO_4-fixed, uranyl acetate/lead citrate-contrasted). The inner area of the sheath is weakly stained, the outer layer heavily. *CW* cell wall; *Sh* sheath. Bar represents 0.5 μm

carbazole/H_2SO_4 (Galambos 1967), amino sugars and amino acids determined in an automatic amino acid analyzer, fatty acids gas-chromatographically as methyl esters and phosphorus according to the Lowry method (for references see Adhikary et al. 1986).

Results

Interference-contrast micrographs of *Gloeothece* sp. PCC 6501 showed single cells and cell aggregates after cell division surrounded by distinctly visible sheath layers (Fig. 1). In electronmicrographs, the inner sheath area is weakly stained showing few fibers oriented parallel to the cell surface (Fig. 2). The outer area is heavily stained.

Isolation of sheath from cell homogenates

Homogenization of cells for separation of large sheath fragments was most effective by applicating a Vibrogen shaker among the various methods used (e.g. Ultra-turrax; ultrasonication; Braun dismembrator II, Braun-Melsungen, FRG), as controlled by light-microscopy. Differential centrifugation (120 × *g*, 4°C, 15 min; then sedimentation of the supernatant at 3,000 × *g*, 4°C, 15 min) yielded in the latter sediment unbroken cells covered by a thick, gel-like layer of sheath material. A four times purification (3,000 × *g*, 4°C, 15 min) of the sheath material, followed by sucrose gradient centrifugation (60, 55, 50, 45, 40% sucrose, w/w, in Tris-HCl-buffer) sedimented the sheath, again, as a layer directly overlying the residual unbroken cells at the tube bottom. The yield was 6% of whole cells (dry weight basis) after a seven-fold washing (48,000 × *g*, 4°C, 30 min). Further purification was achieved by lysozyme treatment followed by SDS-extraction. The yield was 2% of whole cells (dry weight basis). SDS-extracted sheath fractions still showed methylene-blue Azur II stainable structures on semi-thin sections (Fig. 3).

Galactose, glucose, mannose, rhamnose, xylose and an unknown sugar were found in the isolated sheath (Table 1). The latter behaved lipophilic on thin-layer chromatography ($R_{Rh} = 1.13$ in solvent system ethylacetate-pyridine-water = 12:5:4, v/v/v) and stained red with anilinium hydrogen phthalate. On mass spectrometric fragmentation of the sugar alditol acetate (reduction with $NaBD_4$), the primary fragments m/e 261 and 217 and secondary fragments at m/e 201, 141, 159, 117, 127, 99 and 187, 127, respectively, were obtained indicating a 2-O-methyl pentose (Jansson et al. 1976). Xylose was found after demethylation of the isolated sugar, as proven by thin-layer chromatography (solvent as indicated above) and by simultaneous injection of the demethylation product and authentic xylose (as alditol acetates) into the gas-liquid-chromatograph. Optical configuration of 3-O-methyl-D-xylose was reported by Laidlaw (1954) to be $\{\alpha\}_D^{25}$ +37°C. Since only about 1 mg of the purified 2-O-methyl sugar from *Gloeothece* sp. PCC 6501 was available, only the (positive) direction was measured, allowing to identify it as 2-O-methyl-D-xylose.

Both, glucuronic and galacturonic acid were found in the isolated sheath of *Gloeothece*. The fatty acid content of all fractions did not exceed 0.4% of dry weight (Table 1), C_{16} and C_{14}:1 being dominating among the small amounts found. The phosphorus content was negligible. The only amino sugar found was little glucosamine which together with the small amounts of diaminopimelic acid found represents a peptidoglycan contamination. The protein moiety of

Table 1. Chemical analyses (μg/100 μg fraction dry weight) of sheath fractions from *Gloeothece* sp. strain PCC 6501. The sheath was isolated from cell homogenates by differential and sucrose gradient centrifugation followed by lysozyme and SDS-treatments

Compound	Subsequent purification by		
	Sucrose gradient centrifugation	Lysozyme digestion	SDS-treatment (2%, 100° C, 5 min)
Total neutral sugars	34.9	42.0	44.0
2-O-Methyl-D-xylose	3.9	4.9	5.2
Rhamnose	3.6	4.0	4.2
Xylose	1.2	1.5	1.8
Mannose	6.4	7.6	8.0
Galactose	10.8	13.6	14.2
Glucose	9.0	10.4	10.6
Uronic acids (glucuronic and galacturonic acids)	2.3	2.4	2.3
Protein	3.8	11.2[a]	2.6
Diaminopimelic acid	0.2	–[b]	–[b]
Glucosamine	0.2	–[b]	–[b]
Fatty acids	0.4	0.4	0.3
Phosphorus	0.06	0.06	0.03

[a] Due to incomplete removal of lysozyme
[b] Absent

about 2% of sheath dry weight was not removed by the hot SDS-treatment.

Discussion

Isolation of sheaths from cyanobacteria including *Gloeothece* sp. PCC 6501 is facilitated by high density and mechanical stability of the sheath. Accordingly, the sheath isolated from *Gloeothece* sp. PCC 6501 cell homogenates by centrifugation steps contains only little cell wall contamination. They could be removed by lysozyme and SDS-treatment. Resistance against strong detergents, with even retaining the fine-structure, has also been observed with the sheaths of *Chlorogloeopsis* sp. PCC 6912 (Schrader et al. 1982) and *Chroococcus minutus* (Adhikary et al. 1986). However, in contrast to *Chlorogloeopsis* sp. PCC 6912, sheath material of *Gloeothece* sp. PCC 6501 is also extracted into the water-phase of hot phenol-water extracts (T. Lukas, unpublished work). Probably, this is due to the lower protein content of the *Gloeothece* sheath, although the SDS-insolubility remains to be explained. A lower protein content may also be responsable for the weak staining of inner area of this sheath on ultrathin sections for electron microscopy. It deserves connotation, however, that protein is not completely removed from sheath fractions of all cyanobacteria studied so far even on application of drastic detergent treatments. The chemical nature of the association of protein and carbohydrate remains to be characterized.

The sheath of *Gloeothece*, like those of other cyanobacteria (Drews and Weckesser 1982; Jürgens and Weckesser 1985) has a major sugar moiety containing neutral sugars and uronic acids. The 2-O-methyl-D-xylose (identified here for the first time in a natural product, to our knowledge) represents another example of sugar O-methyl ethers, frequently found in cell envelope polymers, including lipopolysaccharides, from cyanobacteria and phototrophic bacteria as well (Weckesser et al. 1979). They contribute sugar specificity to the cell surface: in case of lipopolysaccharide to the outer membrane, in case of sheath to external cell envelope layers. Complex sugar composition with rare sugar O-methyl ethers included, together with insolubility and mechanical stability, may render cyanobacterial sheaths resistant to enzymatic degradation (Schrader et al. 1982; Drews and Weckesser 1982; Adhikary et al. 1986). Preservation of cyanobacterial sheaths in geological sediments and fossils is a well known phenomenon (Schopf and Walter 1982).

About half of sheath material was not identified in this study. This seems not to be caused by degradation of identified compounds during hydrolysis. In addition, protein and lipids can be excluded as major compounds. Thus, the residual sheath material, aside from its water content, may be composed of nonidentified compounds. The sheath of *Gloeothece* ATCC 27152 has recently found to contain sulfate (B. Tease, personal communication).

Acknowledgements. The authors thank D. Borowiak for mass-spectra, J. R. Golecki for electron microscopy as well as H. Falk for taking light-micrographs. The work was supported by the Deutsche Forschungsgemeinschaft.

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Received December 19, 1986/Accepted January 20, 1987