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Structural investigation of a polysaccharide released by the cyanobacterium *Nostoc insulare*

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Abstract The structural investigation of an extracellular polysaccharide released during photoautotrophic growth by the cyanobacterium *Nostoc insulare* is reported. After 60 days of cultivation, an average yield of purified, desalted, and freeze-dried released polysaccharide (RPS) of 0.9 g L^{-1} medium was obtained. The apparent hydrodynamic volume, determined for RPS, was 1.1×10^6 Da, and the average molecular weight was 2.8×10^6 Da. No sulfate and only traces of pyruvate and acetate groups were detectable. A protein content of only 0.7% indicates a high degree of purity of RPS. The following constituent uronic acids and sugars were identified: glucuronic acid (GlcA), glucose (Glc), arabinose (Ara), and for the first time, cyanobacterial RPSs 3-O-methyl-arabinose (3-O-Methyl-Ara). Adapted from linkage analyses of untreated RPS and of RPS treated by means of reduction of uronic acids, mild acid hydrolysis with oxalic acid, or lithium degradation, respectively, the following partial structure of RPS is proposed, which possesses an arborisation built by 1,3,4-Glc*p* and a side chain built by 3-O-Methyl-Ara*f*: →1)-Glc*p*-(3→1)-Glc*p*-[(3→1)-3-O-Methyl-Ara*f*] (4→1)-GlcA*p*-(4→).

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Introduction

Cyanobacteria are photosynthetic prokaryotic organisms that have been known for a long time to produce large amounts of exopolysaccharides (Drews and Weckesser 1982; Hellebust 1974). Different types of cyanobacterial exopolysaccharides are formed: e.g., as a mucilaginous external layer around the cell, either being organised into a well-defined sheath or a capsule [capsular polysaccharides (CPSs)] that is intimately associated with the cell surface, or as slime that is only loosely associated with the cell surface; or as soluble polysaccharides [released polysaccharides (RPSs)], released into the environment (culture medium) during cell growth (Li et al. 2001). Such microbial exopolysaccharides, in particular the CPSs, serve as a boundary to the immediate environment and play a protective role against desiccation or antimicrobial agents (De Philippis and Vincenzini 1998; Potts 1994). Compared with CPSs, the RPSs are easily extractable from culture media, for instance, by precipitation with alcohol. Consequentially, over the past years in particular, this type of cyanobacterial exopolysaccharide has received increasing attention in view of a potential industrial application; e.g., as viscosifying or suspending agents, as additives for removal and recovery of dissolved

heavy metals, or, in the case of sulfated polysaccharides, as bioactive substances (De Philippis and Vincenzini 1998; De Philippis et al. 2001; Li et al. 2001).

Compared with many macroalgal and other microbial polysaccharides, cyanobacterial RPSs are less well characterised. Reports in the literature focus mainly on their monosaccharide composition (De Philippis and Vincenzini 1998; De Philippis et al. 2000a; Huang et al. 1998; Li et al. 2001; Moreno et al. 2000), but only very few structures have been proposed, e.g., for the extracellular polysaccharide from *Cyanospira capsulata* (Garozzo et al. 1995, 1998), for the CPS produced by *Mastigocladus laminosus* (Gloaguen et al. 1997, 1999), and for the RPS of *Nostoc commune* (Brüll et al. 2000; Helm et al. 2000). However, for potential industrial application of natural products, their chemical characterisation needs to be defined as accurately as possible.

The cyanobacterial genus *Nostoc* includes a wide variety of strains possessing different physiological and biochemical features. Many *Nostoc* strains release significant amounts of polysaccharidic material into their surroundings (De Philippis et al. 2000a, b) as, for instance, previously described for *N. insulare* (Fischer et al. 1997). Therefore, strains of this genus are particularly promising as sources for new hydrophilic biopolymers with potential commercial application. The present study was undertaken to characterise not only the constitutive sugars but also the structure of the RPS of *N. insulare* to enhance the currently low knowledge in this field.

Materials and methods

The strain *N. insulare* 54.79 was obtained from the Sammlung von Algenkulturen Göttingen (SAG), (Schlösser 1994). Cultivation was carried out as previously described (Volk 2005). Briefly, *N. insulare* was cultivated in 10-L conical shoulder flasks under axenic conditions, continuous aeration, and continuous illumination (25–30 μmol photons m^{-2} s^{-1}) in a constant-temperature room at 27°C. The culture medium consisted of 10% artificial seawater (Instant Ocean by Aquarium Systems Inc., Sarrebourg, France) and 90% demineralised water with added phosphate, nitrate, and trace elements, according to Pohl et al. (1987). Cultivation time was about 60 days up to the end of the exponential growth phase. Sterile filtered water was added to compensate the loss of water caused by aeration and resultant increased evaporation. To examine cultures for possible contamination with other organisms, samples were taken during cultivation and after harvesting and were examined microscopically. Contaminated cultures were excluded from the subsequent investigations. In addition, the samples were used to ascertain the biomass content of the culture (gravimetrically by centrifugation and freeze-drying) as well as the carbohydrate content of the biomass-free culture medium (photometrically by the phenol-sulfuric acid method (Dubois et al. 1956)).

Isolation of RPSs

For isolation of RPSs, biomass and culture medium were separated by centrifugation [10,000 g, continuous-flow centrifuge (Contifuge 17S, Heraeus Instruments, Germany) RT]. The culture medium was reduced to a volume of 1 L at 35°C using a rotating evaporator. To precipitate free proteins, the medium was heated (94°C, 10 min) and centrifuged. The still dissolved RPSs were precipitated from the supernatant by addition of ethanol [final concentration 80% (v/v)] at 4°C overnight, centrifuged (3,000 g, 10 min), washed with cold ethanol 96%, and dissolved in demineralised water for subsequent dialysis (membrane tubing, 12–14 kDa, Spectra/Por, USA). The obtained purified and desalted RPS was freeze-dried.

Chemical characterisation of isolated RPS

The molecular weight and hydrodynamic volume of the RPS were determined by size-exclusion chromatography on a Sephacryl S-400 HR, XK 16/60 column (Pharmacia) using 1 M NaCl as eluant. Detection with multiple-angle laser-light-scattering (MALLS) Detektor miniDAWN (Wyatt Technology Corp., USA) provided molecular weight. The hydrodynamic volume was estimated based on refractive index (RI) detection (ERC-7515A, Erma, Japan) using pullulans of known molecular weight (P10-P800 Shodex, Macherey & Nagel, Germany) as standards.

Detection of substituents. The sulfate content of the RPS was estimated photometrically according to Craigie et al. (1984) in the following manner: release

of sulfate groups by acid hydrolysis (2N HCl, 2 h, 100°C) of the RPS and subsequent precipitation of free sulfate as barium sulfate. Potassium sulfate was used as standard. The result was verified by elemental analysis using a HEKAtech CHNS analyser. The pyruvate content was estimated by two different photometrical methods: according to Sloneker and Orentas (1962) by acid hydrolysis of the RPS (1N HCl, 3 h, 100°C) and converting the released pyruvate by addition of 2,4-dinitrophenylhydrazine into a coloured product using different concentrations of pyruvate acid as reference or, in a more specific manner, according to Hirase and Watanabe (1972) by acid hydrolysis (0.04N oxalic acid, 4 h, 100°C) and converting the pyruvate by lactate hydrogenase into lactate. The acetate content was calculated photometrically according to McComb and McCready (1957) by formation of acetohydroxamic acid. α-D-glucose-pentaacetate was used as standard.

Amino acid analysis and estimation of protein content. RPS samples were hydrolyzed in 6 M hydrochloric acid (110°C, 22 h). The carbonised carbohydrates were removed by centrifugation, and the amino-acid-rich supernatant was evaporated to dryness, redissolved in water, and freeze-fried. The residue was dissolved in sodium borate buffer (pH 2.2) and analysed by high-performance liquid chromatography (HPLC) (Amino Sys, Germany). Subsequent protein quantification based on this analysis.

Analysis of sugar components. The neutral monosaccharides were analysed after acid hydrolysis with trifluoroacetic acid (2 M TFA, 1 h, 121°C) as their alditol acetates by gas chromatography (Blakeney et al. 1983). The total content of uronic acids was determined photometrically according to the method of Blumenkrantz and Asboe-Hansen (1973). For a closer examination, the uronic acids were reduced to neutral sugars using two different methods: according to Taylor and Conrad (1972) by activating the carboxyl groups with 1-cyclohexyl-3-[2-methylmorpholinoethyl]-carbodiimide-metho-4-toluolsulfonate and subsequent reduction with sodium borodeuteride, and according to Fontaine et al. (1994) by activating the carboxyl groups by esterification with diazomethane and subsequent reduction with sodium borodeuteride. The resulting neutral sugars were analysed as their alditol acetates by gas chromatography, as described above.

Splitting of RPSs into fragments (poly-, oligo-, monomers). Partial acid hydrolysis according to Gleeson and Clarke (1979): RPS was hydrolysed with oxalic acid (12.5 mM at 100°C for 5 h). Ethanol was added to the hydrolysate up to a final concentration of 80% (v/v). For precipitation and separation of remaining insoluble polymers, the solution was stored at 4°C overnight and centrifuged (20,000 g for 10 min). After two washing steps of the precipitate with ethanol 80% (v/v), the supernatant, combined with the washing solutions, and the purified precipitate were freeze-dried separately. Alternatively, a lithium degradation of uronic acids was carried out according to Lau et al. (1987a, b) to obtain defined fragments of RPS (segments between two uronic acids of the polymer).

Linkage analyses of the RPS. The polysaccharide was subjected to linkage analyses by the method of Harris et al. (1984) in the following manner: Methylation was accomplished with potassium methylsulfinyl carbanion and methyl iodide followed by hydrolysis and acetylation. The following gas chromatography–mass spectrometry (GC-MS) analysis of partially methylated alditol acetates (see above) was performed on fused silica capillary column (0.25 mm i.d.×25 m, OV-1701, Macherey & Nagel, Germany) using helium as eluant. Mass spectra were recorded on an HP MS Engine 5898 A instrument. The procedure was carried out with and without previous reduction of carboxyl groups or with and without previous splitting of the polymer, as described.

Results

Biomass and carbohydrate content in the medium of cultures of *N. insulare* increased continuously during cultivation (Figure 1). At the 60th day (day of harvesting), the averaged yield of purified, desalted and freeze-dried RPS was 0.9 g L^{-1} of medium.

Chemical characterisation of isolated RPS

Using size-exclusion chromatography and pullulans with known molecular weights as standards (0.01×10^6–0.8×10^6 Da), an average hydrodynamic volume of over 0.8×10^6 Da was found for the RPS of *N. insulare*.

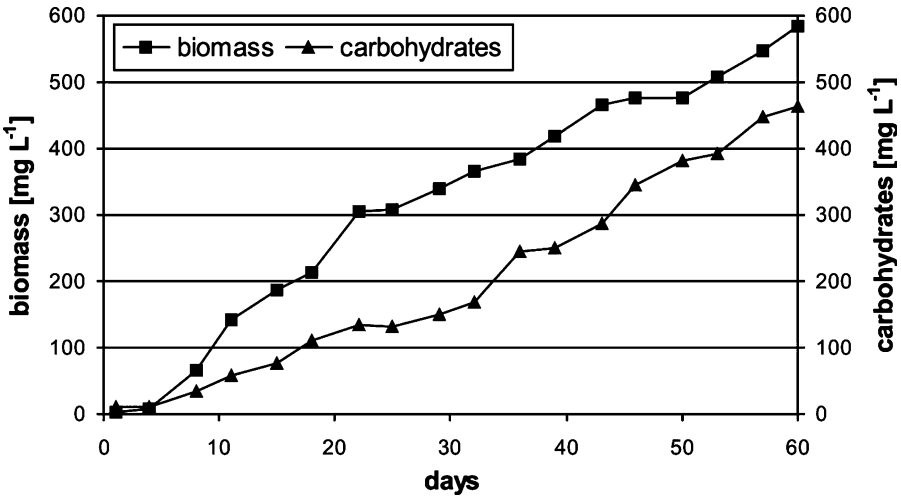

Figure 1 Biomass and carbohydrate content in an 8-L culture of the cyanobacterium *Nostoc insulare* during 60 days of batch cultivation.

By extrapolation of the standard values, an apparent hydrodynamic volume of 1.1×10^6 Da was estimated. The average molecular weight of the RPS, calculated on the basis of the MALLS detector signal, was 2.8×10^6 Da.

Detection of substituents. In elemental analysis no sulfur was found. Furthermore, no sulfate was detected photometrically according to Craigie et al. (1984). Therefore, it is assumed that the RPS of *N. insulare* is free of sulfate groups. The determined pyruvate content was 0.2% (m/m) according to the method of Sloneker and Orentas (1962), and 0.1% (m/m) according to Hirase and Watanabe (1972). The acetate content, calculated photometrically according to McComb and McCready (1957), was 0.7% (m/m).

A protein content of 0.7% (m/m) was calculated adapted from HPLC amino acid analysis.

After acid hydrolysis with trifluoroacetic acid (TFA), reduction and acetylation (according to Blakeney et al. 1983), the following neutral sugars were found via gas chromatography: arabinose (Ara; 34.0%), 3-O-methyl-arabinose (3-O-Methyl-Ara; 22.9%) and glucose (Glc; 43.1%) (Table 1, data are given in mol %).The total content of uronic acids, determined photometrically according to the method of Blumenkrantz and Asboe-Hansen (1973), was 26.4% (m/m).

For a closer examination of uronic acids, they were reduced to neutral sugars in one sample of the RPS according to Taylor and Conrad (1972) and, alternatively, in another sample according to Fontaine et al. (1994). Subsequent analyses of the resulting neutral

Table 1 Neutral monosaccharide composition of the *Nostoc insulare* released polysaccharide (RPS) and its degradation products after partial acid hydrolysis with oxalic acid (in mol %)

Neutral monosaccharide	RPS (untreated)	RPS after partial acid hydrolysis (with oxalic acid)		
		Ethanol-insoluble fraction	Ethanol-soluble fraction	
			Not hydrolysed[a]	Hydrolysed with TFA[b]
3-O-Methyl-Ara	22.9	0.0	82.7	23.2
Ara	34.0	53.2	13.2	31.6
Gluc	43.1	46.8	4.1	45.2

A sample of the ethanol-soluble fraction was also hydrolysed with trifluoroacetic acid (TFA) for detection of not only the monomers but additionally of constituents of the oligomers

[a] Only monomers were detected

[b] Also constitutive sugars of oligomers were detected

sugars as their alditol acetates revealed a significant increase of glucose content. The reduction was repeated with simultaneous integration of deuteride during the procedure. The resulting neutral sugar could be subsequently identified by mass spectroscopy as 1,4-glucose in pyranose form (1,4-Glc*p*). From this, it follows that glucuronic acid is the unique uronic acid in *N. insulare* RPS because only this one is transformed into glucose via reduction.

Samples of the RPS were split into fragments by mild partial acid hydrolysis. After precipitation with ethanol, the precipitate (containing polymers) and the supernatant (containing mono- and oligomers) were analysed for their neutral sugar composition. A sample of the supernatant was also hydrolysed with TFA before analysis of neutral sugars to detect not only monomers but also the constituents of oligomers obtained during partial hydrolysis with oxalic acid. The results are given in Table 1. Precipitate and supernatant were obtained in a mass ratio of 1:13. Thus, the RPS was split mostly in ethanol-soluble mono- and oligomers. The ethanol-insoluble precipitate consisted of Ara and Gluc in nearly equal amounts. 3-O-Methyl-Ara was missing in this fraction, but this sugar dominates the monosaccharide fraction of the ethanol-soluble supernatant [83%, accordant to an approximate molar ratio of 20:3:1 (3-O-Methyl-Ara:Ara:Glc)]. When the oligomers of the supernatant were split into detectable monomers by hydrolysis with TFA before analysis of neutral sugars, the portion of 3-O-Methyl-Ara was conspicuously lower [accordant to an approximate molar ratio (3-O-Methyl-Ara:Ara:Glc) of 2:3:4].

For linkage analyses of monomers, the RPS was subjected to methylation, hydrolysis and acetylation. The resulting partially methylated alditol acetates (PMAAs) were analysed by GC-MS. To obtain more differentiated results, the treatment was carried out with and without previous reduction of carboxyl groups or with and without previous splitting of the polymer. Results of linkage analyses are given in Table 2.

Reduction of uronic acids increased the content of 1,4- and 1,3,4-Glucose (*p*) (1,4- and 1,3,4-Glc*p*). Mild, partial acid hydrolysis with oxalic acid followed

Table 2 Linkage analyses of the *Nostoc insulare* released polysaccharide (RPS)

PMAA	RPS	RPS after reduction of uronic acids[a]		RPS after partial hydrolysis with oxalic acid[b]		RPS-Red.$_{TC}$ after partial hydrolysis with oxalic acid[b]		RPS after Li-degradation of uronic-acids[c]
		Red.$_{F}$	Red.$_{TC}$	Ethanol-insoluble fraction	Ethanol-soluble fraction	Ethanol-insoluble fraction	Ethanol-soluble fraction	
1- Ara*f*	28.3	19.1	19.1	4.5	19.3	10.1	19.4	23.6
1-Ara*p*	3.7	0.0	2.9	0.0	15.6	1.8	7.4	0.0
1,5-Ara*f*	23.9	19.3	21.6	24.2	18.2	22.3	19.2	0.0
1-Glc*p*	0.0	0.0	0.0	23.5	20.7	7.0	7.2	0.0
1,3-Glc*p*	29.1	13.9	25.6	36.9	16.3	27.8	19.4	51.0
1,4-Glc*p*	1.8	20.3	14.2	10.9	6.0	23.4	21.3	0.0
1,6-Glc*p*	0.0	4.5	1.2	0.0	1.8	0.0	0.0	25.4
1,3,4-Glc*p*	13.2	22.9	15.4	0.0	2.1	7.6	6.1	0.0

Partially methylated alditol acetates (PMAAs) of monosaccharides of the untreated RPS, of the RPS after reduction of carboxyl groups and after splitting of the untreated or the reduced RPS either with oxalic acid or by lithium degradation were analysed. Concentrations are given in mol %

Ara arabinose, *Glc* glucose, *f* furanose form, *p* pyranose form

[a] Reduction of uronic acids to neutral sugars: Red.$_{F}$, according to Fontaine et al. (1994); Red.$_{TC}$, according to Taylor and Conrad (1972)

[b] Mild partial acid hydrolysis with oxalic acid, subsequent precipitation with ethanol (80%), followed by division in an ethanol-soluble and an ethanol-insoluble fraction (by centrifugation)

[c] A lithium degradation of uronic acids was carried out according to Lau et al. (1987a, b) to obtain defined fragments of RPS (segments between two uronic acids of the polymer)

by precipitation with ethanol (80%) led to an ethanol-soluble and an ethanol-insoluble fraction. For the ethanol-insoluble fraction, a significant decrease in terminal arabinose content (particularly of 1-Ara*f*) but a significant increase in 1,4-Glc*p* and terminal glucose (1-Glc*p*) content was found. When the uronic acids were reduced to neutral sugars before partial acid hydrolysis with oxalic acid, the increase in 1,4-Glc*p* content was much higher. The ethanol-soluble fractions were dominated on a quantity basis by terminal sugars.

Lithium degradation of the RPS led to segments located between two uronic acids of the polymer. Linkage analysis of the products showed terminal arabinose (1-Ara*f*), 1,3-Glc*p* and 1,6-Glc*p*.

Discussion

The above results show that the RPS produced by *N. insulare* is a complex heteropolysaccharide composed of three neutral sugars (arabinose, 3-O-methyl-arabinose, glucose) and one uronic acid (glucuronic acid). However, the presence of fucose, reported by Fischer et al. (1997) earlier, was not confirmed. Substituents, such as sulfate, pyruvate and acetate groups, were not – or were at most in traces – detectable, and a protein content of only 0.7% indicates a high degree of purity of RPS. The investigation of RPSs of 25 different *Nostoc* strains from the Pasteur Culture Collection, by De Philippis et al. (2000a), revealed a number of constitutive monosaccharides ranging from six to nine. Moreover, a large number of those *Nostoc* RPSs showed the presence of uronic acids (glucuronic acid, galacturonic acid) and of substituents such as pyruvate or sulfate. Consequently, the characteristics of the RPS of *N. insulare* are the absence of sulfate and the limited number of different sugars as constituents. However, the most outstanding characteristic is the occurrence of a high percentage of 3-O-methyl-arabinose. The presence of methylated sugars has so far only been reported for a few cyanobacterial RPSs, e.g., 2-O-methyl-glucose for the RPSs of *N. commune* (Brüll et al. 2000) and of another *Nostoc* species (Hokputsa et al. 2003), or 2-O-methyl-rhamnose for the RPSs of *Microcoleus vaginatus* and *Scytonema javanicum* (Hokputsa et al. 2003). Our report is, to our knowledge, the first of a cyanobacterial RPS containing 3-O-methyl-arabinose as constitutive sugar.

The yield of purified RPS after 60 days of cultivation of *N. insulare* was 0.9 g L^{-1}, giving an average of 15 mg L^{-1} d^{-1}. In a total of 40 *Nostoc* strains from the Pasteur Culture Collection, De Philippis et al. (2000b) found 25 excreting RPS in significant amounts and detected mean RPS productivities in a range of 3–47 mg L^{-1} d^{-1}. The RPS productivity found for *N. insulare* aligns with these findings.

There was a discrepancy between the amount of the purified RPS (0.9 g L^{-1}), which was obtained after 60 days of cultivation and the carbohydrate content of the biomass-free culture medium at the 60th day determined photometrically by the phenol-sulfuric acid method according to Dubois et al. (0.46 g L^{-1}). By using the same photometrical method for the determination of time courses of total carbohydrates in cultures of *Cyanospira capsulata*, Vincenzini et al. (1990) found a multiplying factor of two to convert soluble carbohydrates into actual RPS. Data of the present study confirm this factor and emphasise the assumption that the sensitivity of the phenol-sulfuric-acid method to polymeric RPSs is low.

The difference, detectable between average hydrodynamic volume and molecular weight of the RPS of *N. insulare* (1.1×10^6 Da and 2.8×10^6 Da, respectively) indicates the presence of branching in the structure of the RPS. The hydrodynamic volume was estimated using pullulans of known molecular weights as standards. Pullulans are linear-linked maltotriose polymers produced by the fungus *Aureobasidium pullulans*. Branching polymers are considerably more consolidated than the solely linear-linked pullulans. Using size-exclusion chromatography, such consolidated polymers were eluted together with pullulans of a lower molecular weight. This causes a difference between estimated hydrodynamic volume and the actual molecular weight of the polymer.

Hokputsa et al. (2003) determined for the RPSs of different cyanobacteria (*Microcoleus vaginatus*, *Nostoc* sp. and *Phormidium tenue*) average molecular weights in a range of 4×10^3 Da to 250×10^3 Da.

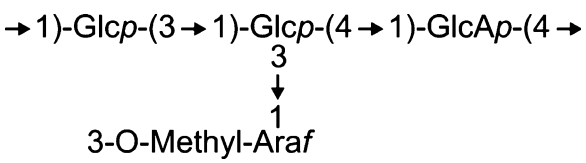

Figure 2 Proposed partial structure of the released polysaccharide (RPS) of *Nostoc insulare*.

Moreno et al. (2000) reported an average molecular weight of 1.35×10^6 Da for the RPS of *Anabaena* sp. ATCC 33047. The molecular weight found for the RPS of *N. insulare* aligns more with the latter example.

Because the reduction of uronic acids resulted not only in an increase of 1,4-Glc*p* but also in an increase of 1,3,4-Glc*p*, it is assumed that 1,3,4-Glc*p* was partly linked to 1,4-GlcA*p* in the polymer. Without a previous reduction of uronic acids, this part was masked by 1,4-GlcA*p*. The conditions of mild hydrolysis by the use of oxalic acid primarily led to a cleavage of weak bonds, such as most of the Ara-bonds. After subsequent precipitation with ethanol, 3-O-Methyl-Ara was found to dominate the monosaccharide fraction of the supernatant, indicating a terminal linkage of this sugar. This assumption was confirmed by analysis of PMAAs of the ethanol-insoluble precipitate in which not only a significant decrease of terminal Ara but also of arborisations (1,3,4-Glc*p*) and, coevally, an increase of 1,4-Glc*p* was detectable. The latter finding leads to the conclusion that terminal 3-O-Methyl-Ara formed the side chains of the polymer, which were bound to position 3 of 1,3,4-Glc*p*.

After lithium degradation of the RPS, a significant amount of 1,6-Glc*p* (25%) was detectable. This sugar was absent in the untreated RPS and was only detectable in traces in other hydrolysed fractions. It is therefore assumed that 1,6-Glc*p* was formed by conversion of 1,4-GlcA*p* during the degradation procedure. Unfortunately, an explicit reaction mechanism of lithium degradation of uronic acids is missing so far (Lau et al. 1987a, b). Besides 1,6-Glc*p*, the constituents 1-Ara*f* and 1,3-Glc*p* were detectable, approximately in a ratio of 1:1:2. The amount of 1,3-Glc*p* was significantly higher than in the untreated RPS. This and the assumption that 1,4-GlcA*p* was bound to 1,3,4-Glc*p* (see above) leads to the conclusion that, by separation of the uronic acid, 1,3-Glc*p* was formed from 1,3,4-Glc*p*, which possessed a terminal 3-O-Methyl-Ara bound to position 3 (see above) and another 1,3-Glc*p* bound to position 1. The proposed partial structure of the RPS of *N. insulare*, deduced from these assumptions, is given in Figure 2.

Cyanobacterial RPSs are, in general, characterised by a great variety both in number and type of constitutive sugars (De Philippis and Vincenzini, 1998), causing a great structural diversity of RPSs. The outcome of this is the possibility of discovering new biopolymers with outstanding properties suited to industrial applications or to other fields of interest. On the other hand, such a variety of constituents complicates structure determinations of these polymers. Due to the latter, until now, most reports that characterise cyanobacterial RPSs focus mainly on their monosaccharide composition. Only very few data are available about structure determinations of cyanobacterial RPSs, such as the reports of Helm et al. (2000) and Brüll et al. (2000) about the RPSs of different strains of *N. commune*. The present study enhances the currently knowledge in this field. However, the detection of other constitutive sugars in the RPS of *N. insulare*, such as 1,5-Ara*f*, indicates that the present structural proposal represents only a partial structure of the repeating unit of the total RPS. This confirms once more the complexity of the chemical structure of cyanobacterial RPSs.

The high viscosity of the RPS of *N. insulare*, comparable to that of xanthan gum (Fischer 1996), emphasised the possible usability of this polymer for industrial applications. However, further chemical and physico-chemical characterisations of the RPS are necessary to confirm this.

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