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Structural characterization of the members of a polymer series, compatible solutes in *Anabaena* cells exposed to salt stress

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Abstract

In response to salinity cyanobacteria synthesize different osmoprotective compounds. We had reported that *Anabaena* cells accumulate not only sucrose (Suc) but also fructose-containing oligosaccharides in the presence of NaCl and at stationary phase (Plant Sci. 167 (2004) 1003–1008). In the present study, using NMR and GC–MS we showed that the oligosaccharides previously described are members of a new series of non-reducing Suc derivatives, where glucose (Glc) is linked through its hemiacetalic hydroxyl to the 2 position of the Glc moiety of Suc, resulting in the general structure: [α-D-Glc*p*-(1 → 2)]$_n$-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*. In the continuous series, it was possible to identify members with up to 10 hexose residues, depending on the cell analysed. As each member of the series has only one Fru, that of the terminal Suc molecule, we called these polymers with the general name of sucroglucans. Their occurrence was investigated in representative cyanobacteria of different taxonomic groups either unicellular or filamentous strains, including nitrogen-fixing and non-fixing strains. The oligosaccharides were mainly detected in salt-treated cells of Section 4 strains. Therefore, together with Suc, the members of the sucroglucan series should be included among the compatible solutes synthesized in response to salt stress in filamentous cyanobacterium strains.

Keywords: Cyanobacteria; *Anabaena*/*Nostoc*; Salt-response; Compatible solutes; Oligosaccharides; Sucroglucans

1. Introduction

In plants, environmental stresses elicit a complex of responses at the cellular, physiological and developmental levels [1]. Many plant species synthesize sucrosyl oligosaccharides to cope with abiotic stresses. In all known cases, the biosynthesis of these oligomers is brought about by the transfer of one or more galactopyranosyl, glucopyranosyl or fructofuranosyl residue to either the glucosyl or the fructosyl moiety of sucrose (Suc), producing the series of raffinose, gentianose, planteose, and fructan (fructofuranosyl added at one or more of the three primary alcohol group of Suc) [2]. These oligomers are found in many plant families and, in addition to their obvious function in partitioning and storage of assimilates, they may play a role in osmoregulation. In contrast to starch, they share many chemical characteristics with Suc such as good water solubility and a non-reducing nature [3,4].

Cyanobacteria, photosynthetic oxygen-evolving prokaryotes, also synthesized osmoregulatory molecules to adapt to environmental changes [5,6]. Salinity is an abiotic factor crucial in aquatic ecosystems. An increase in salt concentration in the external medium causes a rise in the osmotic potential and in the cell ion concentration as a consequence of the loss of water. To tolerate salt stress, cyanobacteria can operate two basic processes: (i) the enhancement of active ion export systems, and (ii) the accumulation of organic osmoprotective compounds that are of low molecular mass with no net charge, known as compatible solutes as they do not interfere with the cell metabolism [7]. These osmolytes help to reduce the internal osmotic cell potential and maintain membrane integrity and protein stability. Particularly, strains belonging to the genera *Anabanea* sp. and *Nostoc*, filamentous heterocyst-forming cyanobacteria, have been reported to accumulate Suc as their main organic osmolyte [6]. Recently, we have demonstrated that cells of *Anabaena* (*Nostoc*) sp. PCC 7120 and PCC 7119, and of *A. variabilis* accumulate not only Suc but also oligosaccharides that contain fructose (Fru) in the presence of increasing NaCl concentrations. It has been also shown that it is a reversible, salt-concentration and time-dependent effect [8]. Tsusué and co-wokers had reported the presence of a homologous series of non-reducing oligosaccharides (named

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by them glucofructan) in *Tolypothrix tenuis, A. variabilis*, *Anabaenopsis* sp., *Calothrix brevissima*, *N. muscorum* and *N. commune*. They did not find those polymers in green algae, and consequently, they concluded that the oligosaccharides were one of the specific cellular constituents of cyanobacteria in general [9,10]. As we showed that the oligosaccharides containing Fru accumulate in *Anabaena* cells both in response to salt and in cells harvested at the stationary phase of growth, we suggested that the salt-response oligomers may be similar to those previously described in *A. variabilis* and related strains [9]. Tsusué and Yamakawa [10] proposed the chemical structure of the polymers, consisting in a series of glucofructan with a Glc linked with an increasing number of fructosyl units [fructofuranosyl-(3 → 4)$_n$-fructofuranosyl-(2 → 1)-α-glucopyranoside], resulting different to the plant fructan structures [2,4].

In this study, using NMR and GC–MS we showed that the oligosaccharides previously described [8] are members of a new series of non-reducing glucosyl Suc derivatives, with the following general structure: [α-D-Glc*p*-(1 → 2)]$_n$-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*. These oligosaccharides, named in general as sucroglucans, reversibly accumulate in response to the presence of NaCl in filamentous cyanobacteria, mainly of strains belonging to Section 4. Thus, together with Suc, the members of the sucroglucan series should be included among the compounds related to osmotic adjustment during a salt stress.

2. Materials and methods

2.1. Cyanobacterial strains and growth

Cells of the cyanobacteria *Anabaena* (*Nostoc*) sp. PCC 7119, *Anabaena* (*Nostoc*) sp. PCC 7120, *A. variabilis* PCC 7937, *Fischerella* sp. PCC 7520, *Gloeothece* sp. PCC 6909, *Nostoc* sp. PCC 7107, *Nostoc* sp. PCC 7413, *N. ellisosporum* (B1453-7), *Oscillatoria* sp. PCC 6304, *Synechocystis* sp. PCC 6301, *Synechococcus* sp. PCC 7942, *Synechococcus* sp. PCC 6714 and *Synechocystis* sp. PCC 6803 were cultivated in BG-11 liquid medium [11,12] replacing the $NaNO_3$ by KNO_3 [13]. *N. punctiforme* sp. ATCC 29133 cells were grown in Allen and Arnon medium (1/4) as previously described [14]. Salt stress was applied to cultures at exponential phase by adding NaCl up to a final concentration of 80 mM. After 24 h of treatment, cells were harvested, washed as described [12] and stored at −80 °C until sugar extraction was performed. For large amount of sugar preparations, cells were grown under 5% CO_2 enriched air.

2.2. Extraction and determination of sugars

Packed cells (5 g) were extracted three times with boiling alkaline water (2 ml g^{-1}) for 5 min. The extracts were pooled for sugar determination and lyophilized for further analyses. Suc was quantified after hydrolysis with invertase by measuring the formation of NADPH [15] or with the Somogyi–Nelson reagents [16]. Fru-containing oligosaccharides were determined by the thiobarbituric acid method (TBA) after incubation with sucrase and alkaline destruction of monosaccharides [17].

2.3. Thin layer chromatography

Sugar extracts were desalted by passage through mixed bed ion exchange resins (AG 501, 20–50 mesh, BioRad). Mono- and oligosaccharides were separated by thin layer chromatography (TLC) using Schleicher and Schuell F-1500 ready foils by developing three times with 1-butanol:isopropanol:water (3:12:4) [18]. The position of Fru-containing oligosaccharides was determined by development with urea-phosphoric acid reagent [17,19]. Standard sugars were chromatographed in parallel.

2.4. Preparation of oligosaccharides

Sugars from lyophilized extracts were dissolved in 0.5 ml of water and loaded onto a Bio-Gel P2 (200–400 mesh) column (2 cm × 230 cm) [18]. Elution was carried out with water and collected fractions were assayed for Fru-containing oligosaccharides content by the TBA method [17,20]. Fractions under each peak were pooled and frozen for further analysis.

2.5. Methylation analyses

Methylation analyses of the oligosaccharides were performed at the Complex Carbohydrate Research Center (CCRC, University of Georgia, USA). Samples were permethylated as described by Hakomori [21]. They were hydrolyzed with 0.5 M trifluoroacetic acid (TFA) at 60 °C for 30 min and reduced with sodium borodeuteride at room temperature. The products were then hydrolyzed with 2 M TFA at 120 °C, reduced with sodium borodeuteride at room temperature and acetylated with acetic anhydride and TFA. The partially methylated alditol acetates were analysed by gas chromatography–mass spectrometry (GC–MS).

2.6. NMR experiments

NMR experiments were carried out at CCRC, Georgia, USA. Samples were dissolved in D_2O and lyophilized. This procedure was repeated one more time, and the resulting residues were dissolved in 0.5 ml D_2O and analysed by NMR using a Varian 600 MHz instrument. The NMR analysis included obtaining 1D proton, a 2D correlation spectroscopy (2D COSY), 2D total correlation spectroscopy (2D TOCSY), and 2D nuclear overhauser enhancement spectroscopy (NOESY) spectra. The varian software was used to run these NMR experiments.

2.7. Biochemichal analyses of the oligosaccharides

The action of yeast invertase and [α-D-(1 → 4)] glucosidase was tested on the oligosaccharides. Products were characterized by incubation in a mixture containing ATP-Mg^{2+}, NADP, and the auxiliary enzymes hexokinase, phosphoglucoisomerase and glucose-6-phosphate dehydrogenase [22]. Hexoses:Fru ratio for each oligomer was established by determining total hexoses and Fru in each sample using the anthrone method and

the TBA reagent, respectively [16,17,20]. The trisaccharides, tetrasaccharides and pentasaccharides were partially hydrolyzed in the presence of 0.5 M TFA at 70 °C and fully hydrolyzed after 30 min in 0.5 M TFA at 120 °C. The products were freeze-dried and dissolved in water. The monosaccharides liberated were identified after incubating in a reaction mixture containing ATP-Mg^{2+}, NADP and the auxiliary enzymes described above [22].

2.8. Oligosaccharide biosynthesis

Oligosaccharide synthesis was assayed in toluene-permeabilized *Anabaena* cells as previously described [8]. The reaction mixture (50 μl total volume) contained toluene-treated cells [^{14}C]-Fru-6-P (specific activity 2×10^5 cpm μmol^{-1}), UDP-[^{14}C]-Glc (specific activity 7×10^5 cpm μmol^{-1}), 10 mM $MgCl_2$, and 100 mM Hepes-NaOH (pH 7.5). The assays were carried out at 30 °C. After 4 h, the incubation mixtures were desalted, and sugars separated by chromatography and quantified by determining the labeled sugars. Standard sugars (Suc and *Anabaena* sp. PCC 7119 trisaccharide) were chromatographed in parallel [8].

3. Results

3.1. Occurrence of Fru-containing oligosaccharides in salt-treated cyanobacterium cells

It has been shown recently that *Anabaena* (*Nostoc*) sp. PCC 7119, *Anabaena* (*Nostoc*) sp. PCC 7120 and *A. variabilis*, fresh water, filamentous heterocyst-forming cyanobacteria, accumulate no only Suc but also Fru-containing oligosaccharides in response to salt stress [8]. In order to find out whether this was a general response of cyanobacteria to a NaCl treatment, the occurrence of the oligosaccharides was investigated in unicellular and filamentous strains, belonging to different taxonomic groups (Sections 1, 3–5) [11]. The presence of the salt-response oligosaccharides was detected in salt-treated cells of filamentous strains most belonging to Section 4 and of one strain of Section 3 (*Oscillatoria* sp. PCC 6304) [11]. On the other hand, the oligosaccharides were not found in the strains studied of Sections 1 and 4 (*Synechococcus* sp. PCC 7942, *Synechococcus* sp. PCC 6714, *Synechocystis* sp. PCC 6301, *Synechocystis* sp. PCC 6803 and *Fischerella* sp. PCC 7520). When sugar extracts were submitted to TLC, the oligosaccharides migrated in a similar position to those of *Anabaena* sp. PCC 7120 [8], and differently to plant fructans prepared from *Helianthus tuberosus* tubers and *Triticum aestuvum* leaves. As an example, we show the migration of *N. punctiforme* oligomers and those of plants (Fig. 1). Fru-containing oligosaccharide level was higher in *N. ellisosporum* and *N. punctiforme* than in *Anabaena* sp. PCC 7120 and *A. variabilis* cells (Table 1).

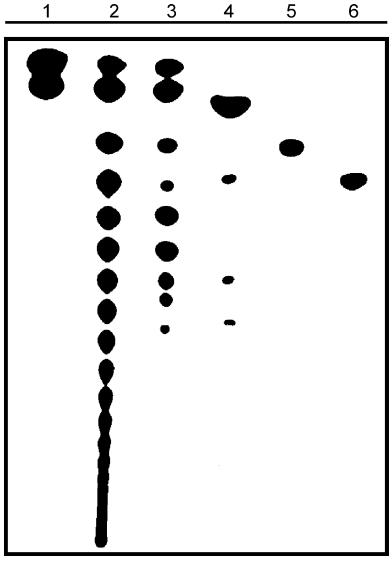

Fig. 1. Thin-layer chromatography of Fru-containing oligosaccharides from *N. punctiforme* and plant fructans. Lanes 1 and 5: standard sugars (Suc and Fru, and 1-kestose, respectively); lanes 2 and 3, *Helianthus tuberosus* and *Triticum aestivum* fructans, respectively; lane 4, soluble sugar extracts from *N. punctiforme* salt-treated cells; lane 6, concentrated fractions corresponding to peak II from Bio-Gel P2 chromatography (see Fig. 2).

Table 1
Effect of NaCl on sucrose (Suc) and Fru-containing oligosaccharide content in cells of different filamentous heterocyst-forming cyanobacterium strains

Cyanobacterium strain	Suc (nmol (mg FW)$^{-1}$)		Fru-oligosaccharides (nmol (mg FW)$^{-1}$)		Reference
	Control	+NaCl	Control	+NaCl	
Anabaena sp. PCC 7120	0.5 ± 0.2	10.3 ± 2.6	0.7 ± 0.2	5.9 ± 1.0	[8]
A. variabilis	0.3 ± 0.1	10.0 ± 1.6	1.2 ± 0.4	5.5 ± 1.3	[8]
N. punctiforme sp.	0.8 ± 0.2	16.0 ± 1.5	3.0 ± 0.6	14.8 ± 2.3	This work
N. ellisosporum sp.	1.5 ± 0.4	15.1 ± 1.7	2.3 ± 1.3	15.5 ± 2.1	This work

Salt (80 mM) was added to cultures at the exponential phase and cells were harvested after 24 h of treatment. Data are the mean and standard deviation of three independent experiments.

3.2. Separation of Fru-containing oligosaccharides by gel filtration chromatography

To study the polymer structures, sugar extracts from salt-treated *Anabaena* sp. PCC 7120 cells were chromatographed in preparative Bio-Gel P2 columns [18]. The oligosaccharides separated in various fractions of increasing molecular mass (Fig. 2A). The fractions under each peak were pooled and concentrated and those pools corresponding to peaks III–V were re-chromatographed individually in Bio-Gel P-2 columns (Fig. 2B–D). Fractions under each major peak were pooled and concentrated for further characterization. Similar procedures were done with sugar extracts from salt-treated cells of *N. ellisosporum*, *A. variabilis* and *N. punctiforme* (not shown).

3.3. Characterization of the different oligosaccharides

Aliquots of each pool were submitted to the action of yeast invertase. The sugar under peak I was fully hydrolyzed by the enzyme (Fig. 3A). The hydrolysis products were characterized

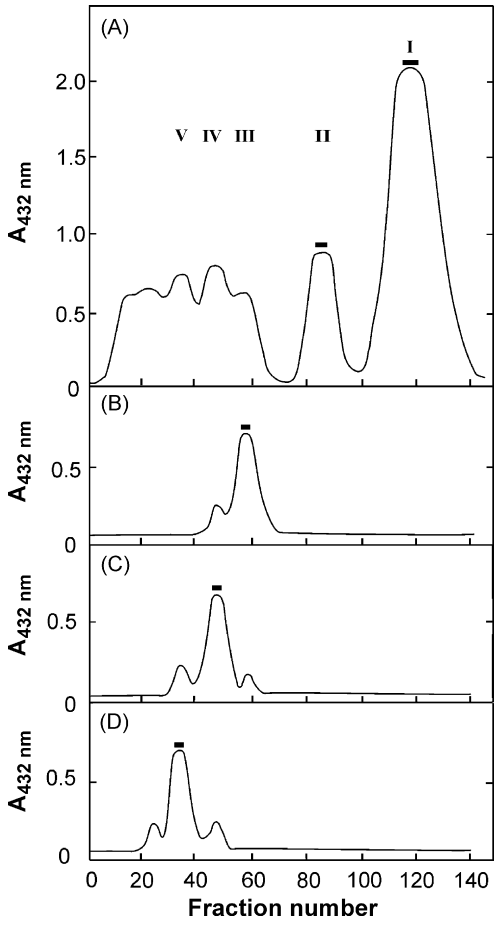

Fig. 2. Bio-Gel P2 chromatography of soluble sugars extracted from salt-treated cells of *Anabaena* sp. PCC 7120. NaCl (80 mM) was added to exponential phase cultures and cells were harvested after 24 h of treatment. (A) Total sugar extract was loaded onto the column. (B–D) Concentrated fractions under peak III–V, respectively, were re-chromatographed individually. Fractions were analysed by the TBA method. Standard Suc eluted in a position similar to fractions of peak I.

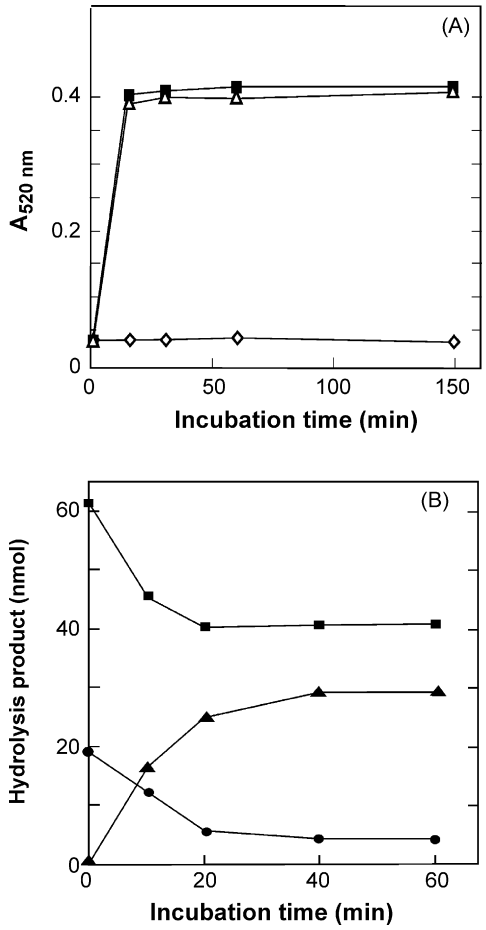

Fig. 3. Hydrolysis of the sugar of Bio-Gel P2 peak synthesized in *Anabaena* sp. PCC 7120 salt-treated cells. Fractions eluted under peak II (Bio-Gel P2 chromatography, Fig. 2) were pooled, concentrated and submitted to enzyme or acid hydrolysis. (A) Incubation of Suc (■), 1-kestose (△) and peak II sugar (◇) with yeast invertase, Reducing sugars were determined by the Somogyi–Nelson reagent. (B) Peak II sugar was hydrolysed with 0.5 M TFA at 70 °C and total hexoses (■), reducing sugars (▲) and Fru (●) were quantified by the anthrone, Somogyi–Nelson and TBA reagents, respectively.

as Fru and Glc in 1:1 ratio after incubating in the presence of ATP-Mg^{2+}, NADP, and auxiliary enzymes. Thus, peak I fractions were ascribed to Suc, which was reconfirmed by NMR analysis (not shown). On the other hand, the sugar under peak II was not hydrolyzed by invertase and did not react with the Somogyi–Nelson reagent indicating that it has no reducing power (Fig. 3A). Similar results were obtained for the oligosaccharides present under peaks III–V (not shown).

Different biochemical analyses were then carried out to determine oligosaccharide compositions. The total-hexose:-Fru ratios resulted 3:1, 4:1, 5:1 and 6:1 for the sugars of peaks II–V, respectively. Therefore, the oligosaccharides present in fractions eluted under peaks II–V seemed to be tri-, tetra-, penta-, and hexasaccharides, respectively. The trisaccharide of peak II was submitted to TFA hydrolysis at 70 °C. At different times, three aliquots of the reaction were taken, freeze dried, dissolved in water and analysed either by Somogyi–Nelson, or anthrone or TBA reagents (Fig. 3B). During TFA hydrolysis, while Fru was liberated and partially destroyed, concomitantly, it appeared a compound composed of two of the three hexoses of the trisaccharide, which had a reducing power lower than that of Glc and seemed to be partially acid resistant. Also, the trisaccharide was fully hydrolyzed in TFA at 120 °C, and the monosaccharides liberated were quantified and identified with coupling auxiliary-enzyme reactions. We confirmed that two Glc and one Fru composed the trisaccharide.

3.4. Structure of the salt-response oligosaccharides

A sample of the trisaccharide was methylated and the resulting alditol acetates were analysed by GC–MS. The partially acetylated derivative analysis showed the presence of 1,3,4,6-tetra-*O*-methyl Fru, 3,4,6-tri-*O*-methyl Glc, and 2,3,4-tetra-*O*-methyl Glc in a ratio 1:1:1 (Table 2), which indicate that the sugar corresponding to peak II is composed by two Glc and one Fru, with a 1-linked Glc, a 1,2-linked Glc and a 2-linked Fru. The sample was also analysed by NMR. The 1D NMR spectrum was consistent with the previous methylation data that showed the

Table 2
Methylation and GC–MS analysis of the Fru-containing trisaccharide of *Anabaena* sp. PCC 7120

Partially acetylated derivative	Retention time (min)	Peak area	%
1,3,4,6-Tetra-*O*-methyl fructose	12.51	1252	31.5
3,4,6-Tri-*O*-metil glucose	16.76	1480	37.4
2,3,4,6-Tetra-*O*-methyl glucose	13.71	1236	31.3

presence of two glucosyl residues, a terminally 1-linked residue and a 1,2-linked residue, and that both glucosyl residues were in the α-configuration. Thus, the 2D NMR data were consistent with the presence of a trisaccharide with the following structure: α-D-glucopyranosyl-(1 → 2)-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside (Fig. 4). The other oligosaccharide fractions (corresponding to fractions under peak III–V of the Bio-Gel P2 chromatography profile) re-chromatographed individually in Bio-Gel P2 columns (Fig. 2B–D), were also submitted to GLC and NMR analysis. The spectrum data confirmed the presence of a tetra-, penta-, and hexasaccharide in the fractions corresponding to peak III–V, respectively (Fig. 5). The assignment for each glucosyl and fructosyl residue was determined as given in Table 3. H1 resonance signals clearly showed the presence of 3, 4 and 5 α-linked glucosyl residues in the tetra-, penta- and hexasaccharide, respectively. The ROESY spectra indicated that the glucosyl residues of the oligomers were α-(1 → 2)-linked and that the glucosyl residue A being α-linked to the terminally linked fructosyl residue at position 2. This was also confirmed by GC–MS analysis of methylated alditol acetates derivatives of the three oligomers (not shown). The analyses confirmed that the members of the polymer series consist of an α-(1→2) chain of D-glucosyl units attached to the 2-COH position of the Glc moiety of Suc, which can be written as: [α-D-glucopyranosyl-(1 → 2)]$_n$-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside, where n means degree of polymerization (DP). The continuous series present in salt-treated *Anabaena* and *Nostoc* cells is composed by oligosaccharides with DP ranging from 1 to 8. Similar structural features were found when the polymers isolated from the other cyanobacterial strains were analysed (not shown). We called these polymers with the general name of sucroglucans.

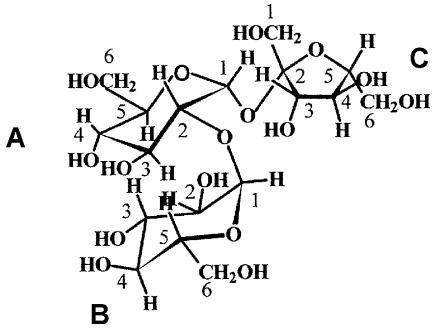

Fig. 4. Structure of the trisaccharide synthesized in salt-treated filaments of *Anabaena* sp. PCC 7120.

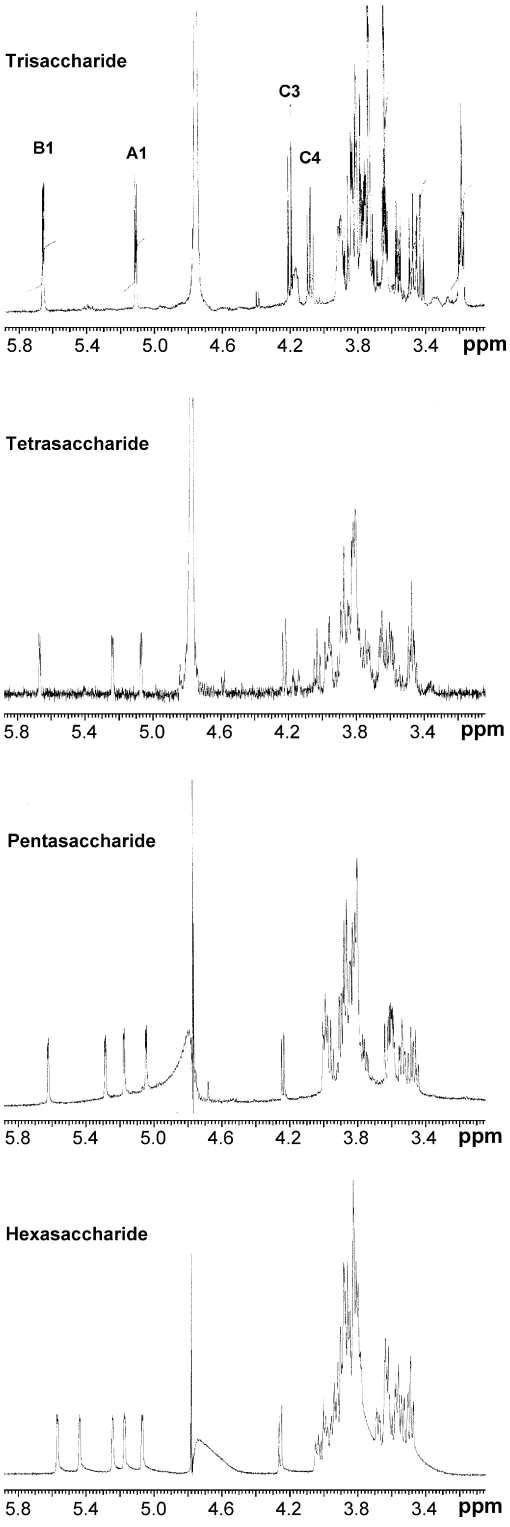

Fig. 5. 1D NMR spectra of the tri-, tetra, penta- and hexaoligosaccharides extracted from salt-treated cells of *Anabaena* sp. PCC 7120.

3.5. Biosynthesis of cyanobacterial sucroglucans

Previously, we had shown that *Anabaena* oligosaccharides seemed not to be synthesized from Suc [8]. On the other hand, the general structure of the polymers suggested that their synthesis would take place by the addition of glucosyl units to

Table 3
^{1}H NMR chemical shifts for salt-stress *Anabaena* oligosaccharides

Oligosaccharide	H1	H2	H3	H4	H5	H6
Tetrasaccharide						
Glc A	5.68	3.61	3.90	3.49	n.d.	n.d.
Glc B	5.25	3.66	3.83	3.49	3.88	n.d.
Glc C	5.08	3.60	3.81	3.47	3.97	n.d.
Fru	(3.25)	–	4.24	4.05	3.83	3.83/3.89
Pentasaccharide						
Glc A	5.63	3.81	3.86	3.52	3.86	n.d.
Glc B	5.29	3.59	3.80	3.44	3.86	n.d.
Glc C	5.17	3.58	3.95	3.53	3.98	n.d.
Glc D	5.04	3.59	3.84	3.47	3.99	n.d.
Fru	(3.78)	–	4.23	3.98	3.88	3.83/3.86
Hexasaccharide						
Glc A	5.58	3.64	3.92	3.59	3.92	n.d.
Glc B	5.45	3.68	3.81	3.49	4.05	n.d.
Glc C	5.26	3.57	3.94	3.53	3.99	n.d.
Glc D	5.19	3.63	3.86	3.56	3.96	n.d.
Glc E	5.08	3.63	3.89	3.49	4.05	n.d.
Fru	(3.88)	–	4.26	4.01	3.89	3.91/3.93

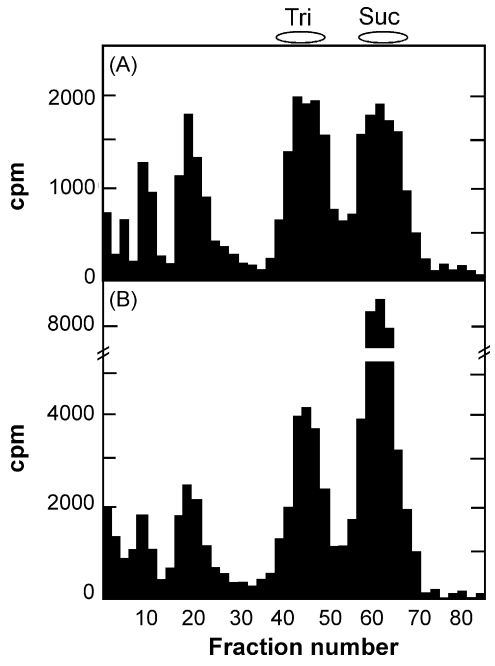

Fig. 6. Biosynthesis of sucroglucans in toluene-permeabilized cells of *Anabaena* sp. PCC 7120. Chromatographic analysis of the products of incubating salt-treated permeabilized cells with [^{14}C]-Fru-6-P and UDP-[^{14}C]Glc. Cells were harvested after 24 h after the addition of NaCl (A) 20 mM or (B) 80 mM. Standard sugars (Suc and the trisaccharide purified from *Anabaena* sp. PCC 7119 salt-treated cells) were localized in parallel strips.

Suc. A very likely candidate to be the glucosyl donor would be a sugar nucleotide, like UDP-Glc. In a preliminary approach we tested the polymers synthesis in situ by incubating toluene permeabilized cells of *Anabaena* sp. PCC 7120 with UDP-[^{14}C]Glc and [^{14}C]-Fru-6-P, substrates for Suc synthesis [23]. The results presented in Fig. 6 indicate that the oligosaccharide synthesis occurs and that it is dependent on the NaCl concentration present in the culture medium.

4. Discussion

In cyanobacteria, the physiological mechanisms responsible for salt tolerance and osmotic adjustment have been widely studied. The synthesis of compatible solutes is one of the main cyanobacterial salt-adaptation strategies to cope with the rise in the osmotic potential and in the cell ion concentration [6]. It has been reported that these molecules are also able to protect the cell from other types of desiccation, as well as from cold and heat stress [6,24]. Recently, we have shown that not only Suc but also Fru-containing oligosaccharides accumulate in cells of *Anabaena* sp. PCC 7119, PCC 7120 and *A. variabilis* in the first hours after exposure to NaCl [8]. As Tsusué and co-workers [11] had described the presence of a homologous series of non-reducing oligosaccharides (named glucofructan) in some cyanobacteria, we suggested that the salt-response oligosaccharides may be similar to those reported by them [8]. The present study shows that the oligomers accumulated in response to a salt treatment in other filamentous cyanobacterial strains, mainly classified in Section 4 [23]. When we investigated biochemical characteristics of members of the series present in *Anabaena* sp. PCC 7120, *A. variabilis*, *N. ellisosporum*, and *N. punctiforme*, we found that the ratio Glc:Fru did not correspond to that of glucofructans [11]. Using NMR and GC–MS we showed that the oligosaccharides belong to a new series of Glc polymers based on Suc. The first member of the series is formed by addition of one Glc unit linked through its hemiacetalic hydroxyl to the 2 position of the Glc moiety of Suc. The remaining members of the series are formed by a similar linkage. Thus, the general structure of the oligosaccharides is: [α-D-Glc*p*-(1 → 2)]$_n$-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*. As each oligomer has only one Fru, that of the terminal Suc molecule, we called these polymers with the general name of sucroglucans. The continuous series present in salt-treated cells of the filamentous cyanobacterial strains studied is composed by oligosaccharides with DP ranging from 1 to 8. Among the oligosaccharides based on Suc found in nature, only umbelliferose [α-D-galactopyranosyl-(1 → 2)-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*], an isomer of raffinose, exhibits a (1 → 2) linkage. However, no higher homologs of this trisaccharide has been found [2].

In addition to their obvious function as carbohydrate reserve, plant oligomers of the raffinose and fructan families, the most widespread oligosaccharides based on Suc, may also play an important role in tolerance to abiotic stress, like low temperature, dehydration and salinity [1–4]. Similarly, cyanobacterial sucroglucans are a series of water-soluble compounds that may be envisaged as involved in stress tolerance. In this respect, we had shown their accumulation after a salt treatment and in stationary phase cells (nutritional stress) [8]. During the writing of the present work, it was reported the occurrence of similar oligosaccharides in cyanobacteria exposed to heat stress and a possible function as thermoprotectants was proposed [26]. Whether the accumulation of sucroglucans is associated with a stress response or is just an indication that growth is ceasing is still a point of discussion. Thus, salt-shocked *Anabaena* sp. PCC 7119 and 7120 cultures exhibited a decline

in growth [Curatti unpublished] as it happens in plants in response to cold stress or drought [25]. The fact that cyanobacterial polymer accumulation could be reverted a few hours after stopping the stress, strongly suggests that they may play a role in osmoregulation, accumulating as osmotically inert polymers that rapidly may be converted into osmotically active monosaccharides, as shown for plant Suc-based oligosaccharides [4].

Cyanobacterial sucroglucans seem to be synthesized by a series of glucosyl transfers from UDP-Glc, first to Suc, and then to each higher homolog. Thus, we suggest that the first step in the biosynthetic pathway would be similar to the biosynthesis of umbelliferose from UDP-galactose and Suc [2]. The formation of [α-(1 → 2)] linkages is likely to require obligatory a sugar nucleotide as donor. On the other hand, sucroglucans are not hydrolyzed by [α-D-(1 → 4)] glucosidase, invertase or sucrase activity, suggesting that a specific hydrolase should be responsible for their degradation. Work is in progress to characterize the enzymes involved in sucroglucan metabolic pathway.

In summary, in addition to Suc, sucroglucans, non-reducing Suc-derived oligosaccharides exhibiting a seldom-found linkage [α-(1 → 2)] among their Glc residues, can accumulate as a response to environmental stress. Particularly, this novel polymer series should be considered as cyanobacterial compatible solutes related to osmotic adjustment during environmental adaptation to salinity.

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