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Characterization of the *Nannochloropsis gaditana* storage carbohydrate: a 1,3-beta glucan with limited 1,6-branching

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Abstract

As a result of a secondary endosymbiosis of a red alga, stramenopiles retained β-1,3 glucans as the primary soluble storage carbohydrate, in contrast to the starch and glycogen of green plants and animals. This storage carbohydrate has been identified and characterized in representative diatoms, brown algae, and oomycetes, but has not been biochemically characterized in species of Nannochloropsis*,* an industrially relevant genus of algae favored for its lipid content and productivity. In this study, the soluble storage carbohydrate of Nannochloropsis is characterized by ^{1}H-NMR, linkage analysis, and size exclusion chromatography. The putative genes encoding enzymes required for synthesis of the carbohydrate oligosaccharide from glucose 6-phosphate are identified. A glycogenin-like glycosyltransferase family 8 (GT8) protein was also identified to be conserved among all species of Nannochloropsis, despite the lack of glycogen in the genus. Homologs were identified in available genomes of brown algae, diatoms, and oomycetes, all documented to utilize β-1,3 glucans for carbon storage. Finally, a set of three likely laminarinases are highlighted from the glycosyl hydrolases by phylogenetic analyses.

Keywords: Nannochloropsis, carbohydrate, glycoside hydrolase, laminarin, laminarinase

Highlights

- *N. gaditana* stores a 1,3-beta glucan carbohydrate with rare 1,6-branching
- Genus-conserved glycogenin-like protein may nucleate glucan polymerization
- Heterokont-wide phylogenetic analysis identifies likely laminarinases

1. Introduction

In response to stress, many algae accumulate chemical energy stores in the form of lipids and/or carbohydrates that can used to maintain cellular viability until growth conditions that support cell proliferation are restored. Nannochloropsis is a genus of algae of biotechnological interest due to its relative stability in outdoor cultivation, and significant lipid production; especially in response to the stress of nitrogen-starvation [1–3]. Species of Nannochloropsis have been shown to accrue lipid to greater than 60% of their dry cell weight [4]. Although much of the stored energy in species of Nannochloropsis is in the form of triacylglyceride (TAG), Nannochloropsis has also been demonstrated to accumulate a water-soluble storage carbohydrate during the day of a diel light-cycle and consuming this carbohydrate throughout the night [5].

While most organisms store carbon in α-1,4-glucans, stramenopiles typically synthesize β-1,3-glucans to fulfill a similar functional role. In brown algae, this polysaccharide is called laminarin, with occasional 1-linked D-mannitol at non-reducing ends, and has short β-1,6-linked branches. Diatoms produce a polysaccharide called chrysolaminarin which is structurally similar to laminarin but lacks mannitol. The mycolaminarin of oomycetes is biochemically closest to the chrysolaminarin of diatoms. Nannochloropsis is a genus within eustigmatophyceae, another class of stramenopiles. Recent work suggests that Nannochloropsis synthesizes laminarin as the storage glucan based on the presence of polymeric mannitol in the intracellular neutral sugars of *Nannochloropsis oceanica* IMET1 as detected after ion chromatography [6], but the storage glucan has not yet been confirmed.

Although the soluble carbohydrates in species of Nannochloropsis have frequently been described as laminarin or chrysolaminarin, little biochemical evidence supporting this assignment or characterizing the nature of the soluble carbohydrate in species of Nannochloropsis has been reported [7]. Here we use a variety of analytical techniques (^{1}H-NMR, glycosyl composition, glycosyl linkage analysis, and size exclusion chromatography) to

characterize the most abundant soluble carbohydrates oligomers in *Nannochloropsis gaditana* CCMP526.

An understanding of the carbohydrate structure and the enzymes involved in its synthesis and consumption will help us to understand energy management and physiological culturing phenotypes, especially during diel growth, in this phototroph and related algae. Additionally, manipulation of carbohydrate accumulation may allow directing increased carbon flux toward TAG, or alternatively into more carbohydrate [8–11].

The presence of β-1,3-glucans as a storage carbohydrate in this evolutionary lineage is peculiar, and a better understanding of all the genes involved, as well as the energetic role within the cell, will likely help efforts to understand how and why this unique structure was maintained. It is believed that the last eukaryotic common ancestor had the tools for synthesizing both α-1,4- and β-1,3-glucans [12], and that the primary endosymbiosis of cyanobacteria, employing an α-1,4-glucan, gave rise to a metabolism built around starch in green and red algae.

2. Materials and Methods

2.1. Carbohydrate extraction

Soluble carbohydrates were extracted from 50 ml of *Nannochloropsis gaditana* CCMP526 culture grown under constant light (90 µmol photons $m^{-2} \cdot s^{-1}$) in a 250 ml flask, shaking at 200 rpm in air-level CO_2 at 24-26 °C as shown in Figure 1. The culture was grown to late exponential phase (two weeks) and centrifuged for 10 min at 3500 rcf and the supernatant decanted. The cell pellet was resuspended in 4.5 ml of deionized water and transferred to six 1.7 ml tubes. These tubes were centrifuged at 15,000 rcf for 3 min and the supernatant was removed. Approximately 100 mg of garnets (BioSpec Products cat. no. 11079103gar) and 0.75 ml 100% methanol was added to each tube. The samples were vortexed for 5 min to lyse the cells. The cells and garnets were centrifuged again, and the supernatant removed. This process was repeated three more times, until the green color was no longer visible in the pellet.

The lysate was resuspended in 0.75 ml of 0.1 M HCl and incubated at 70 °C for 1 h. The insoluble cell debris was pelleted by centrifugation and the soluble supernatant was transferred to 15 ml tubes. The soluble carbohydrate was precipitated by the addition of 9 volumes of 100%

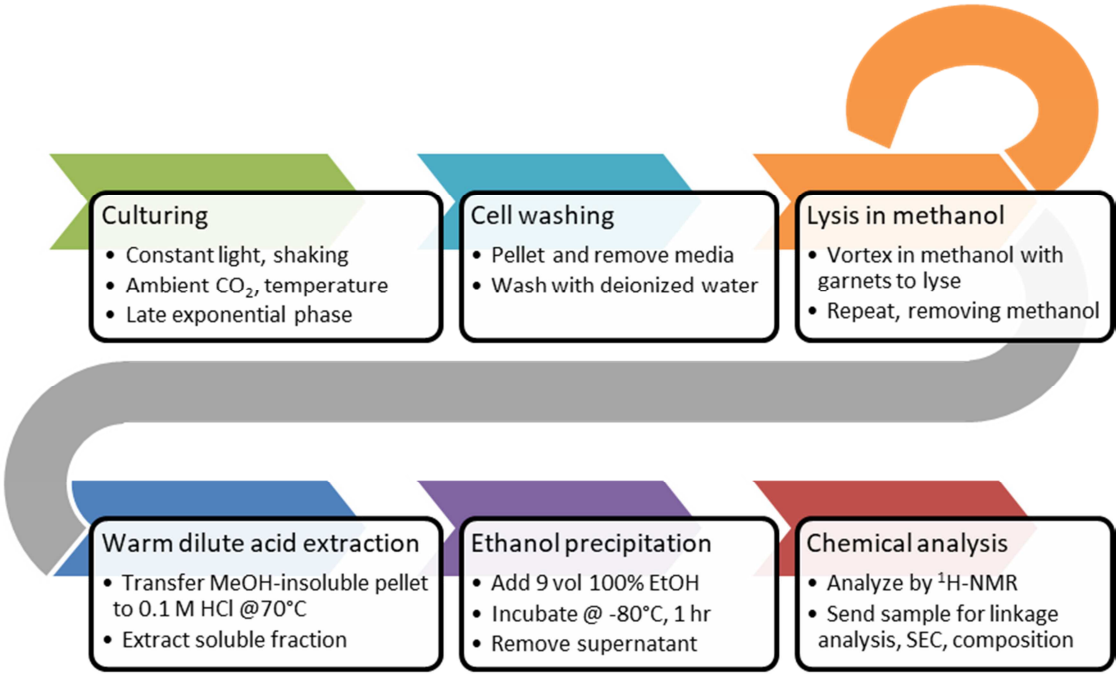

Figure 1. Extraction process for the soluble carbohydrate from *Nannochloropsis* involves harsh lysis in methanol, followed by extraction in dilute HCl.

ethanol and a 1-h incubation at -80 °C. Following this, the carbohydrate was pelleted by centrifugation at 3500 rcf for 30 min. The supernatant was removed, and the remaining solvent was removed using a lyophilizer. The dried sample was subsequently analyzed by proton NMR, size exclusion chromatography, and carbohydrate residue and linkage analysis.

2.2. ^{1}H-NMR analysis

Approximately 6 mg of *N. gaditana* soluble carbohydrate extract or laminarin from *Laminaria digitata* [used as a control (Sigma Aldrich L9634)] was dissolved in 1 ml of 6:1 DMSO-d_6:D_2O solvent, to which 4 drops of trifluoroacetic acid (TFA) were added. Proton scans of the *N. gaditana* sample were taken from 25 °C to 75 °C in increments of 10 °C. The sample temperature of 25 °C showed optimal placement for the water peak in that there was no overlap

with the surrounding peaks of interest. A JOEL AC-500 NMR spectrometer acquired 2800 ^{1}H-NMR scans for each sample at 25 °C, using 5.89 µsec (45°) pulses with a 5.75 s repetition time. The resulting data were line-fitted using MestReNova software (Supplemental Table 1), utilizing a mixed Lorentzian/Gaussian lineshape function with least-squares optimization.

2.3. Size exclusion chromatography

Carbohydrate sizing was performed by the Complex Carbohydrate Research Center (Athens, GA), who also provided residue and linkage analysis on the same 6 mg sample used for ^{1}H-NMR analysis. For size exclusion chromatography, the sample was brought to 1 mg/mL in 50 mM ammonium acetate, pH 5.5 and filtered (0.45 µm) before injection. A volume of 100 µl of sample was resolved first using a Superose 12 PC 3.2/30 column (GE Healthcare Life Sciences) with an ELS detector, which indicated the dominance of short-length carbohydrates (Supplementary Figure 1). A second analysis using a Superose 12 10/300 GL column with refractive index detection was performed for better resolution at the smaller sizes of interest.

2.4. Linkage analysis

For glycosyl linkage analysis, the sample was permethylated, depolymerized, reduced, and acetylated. The resultant partially methylated alditol acetates (PMAAs) were analyzed by gas chromatography-mass spectrometry (GC-MS) as described by Heiss et al. [13].

Linkage analysis was performed on soluble carbohydrate extracts from three biological replicates. The samples were suspended in 200 µl of dimethyl sulfoxide and left to stir for 3 days. Permethylation was effected by two rounds of treatment with sodium hydroxide (15 min) and methyl iodide (45 min). Following sample workup, the permethylated material was hydrolyzed using 2 M TFA (2 h in sealed tube at 121 °C), reduced with $NaBD_4$, and acetylated using acetic anhydride/TFA. The resulting PMAAs were analyzed on an Agilent 7890A GC interfaced to a 5975C MSD (mass selective detector, electron impact ionization mode); separation was performed on a 30 m Supelco SP-2331 bonded phase fused silica capillary column.

2.5. Degrees of polymerization and branching calculations

The degree of polymerization (DP) and degree of branching (DB) were calculated from the ^{1}H-NMR data according to Kim et al. [14], using the Lorentzian/Gaussian line fit data. Formulas for calculating the corresponding statistics from linkage data were arrived at by using the sum of all laminarin-related residues (3-linked glucopyranosyl, terminal glucopyranosyl, terminal mannopyranosyl, and 3,6-linked glucopyranosyl) as the numerator of DP and denominator of DB. Instead of the reducing terminus, the number of particles was calculated by taking the terminal residues and subtracting the branches. The 3,6-linked residues were used to calculate the branching.

2.6. Phylogenetic analysis

The glucan synthase, Kre6-like branching enzyme, and glycogenin-like protein were BLAST searched against the non-redundant protein sequences of 26 selected species, spanning vascular plants, green alga, heterokonts, and fungi (Supplemental Table 2). The best match from each organism with an E-value below 10^{-30} was used to construct a ClustalW alignment for each protein, using a BLOSUM cost matrix. These alignments were used to create PhyML trees with the Le Gascuel substitution method and 100 bootstraps [15].

3. Results and discussion

3.1. Nannochloropsis produces a β-1,3-linked soluble storage glucan with β-1,6 branching

It has been largely assumed that Nannochloropsis species utilize a storage carbohydrate structured similarly to its evolutionary neighbors, diatoms (chrysolaminarin [16]) and brown alga (laminarin [12,17]). However, the storage carbohydrate of Nannochloropsis has not been biochemically characterized. Using ^{1}H-NMR, glycosyl composition, glycosyl linkage analysis, and size exclusion chromatography, we support this general structure of β-1,3-linked glucans with occasional β-1,6 branching and provide approximate degrees of polymerization (DP) and branching (DB).

Sample	DP	DB	Method	Referenced Work
Laminarin	33	0.07	^{1}H-NMR	Kim et al. [14]
Laminarin	28	0.063	^{1}H-NMR	Caballero et al. [18]
P. tricornutum	17	0.015	^{1}H-NMR	Caballero et al. [18]
Laminarin	13	0.043	^{1}H-NMR	This study
N. gaditana	8	0.028 - 0.105	^{1}H-NMR	This study
N. gaditana	8.1 - 9.2	0.0036 - 0.0071	Linkage	This study
Laminarin	30.3	0.052	Linkage	Read et al. [17]

Table 1. The degree of polymerization (DP) and degree of branching (DB) are calculated separately using the ^{1}H-NMR and linkage data reported here. For context, DP and DB measurements of soluble carbohydrates from diatoms and brown alga are provided.

Both our linkage and ^{1}H-NMR data indicate degrees of polymerization much shorter than those reported for other stramenopiles (Table 1). By size exclusion chromatography, greater than 90% of the total soluble carbohydrate peak area was <5 kDa, corresponding to 30 glucose residues or less (Supplementary Figure 1). Roughly, 70% of the peak area corresponded to molecular weights of 1 kDa and less, or carbohydrates of 7 glucose residues and shorter.

Because the ^{1}H-NMR spectra in the region correlating to the proton of the side chain terminus anomeric carbon (4.24-4.29 ppm) is less clear in the *N. gaditana* trace than for the *L. digitata* extract, we provide upper and lower bounds for the area of this signal (Figure 2). This range is carried through the calculation of DB, resulting in the presented possible range.

Results of the linkage analysis showed further evidence of minor cellulose contamination (Figure 3, 4-linked residues), which may have caused an underestimation of DP by increasing the observed terminal

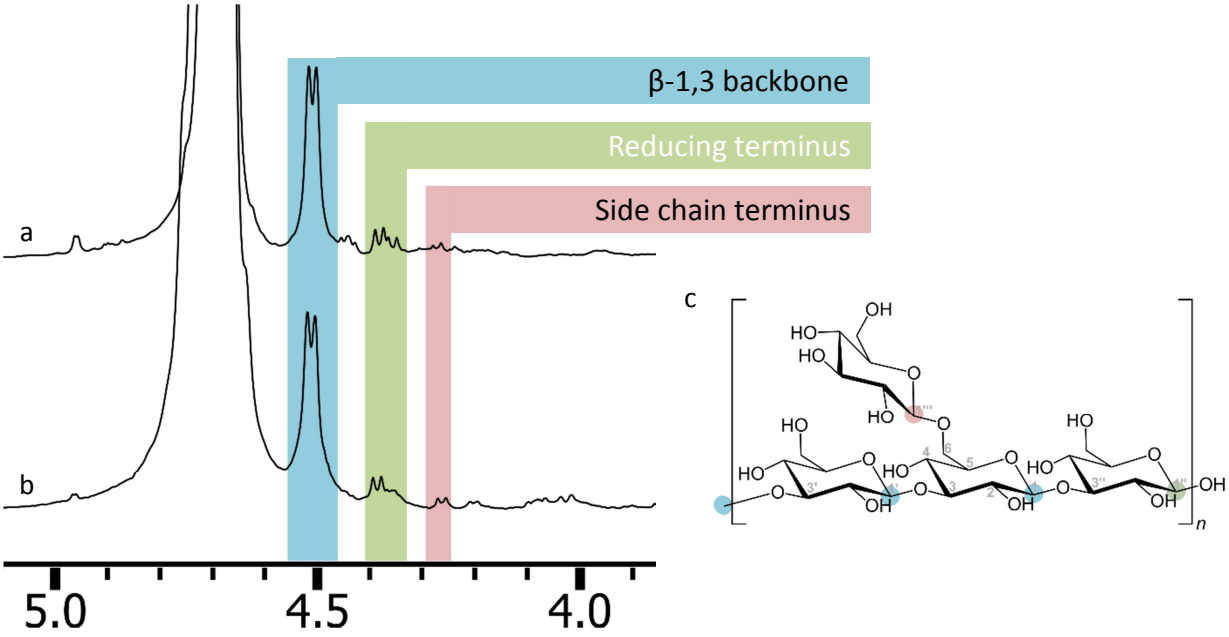

Figure 2. Protons of interest appear between 4.2 and 4.6 ppm, as demonstrated by Kim et al. [14]. Spectra for (a) *N. gaditana* soluble glucan extract and (b) laminarin control are shown. At right (c), the generalized structure is shown, with anomeric carbons highlighted with the colors of their corresponding chemical shifts.

glucopyranosyl residues. However, the high MW of cellulose suggests few terminal residues relative to the number of 4-linked residues, and SEC data suggests the cellulose contamination to be less than 10% of the sample.

Putative cellulose contamination prevents us from declaring the presence or absence of mannitol in the storage carbohydrate. As a result, we present a possible range for values of DP and DB from linkage analysis, depending on how much of the observed terminal mannopyranosyl, if any, is included as carbohydrate termini. When all the terminal mannopyranosyl signal is presumed to be a component of the storage carbohydrate (laminarin), the calculated DP is in closer agreement with the value calculated from our ^{1}H-NMR analysis. Linkage analysis of extracts from three additional biological replicates yielded values that agreed when terminal mannopyranosyl residues were presumed components of the storage

carbohydrate, with DP = 7.7±1.3 hexose residues (mean±stdev of 4 replicates). When terminal mannopyranosyl are not included, the DP has greater variability (44.8±31.1)

Trehalose is a disaccharide produced in many organisms, inherited in stramenopiles from the red algal endosymbiont [12]. The presence of trehalose metabolism in *N. gaditana* may have caused an underestimation of the degree of polymerization by linkage analysis. The α-1,1-linked glucose units of trehalose will likely have appeared as non-reducing terminal glucose units following permethylation, hydrolyzation, and acetylation. However, while the ^{1}H-NMR method is similarly incapable of discriminating the α-1,1-linked glucose units of trehalose from the non-reducing terminus, the signal from the anomeric carbon of the reducing terminus is used to calculate degree of polymerization, reversing the effect on DB calculation.

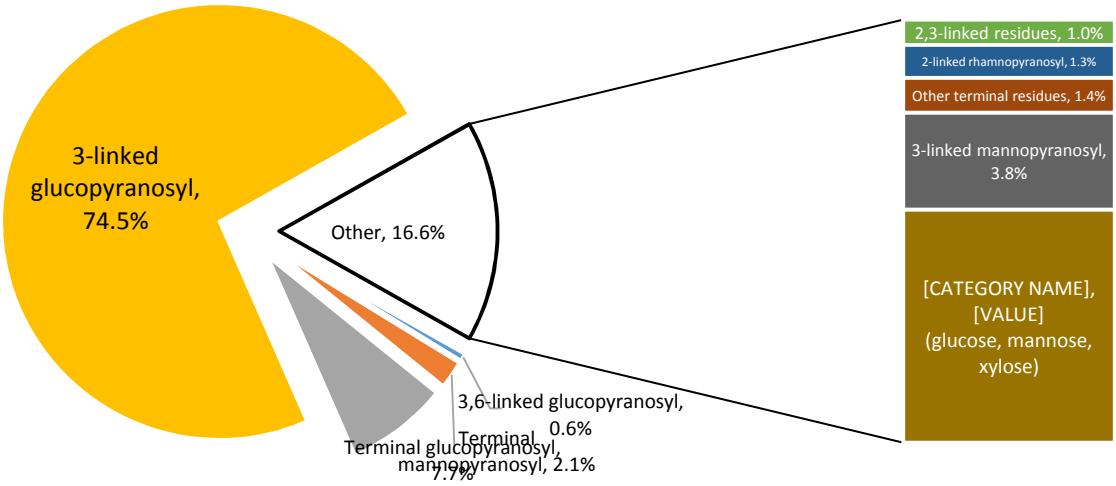

Figure 3. Results of linkage analysis showing a majority of laminarin-related residues, with evidence of some putative cellulose (cell wall) contamination. Relative abundances of each residue were used to estimate degree of polymerization and degree of branching.

3.2. Glucan synthase and Kre6-like branching enzyme are conserved among stramenopiles

It is reasonable to assume that there exists a means of regulating storage carbohydrate production in Nannochloropsis, and that it is at the generation of UDP-glucose from glucose-1-phosphate, analogous to the mechanism of starch regulation in green plants (Figure 4). In green

plants, this regulation is demonstrated to be largely allosteric, such that overexpression of the wild-type gene had little impact on starch content [19].

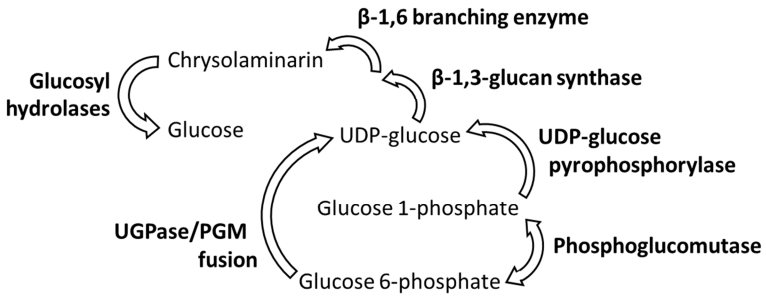

Figure 4. Proposed conserved glucan metabolism of investigated stramenopiles, including the UGPase/PGM fusion that strongly affects β-1,3-glucan synthesis in diatoms. The specific glucosyl hydrolases active on laminarin are not yet verified.

The Nannochloropsis genomes sequenced to date all contain a UDP-glucose pyrophosphorylase (UGPase), phosphoglucomutase (PGM), and a UGPase-PGM fusion polypeptide (Figure 4). The reversible conversion from glucose 6-phosphate to glucose 1-phosphate is catalyzed by phosphoglucomutase. The UGPase enzyme transfers uracil diphosphate to the 1-position of glucose using the energy from the release of a phosphate from each substrate. This charged UDP-glucose is the energetic substrate of β-1,3-glucan synthase, which frees UDP and catalyzes the addition of glucose units to the growing β-glucan polymer.

A glucosyltransferase family 48 (GT48) enzyme and a Kre6-like enzyme are hypothesized to be responsible for generating the linear β-1,3-backbone and β-1,6-branching linkages of laminarin, respectively [12]. Two genes with high homology to GT48 and Kre6-like enzyme are found within sequenced Nannochloropsis genomes, with high conservation and transcript support where available. Functionality of the Kre6-like protein in *P. tricornutum* was demonstrated when two of the three Kre6-like proteins from *P. tricornutum* were shown to functionally complement a Kre6 knockout of *S. cerevisiae* (the third was not investigated) [20]. Considering the very low degree of branching calculated here, it is unclear what benefit this rare

branching may confer to the organism. It is surprising that this branching enzyme appears so highly conserved throughout this lineage, suggesting an additional functionality for this enzyme.

3.3. Nannochloropsis species may utilize a glycogenin-like protein to nucleate laminarin

Glycogenin is the enzyme responsible for initiating the synthesis of glycogen, the storage carbohydrate of animals and fungi [21]. Resulting from efforts to understand the storage carbohydrate metabolism of Nannochloropsis (and Microchloropsis) species, a glycosyltransferase family 8 (GT8) protein with high similarity to glycogenin was found conserved among all species. Homologs were identified in available genomes of brown algae, diatoms, and oomycetes, all documented to utilize β-1,3 glucans for carbon storage [22–24]. Near neighbors were found in multiple apicomplexans and ciliates, and an individual of cryptophyte, labyrinthulomycete, and pelagophyceae.

Because Nannochloropsis lacks glycogen, it is possible that this enzyme is responsible instead for the nucleation of its own storage carbohydrate, chrysolaminarin. Its conservation among many organisms with similar storage carbons supports this hypothesis, but it is notably absent in a few genomes (i.e. *P. tricornutum*, *E. huxleyi*). One explanation is that this nucleation function was taken up by another enzyme in these exceptions, allowing for the loss of this otherwise conserved gene. For some, it may also be that these genes are not being detected due to incomplete genomes. Finally, it is possible that this glycogenin-like gene is not a necessary component of β-1,3 glucan biosynthesis, but instead involved in protein glycosylation or another unknown function.

Phylogenetic analysis was conducted on the glycogenin-like protein of Nannochloropsis using the nearest homologous predicted proteins from an array of vascular plants, alga, fungi and oomycetes. The resulting maximum likelihood tree clusters the vascular plants, in which glycogenin functions to nucleate the formation of glycogen. Outside of this cluster, there is a grouping of heterokonts, including oomycetes, diatoms, brown algae, and Nannochloropsis – all

of which synthesize structurally similar β-1,3 glucans for carbon storage. Separate from these groupings are the glycogenin-like homologs of fungi.

In an analysis of the light/dark transcriptional coordination, Poliner et al. found the glucan synthase transcript to be upregulated in anticipation of dusk, with peak transcription after the beginning of the dark phase [5]. Interestingly, the Kre6-like branching enzyme is maximally expressed in the middle of the light-phase, with minimum transcription at dusk. The glycogenin-like protein is also cyclic, with higher transcription during the light and a swift downregulation at dusk.

3.4. Bioinformatics suggests three potential laminarinases from N. gaditana

The Chromalveolate hypothesis proposes that both stramenopiles and alveolates descended from the same genetic parentage, meaning one instance of the secondary endosymbiosis of a red alga [25]. This theory has the advantage of being much simpler than the alternate theories of as many as five independent endosymbiosis events for chromalveolates [26], but does not easily explain the significantly divergent storage carbohydrate metabolisms. A more complete understanding of the genes involved in β-1,3-glucan metabolism will better inform phylogenetic studies into this evolutionary question.

To identify likely candidates for the breakdown of the Nannochloropsis storage glucan, a list was made of the predicted proteins from glycoside hydrolase families with potential activity on β-1,3 glucans (GH1, GH2, GH3, GH9, and GH81). These proteins were evaluated phylogenetically for clustering among organisms utilizing β-1,3 storage glucans. Among these proteins, only three resulted in a clear separation of these organisms, one GH1 family and two GH3 (Figure 5). Homologs of the two GH3 enzymes (Nga00051 and Nga03687) were found in eight of the ten in-group organisms, and GH1 homologs were found in seven (absent from *Albugo laibachii*). The two *Thalassiosira* genomes were not found to have any of these three enzymes. We hypothesize

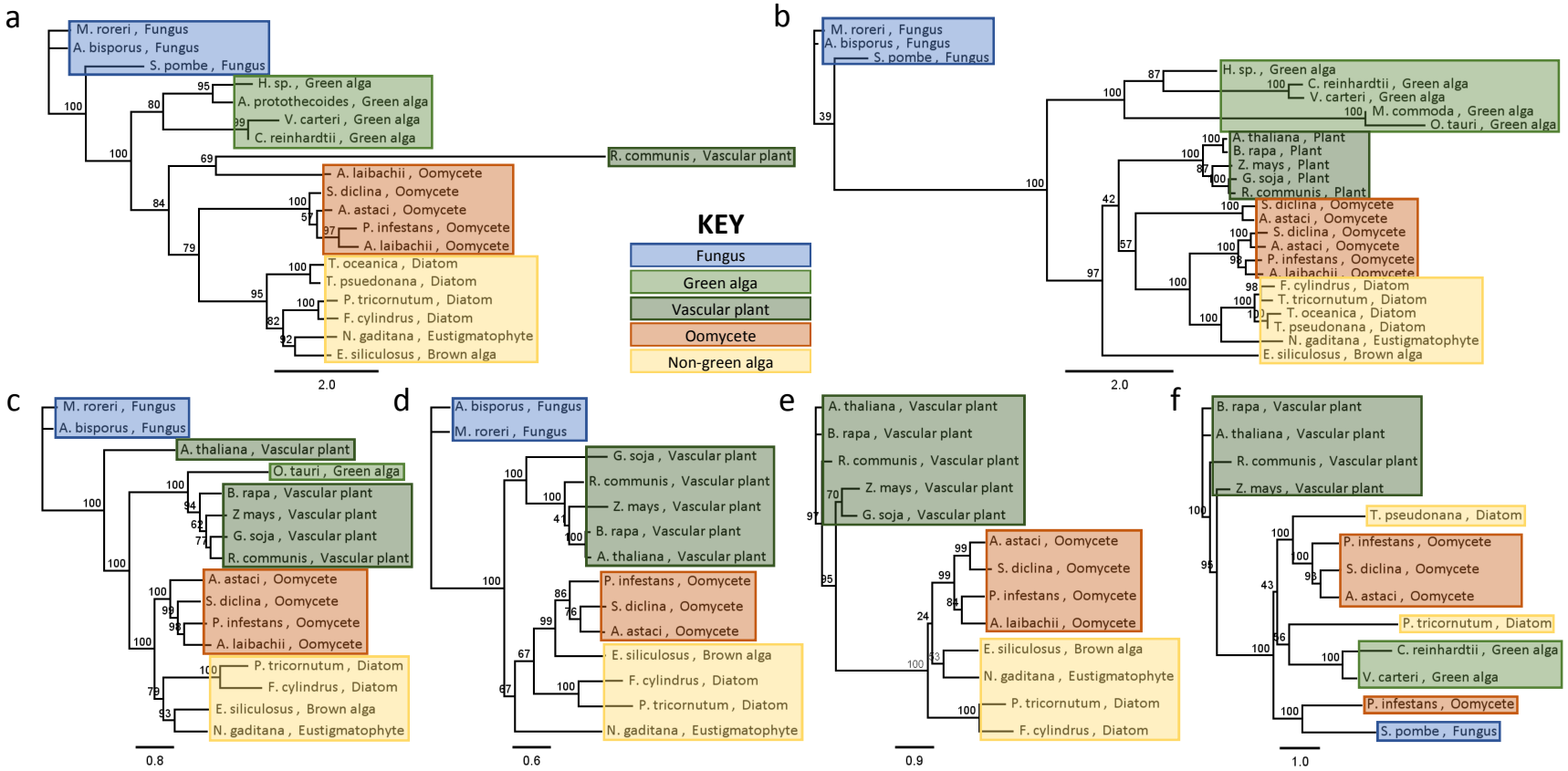

Figure 5. Phylogenetic trees generated for the (a) branching enzyme, (b) glucan synthase, and (c-f) glycoside hydrolases. Both branching enzyme and glucan synthase clustered as expected, with stramenopiles separating well from green plants and fungi. Of the glycoside hydrolases, (c) Nga03687, (d) Nga00952, and (e) Nga00051 clustered similarly, unlike other considered genes. (f) Nga05229 is included as a representative example of rejected glycoside hydrolase

that *Thalassiosira* acquired a different enzyme, perhaps through horizontal gene transfer, capable of degrading their storage glucans. This functional redundancy would have allowed for the loss of the original enzyme.

Additional GH families with potential activity on β-1,3 glucans, as well as undiscovered GH families may also be present that were not identified in this analysis. The *Thalassiosira* genomes necessarily have enzymes capable of hydrolyzing their storage glucans, and the identification of these enzymes would help to further illuminate the structures which dictate this function.

3.5. Manipulating carbohydrate synthesis increases lipid accumulation in diatoms

In the diatom *T. pseudonana*, the glucan synthase gene (Thaps3_12695) was knocked down by RNA interference [8], resulting in reduced chrysolaminarin content, as much as 54% after 24 hours of silicon starvation. In the diatom *P. tricornutum*, a knockout of the bifunctional UGPase-PGM fusion transcript resulted in a dramatic 45-fold increase in nutrient-replete TAG accumulation [9]. This mutant also accumulated two-fold more than wildtype in nitrogen-depleted medium. More recently, a knockdown of this same gene resulted in significantly lower chrysolaminarin content, increased total lipid content (19.5% dry weight), and slightly slower growth rate [10].

It is possible that a resulting buildup of precursor metabolites might generate product feedback inhibition on upstream reactions. On one hand, this accumulation of metabolites has the potential to redirect carbon to preferred metabolic pathways but may instead reduce the rate of carbon fixation. When the beta glucan synthase gene from *P. tricornutum* was knocked down, TAG levels increased, but growth and oxygen evolution rates decreased [11]. For the knockdown exhibiting 22% wildtype level of beta glucan synthase transcript, TAG content nearly doubled following nitrogen deprivation. In that study, the knockdown generated surprising effects on the thylakoids, which may or may not translate to Nannochloropsis, allowing hope that the growth defects might be avoided.

4. Conclusion

N. gaditana CCMP526 produces a soluble β-1,3-linked storage glucan with extremely sparse β-1,6 branching and a surprisingly short average length of 10-20 glucose residues per molecule. The glucan synthase and branching enzyme likely to be responsible for the formation β-1,3 and β-1,6 glycosidic bonds have been identified in the available *N. gaditana* and *salina* genomes (CCMP526, B-31, CCMP527, HH-1, CCMP1776, CCMP537), but knockout/complementation or enzymatic studies are necessary to definitively assign these biochemical activities. Similarly, three genes have been identified as high potential candidates for laminarinase activity by phylogenetic analysis and structural homology. Finally, a gene encoding a glycogenin-like protein is conserved among Nannochloropsis genomes, despite a lack of glycogen, perhaps performing a novel function, such as the nucleation of the storage beta glucan.

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7. Supplemental materials

Supplemental Table 1. Table of peaks and areas for *Nannochloropsis* sample resulting from line-fitting. Peaks attributed to relevant anomeric carbons are noted in the right column.

Name: 6 mg Nannochloropsis in 6:1 DMSO/D2O w/ added TFA
From: 0.344 ppm
To: 6.434 ppm
Residual Error:5.88e-05

#	ppm	Height	Width(Hz)	L/G	Area	Anomeric C
1	6.34	0	7.45	1	0.012	
2	6.24	0	5.94	1	0.012	
3	6.24	0	3.85	1	0.007	
4	6.19	0	5.64	1	0.016	
5	6.18	0	6.02	1	0.022	
6	6.16	0	4.19	1	0.04	
7	6.14	0	3.53	1	0.061	
8	6.14	0	3.73	1	0.063	
9	6.12	0	3.29	1	0.055	
10	6.09	0	7.97	1	0.052	
11	6.08	0	6.03	1	0.044	
12	6.04	0	4.27	1	0.036	
13	6.02	0	4.96	1	0.044	
14	6.00	0	4.13	1	0.048	
15	5.98	0	8.79	1	0.17	
16	5.97	0	3.78	1	0.07	
17	5.86	0	3.66	1	0.069	
18	5.85	0	3.51	1	0.065	
19	5.83	0	5.26	1	0.021	
20	5.81	0	7.57	1	0.054	
21	5.80	0	5.24	1	0.039	
22	5.78	0	3.47	1	0.052	
23	5.77	0	3.59	1	0.054	
24	5.69	0	5.61	1	0.044	
25	5.67	0	7.16	1	0.063	
26	5.65	0	8.8	1	0.05	

27	5.38	0	20.79	1	0.157	
28	5.14	0	79.35	1	0.697	
29	5.13	0	5.09	1	0.019	
30	5.11	0	10.88	1	0.063	
31	5.10	0	8.17	1	0.016	
32	5.07	0	24.89	1	0.075	
34	5.05	0	64.12	1	0.296	
33	5.05	0	21.58	1	0.113	
35	4.99	0	36.06	1	0.559	
36	4.96	0	4.38	1	0.218	RT-alpha
37	4.96	0.01	4.72	1	0.259	RT-alpha
38	4.93	0	7.82	1	0.044	
39	4.91	0	3.98	1	0.076	
40	4.90	0	4.46	1	0.063	
41	4.89	0	6.67	1	0.106	
42	4.87	0	5.88	1	0.125	
44	4.80	0	61.33	1	2.55	HDO
43	4.77	0	68.28	1	2.152	HDO
45	4.75	0.01	8.26	1	0.855	HDO
46	4.72	0.12	6.14	1	8.396	HDO
47	4.69	3.81	4.75	0.79	185.36	HDO
48	4.66	0.12	4.87	0	4.326	HDO
49	4.62	0	2.8	1	0.038	HDO
50	4.59	0	0.43	1	0.001	HDO
51	4.59	0	0.43	1	0.001	HDO
52	4.58	0	6.26	1	0.021	HDO
53	4.55	0	0.93	1	0.003	HDO
54	4.52	0.05	7.37	1	4.141	BC
55	4.50	0.05	7.09	1	3.877	BC
56	4.47	0	13.56	1	0.313	BC
57	4.45	0	4.4	1	0.131	NRT
58	4.44	0	4.47	1	0.13	NRT
59	4.44	0	4.66	1	0.167	NRT
60	4.43	0	4.97	1	0.159	NRT
67	4.41	0	10.63	1	0.021	

61	4.39	0.01	4.13	1	0.367	SRT
62	4.37	0.01	3.96	1	0.345	SRT
63	4.36	0	4.49	1	0.216	SRT
66	4.36	0	5.48	1	0.023	SRT
65	4.35	0.01	4.9	0.96	0.28	RT-beta
68	4.34	0	4.55	1	0.053	RT-beta
64	4.32	0	15.96	1	0.291	SC
69	4.31	0	6.57	1	0.115	SC
70	4.30	0	4.86	1	0.051	SC
71	4.29	0	7.23	1	0.129	SC
72	4.28	0	4.44	1	0.118	SC
73	4.26	0	7.51	1	0.361	SC
74	4.25	0	5.83	1	0.094	TSC
75	4.24	0	4.38	1	0.114	TSC
76	4.23	0	7.53	1	0.149	TSC
77	4.22	0	7.55	1	0.115	TSC
78	4.20	0	6.52	1	0.124	TSC
79	4.19	0	6.02	1	0.079	TSC
80	4.19	0	6.03	1	0.083	TSC
81	4.18	0	7.32	1	0.134	TSC
82	4.17	0	7.51	1	0.092	TSC
83	4.15	0	5.09	1	0.035	TSC
84	4.14	0	11.61	1	0.185	TSC
85	4.14	0	4.73	1	0.033	TSC
86	4.12	0	11.93	1	0.113	
87	4.10	0	14.58	1	0.076	
88	4.08	0	11.3	1	0.085	
89	4.07	0	4.58	1	0.03	
90	4.06	0	3.75	1	0.018	
91	4.05	0	4.01	1	0.026	
92	4.05	0	3.81	1	0.023	
93	4.04	0	3.72	1	0.024	
94	4.03	0	4.55	1	0.033	
95	4.03	0	7.2	1	0.047	
96	4.01	0	5.48	1	0.038	

97	3.97	0	14.19	1	0.24
98	3.95	0	11.06	1	0.128
99	3.92	0	122.15	1	2.26
100	3.89	0	1.58	1	0.002
101	3.88	0	3.79	1	0.011
102	3.87	0	4.73	1	0.013
103	3.81	0	14.95	1	0.509
104	3.77	0	18.06	1	0.863
105	3.73	0.01	25.72	0	1.751
106	3.69	0.06	7.72	1	5.11
107	3.67	0.07	7.22	1	5.577
108	3.63	0.01	10.59	1	1.726
109	3.61	0.01	6.53	1	0.47
110	3.59	0.01	13.23	1	1.617
111	3.57	0.01	10.34	1	1.048
112	3.55	0.01	8.71	1	0.634
113	3.53	0.03	3.59	1	1.059
114	3.49	0.03	10	1	3.225
115	3.47	0.06	8.03	1	4.929
116	3.46	0.04	7.41	1	3.53
117	3.44	0.1	4.14	1	4.764
118	3.42	0.24	3.34	1	8.831
119	3.41	0.24	3.28	1	8.511
120	3.40	0.09	3.66	1	3.542
121	3.38	0.01	5.07	1	0.821
122	3.37	0.01	8.39	1	1.185
123	3.34	0.01	20.01	0	2.665
124	3.28	0.04	7.3	1	3.154
125	3.27	0.06	7.48	1	5.191
126	3.25	0.05	7.56	1	3.778
127	3.23	0.05	13.75	1	7.531
128	3.22	0.02	7.73	1	1.485
129	3.20	0.05	7	1	3.813
130	3.19	0.07	7.69	1	6.147
131	3.17	0.03	6.96	1	2.459

132	3.14	0.02	3.08	1	0.655
133	3.13	0	5.04	1	0.267
134	3.11	0.01	5.43	1	0.345
135	3.09	0	8.4	1	0.31
136	3.07	0.01	5.49	1	0.611
137	3.05	0.02	7.26	1	1.306
138	3.04	0.01	5.22	1	0.729
139	3.02	0	5.3	1	0.272
140	2.99	0	7.16	1	0.046
141	2.98	0	5.58	1	0.014
142	2.96	0	4.36	1	0.037
143	2.94	0	4.57	1	0.053
144	2.93	0	3.17	1	0.019
146	2.91	0	2.26	1	0.017
145	2.90	0	4.52	1	0.082
147	2.88	0	4.74	1	0.068
148	2.87	0	3.23	1	0.032
149	2.85	0	4.36	1	0.039
150	2.84	0	2.1	1	0.009
154	2.80	0	2.54	1	0.007
155	2.78	0	4.32	1	0.038
156	2.77	0	1.88	1	0.002
157	2.77	0	3.14	1	0.007
151	2.76	0	3.01	1	0.011
158	2.73	0	19.87	1	0.801
152	2.65	0	4.68	1	0.021
153	2.64	0	4.75	1	0.101
159	2.64	0	2.57	1	0.031
161	2.63	0	3.06	1	0.028
162	2.63	0	2.44	1	0.033
163	2.62	0	4.3	1	0.014
164	2.59	0	4.73	1	0.009
160	2.56	0	7.83	1	0.069
165	2.53	0.02	6.19	1	1.276
166	2.50	0.27	3.45	1	10.139

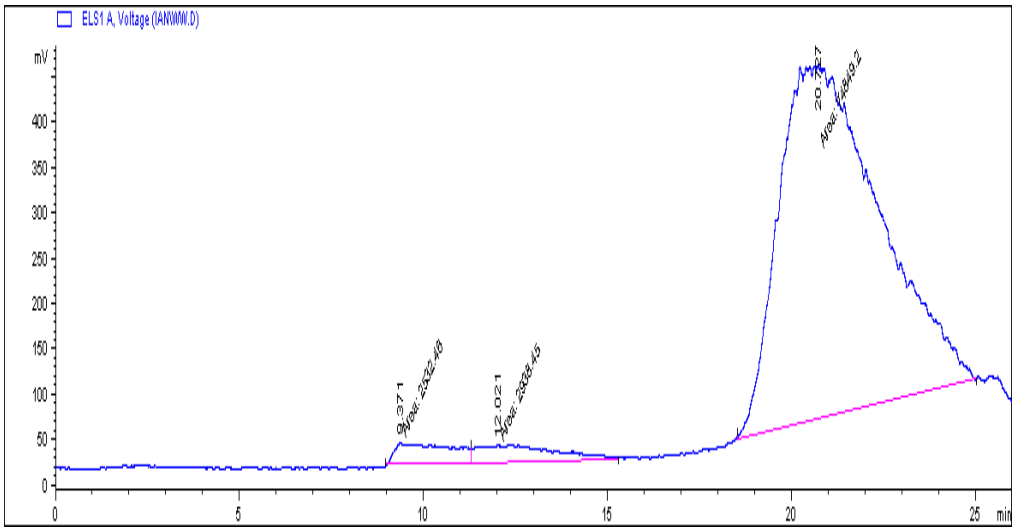
167	2.50	0.64	4.23	1	29.821
168	2.50	0.25	2.82	1	7.761
169	2.47	0.02	4.52	1	1.158
170	2.43	0	6.54	1	0.051
171	2.36	0	3.41	1	0.126
172	2.33	0	4.71	1	0.014
173	2.24	0	26.51	1	0.146
174	2.16	0	9.74	1	0.007
175	2.15	0	3.65	1	0.004
176	2.13	0	6.29	1	0.007
177	2.12	0	16.56	1	0.014
179	2.08	0	4.3	1	0.001
178	2.05	0	19.96	1	0.053
180	2.02	0	5.06	1	0.081
182	2.01	0	10.01	1	0.068
181	1.98	0	13.37	1	0.136
183	1.94	0	15.98	1	0.121
184	1.92	0	4.47	1	0.04
185	1.91	0	10.49	1	0.059
186	1.88	0.02	2.45	1	0.647
187	1.86	0	5.92	1	0.047
188	1.85	0	54.8	1	0.91
189	1.85	0	7.48	1	0.148
190	1.82	0	5.51	1	0.091
191	1.81	0	6.87	1	0.15
192	1.80	0	7.16	1	0.065
193	1.80	0	7.36	1	0.054
194	1.79	0	9.29	1	0.102
195	1.76	0	8.93	1	0.112
196	1.74	0	22.65	1	0.143
197	1.71	0	10.16	1	0.094
198	1.69	0	10.49	1	0.097
199	1.68	0	10.39	1	0.101
200	1.64	0	10.98	1	0.074
201	1.58	0	22.81	1	0.309

202	1.51	0	44.73	1	2.397
203	1.31	0	56.86	0	0.985
204	1.27	0	2.67	1	0.013
205	1.26	0	2.74	1	0.031
206	1.25	0	1.91	1	0.01
207	1.22	0	34.22	0	1.299
208	1.18	0	6.57	1	0.257
209	1.17	0	4.68	1	0.123
210	1.15	0	4.29	0	0.161
211	1.14	0	2.56	0	0.037
212	1.13	0	7.48	1	0.239
213	1.10	0	26.15	1	1.265
214	1.08	0	3.58	0	0.109
215	1.07	0.01	3.25	1	0.235
216	1.06	0.01	5.02	0	0.78
217	1.03	0.43	2.49	1	11.678
218	1.01	0.22	2.62	1	6.21
219	1.00	0.01	2.99	1	0.375
220	0.98	0.01	2.15	1	0.144
221	0.96	0	5.3	1	0.031
222	0.95	0	11.13	1	0.116
223	0.92	0	2.73	1	0.036
224	0.90	0	1.95	1	0.038
225	0.89	0	1.34	1	0.007
226	0.79	0.01	43.82	1	3.407

Supplemental Table 2. The reference organisms and TXIDs used for identifying homologs to biosynthetic genes and potential laminarinases.

TXID	Organism	Taxonomy	Notes
3075	Auxenochlorella protothecoides	Green alga	
145474	Helicosporidium sp.	Green alga	non-photosynthetic, parasitic
3055	Chlamydomonas reinhardtii	Green alga	
70448	Ostreococcus tauri	Green alga	

38832	Micromonas sp.	Green alga	
3067	Volvox carteri	Green alga	multicellular
3848	Glycine soja	Vascular plant	wild soybean
3988	Ricinus communis	Vascular plant	castor bean
3702	Arabidopsis thaliana	Vascular plant	
4577	Zea mays	Vascular plant	maize
3711	Brassica rapa	Vascular plant	field mustard
2850	Phaeodactylum tricornutum	Diatom	
186039	Fragilariopsis cylindrus	Diatom	
29204	Cyclotella cryptica	Diatom	
35128	Thalassiosira pseudonana	Diatom	
159749	Thalassiosira oceanica	Diatom	
2880	Ectocarpus siliculosus	Brown alga	multicellular
4787	Phytophthora infestans	Oomycete	potato late blight agent
653948	Albugo laibachii	Oomycete	white rust
112098	Saprolegnia diclina	Oomycete	infects wide variety of aquatics
112090	Aphanomyces astaci	Oomycete	infects crawfish
5341	Agaricus bisporus	Fungi	button/portabello
4896	Schizosaccharomyces pombe	Fungi	fission yeast
221103	Moniliophthora roreri	Fungi	frosty pod rot of cacao
130081	Galdieria sulphuraria	Red alga	hot sulphur springs
45157	Cyanidioschyzon merolae	Red alga	ultrasmall



Supplementary Figure 1. SEC chromatogram of *N. gaditana* soluble glucan extract on Superose 12 column, indicating a dominance of low MW material.