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User-friendly extraction and multistage tandem mass spectrometry based analysis of lipid-linked oligosaccharides in microalgae



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CONTEXT

Protein *N*-glycosylation is initiated within the endoplasmic reticulum through the synthesis of a lipid-linked oligosaccharide (LLO) precursor [1]. This precursor is then transferred en bloc on neo-synthesized proteins through the action of the oligosaccharyltransferase giving birth to glycoproteins. The *N*-linked glycans bore by the glycoproteins are then processed into oligomannosides prior to the exit of the glycoproteins from the endoplasmic reticulum and its entrance into the Golgi apparatus. In this compartment, the *N*-linked glycans are further matured in complex type *N*-glycans. This process has been well studied in a lot of eukaryotes including higher plants. In contrast, little information regarding the LLO precursor structure and synthesis of *N*-linked glycans is available in microalgae.

OBJECTIVE

A user-friendly extraction method combining microsomal enrichment and solvent extractions followed by purification steps has been optimized for the extract of the LLO from microalgae. This strategy is aiming to extract the LLO precursor from microalgae. Then, the oligosaccharide moiety released from the extracted LLO was analysed by multistage tandem mass spectrometry in two models of microalgae namely the green microalga, *Chlamydomonas reinhardtii* and the diatom, *Phaeodactylum tricornutum*.

METHOD

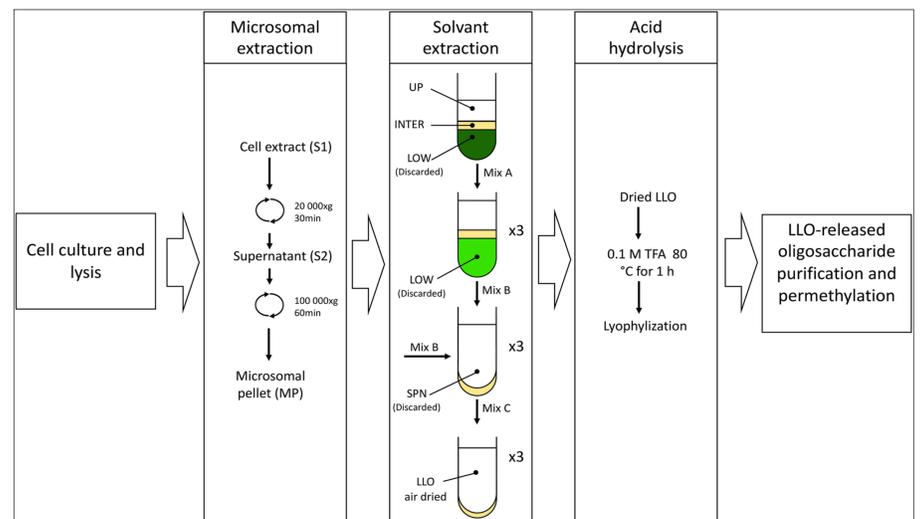


Fig. 1 : Scheme summarizing the Lipid-Linked Oligosaccharide (LLO) extraction method developed in this study to analyse LLO from microalgae.

UP: upper phase; INTER: intermediate phase; LOW: lower phase; SPN: supernatant, Mix: mixture.

C. reinhardtii

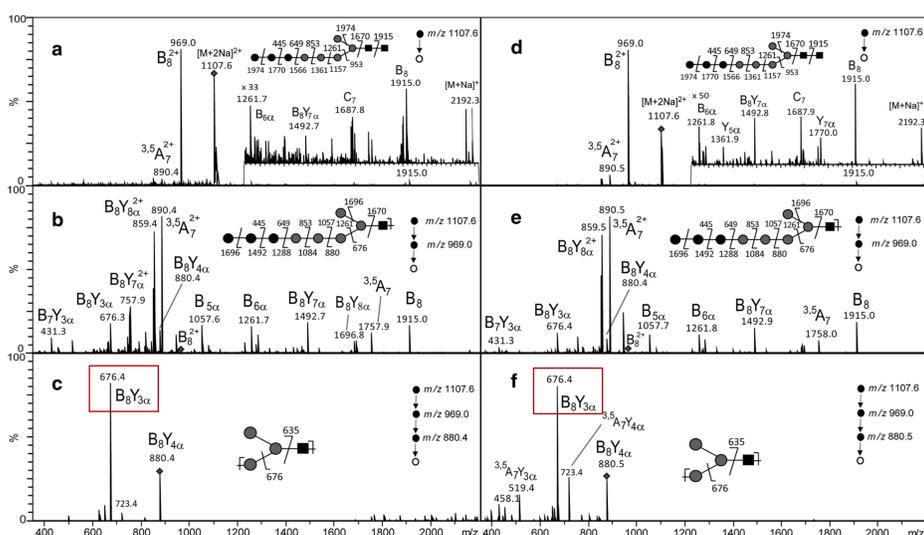


Fig. 2 : Multistage tandem mass spectrometry analysis of the permethylated LLO-released oligosaccharide isolated from *C. reinhardtii* xylosyltransferase mutant (a-c) and from wild-type (d-f).

ESI-MSⁿ spectra with *n* = 2 (a and d), *n* = 3 (b and e), *n* = 4 (c and f) of the [M + 2Na]²⁺ *m/z* 1107.6 precursor ion of the permethylated Hex₃HexNAc₂ derivative isolated from either the *C. reinhardtii* xylosyltransferase mutant or the wild-type cells. The precursor ion selected for the fragmentation analysis is shown with a diamond and its fragmentation pattern is proposed according to [2] Black square: *N*-acetylglucosamine; grey circle: mannose, black circle: glucose. The fragment ions are labelled according to the nomenclature of [3].

The specific fragmentation pathway *m/z* 1107.6 → *m/z* 969.0 → *m/z* 880.4 → *m/z* 676.4 and 1107.6 → *m/z* 1914.8 are consistent with a linear oligosaccharide structure in the green microalga *C. reinhardtii* (Fig. 2).

C. reinhardtii accumulates a linear LLO-released oligosaccharide containing 8 hexose and 2 *N*-acetylglucosamine residues which largely differs from the branched Glc₃Man₉GlcNAc₂ synthesized in most of the eukaryotes [1].

P. tricornutum

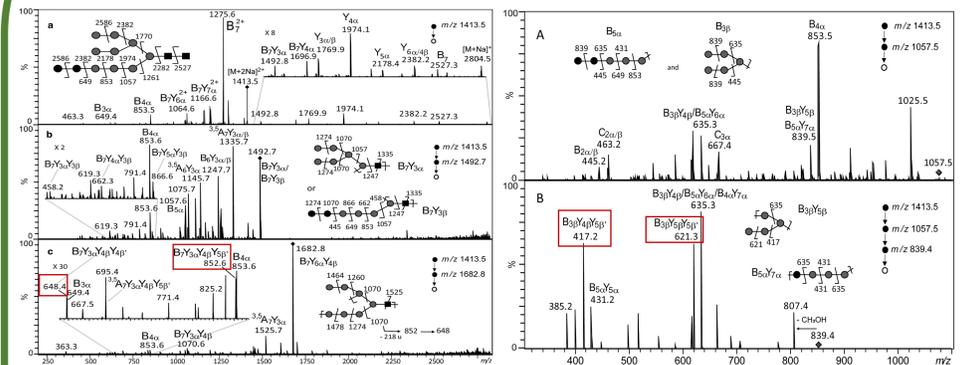


Fig. 3 : Multistage tandem mass spectrometry analysis allowing the determination of the structure of the permethylated LLO-released oligosaccharide isolated from the diatom *P. tricornutum*.

ESI-MSⁿ spectra with *n* = 2 selecting *m/z* 1413.5 ([M + 2Na]²⁺) as the precursor ion (a), *n* = 3 selecting *m/z* 1492.5 ([M + Na]⁺) as intermediate ion (b) and *n* = 3 with *m/z* 1682.5 ([M + Na]⁺) as intermediate ion (c) of permethylated Hex₁₁HexNAc₂ derivative isolated from *P. tricornutum*. On each panel, the ion selected for the fragmentation analysis is shown with a diamond and its fragmentation pattern is proposed according to Prien *et al.* 2009 Black square: *N*-acetylglucosamine; grey circle: mannose, black circle: glucose. The fragment ions are labelled according to the nomenclature of [3].

Fig. 4 : Multistage tandem mass spectrometry analysis allowing the determination of the structure of the permethylated LLO-released oligosaccharide isolated from the diatom *P. tricornutum*.

ESI-MSⁿ spectra with *n* = 3 selecting *m/z* 1057.5 ([M + 2Na]²⁺) as the precursor ion (a) and *n* = 4 selecting *m/z* 839.4 ([M + Na]⁺) as intermediate ion (b) of permethylated Hex₁₁HexNAc₂ derivative isolated from *P. tricornutum*. On each panel, the ion selected for the fragmentation analysis is shown with a diamond and its fragmentation pattern is proposed according to [2] Black square: *N*-acetylglucosamine; grey circle: mannose, black circle: glucose. The fragment ions are labelled according to the nomenclature of [3].

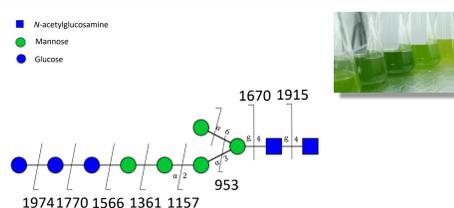
The ESI-MS³ spectrum selecting *m/z* 1682.5 as the intermediate fragment ion revealed the presence of discriminant fragment ions at *m/z* 852.6 and *m/z* 648.4 corresponding respectively to the Hex₃HexNAc and Hex₂HexNAc structures arising from the fragmentation of a branched LLO (Fig. 3).

The presence of a tri-antenna LLO-released oligosaccharide structure was also evidenced by the ESI-MS⁴ spectrum showing the fragmentation pattern *m/z* 1413.5 → *m/z* 1057.6 → *m/z* 839.4 → product ions. The fragment ions *m/z* 417.2 and *m/z* 621.3 can only result from fragmentation of a branched ion *m/z* 839.4 (Fig. 4).

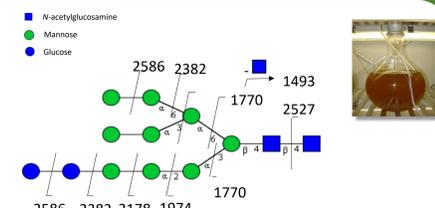
P. tricornutum accumulates a LLO-released oligosaccharide containing 11 hexose and 2 *N*-acetylglucosamine residues which differs from one hexose compared to the LLO structure synthesized in most of the eukaryotes [1].

CONCLUSIONS

1) Detailed structural analysis confirms the presence of a linear precursor type Glc₃Man₉GlcNAc₂ in the green microalga *C. reinhardtii* as previously described [4]. Such results validate the extraction method and analytical procedures.



2) Analysis of the LLO confirms the presence of a branched precursor truncated by one hexose. The Glc₂Man₉GlcNAc₂ structure in *P. tricornutum* is consistent by the absence of ALG10 and glucosidase I gene in the genome of the diatom as previous reported [5]. This work allows us to report for the first time the detailed structure analysis of the LLO released oligosaccharide from the diatom *P. tricornutum* [6].



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Acknowledgments

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