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

Rodolphe Dumontier, Pierre-Louis Lucas, Corinne Loutelier-Bourhis, Alain Mareck, Carlos Afonso, Patrice Lerouge, Narimane Mati-Baouche, Muriel Bardor

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

Rodolphe Dumontier[1] *, Pierre-Louis Lucas[1] * , Corinne Loutelier-Bourhis[2] , Alain Mareck[1] , Carlos Afonso[2] , Patrice Lerouge[1] , Narimane Mati-Baouche[1] and Muriel Bardor[1, 3]

1. Normandie Univ, UNIROUEN, Laboratoire Glyco-MEV EA4358, 76000 Rouen, France,
2. Normandie Univ, UNIROUEN, INSA Rouen, CNRS, COBRA, 76000 Rouen, France,
3. Institut Universitaire de France (IUF), 75000 Paris, France

* Equal contributions of the first authors

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 maturated 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

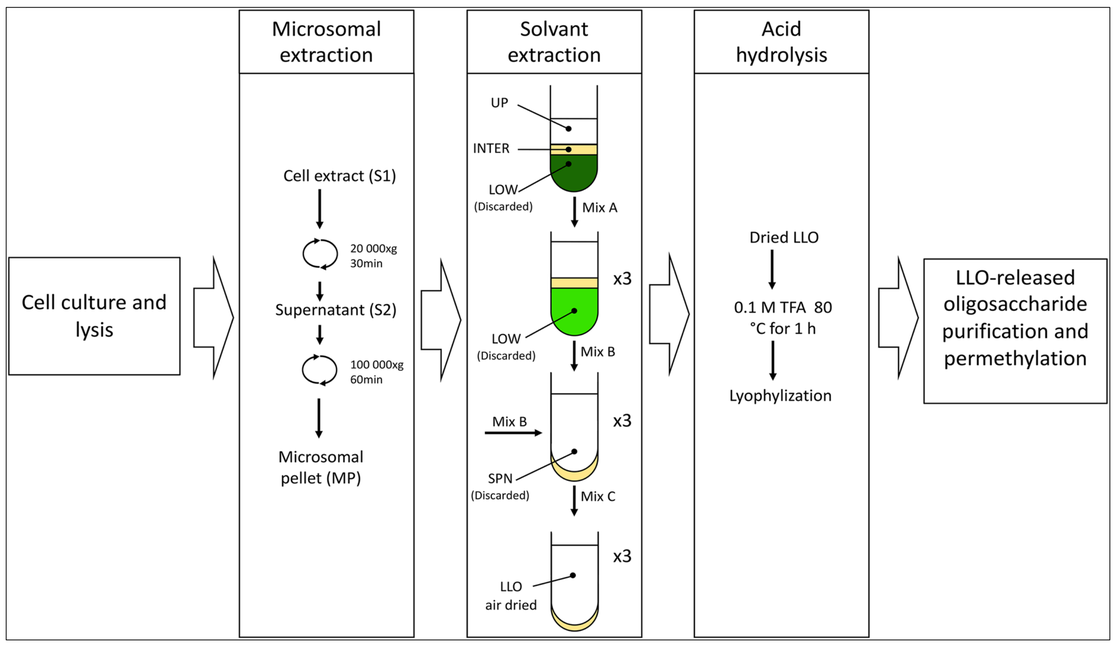


Fig. 1 : Scheme summarizing the Lipid-Linked Oligosaccharide (LLO) extraction method developed in this study to analyse LLO from microalgae.

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

C. reinhardtii

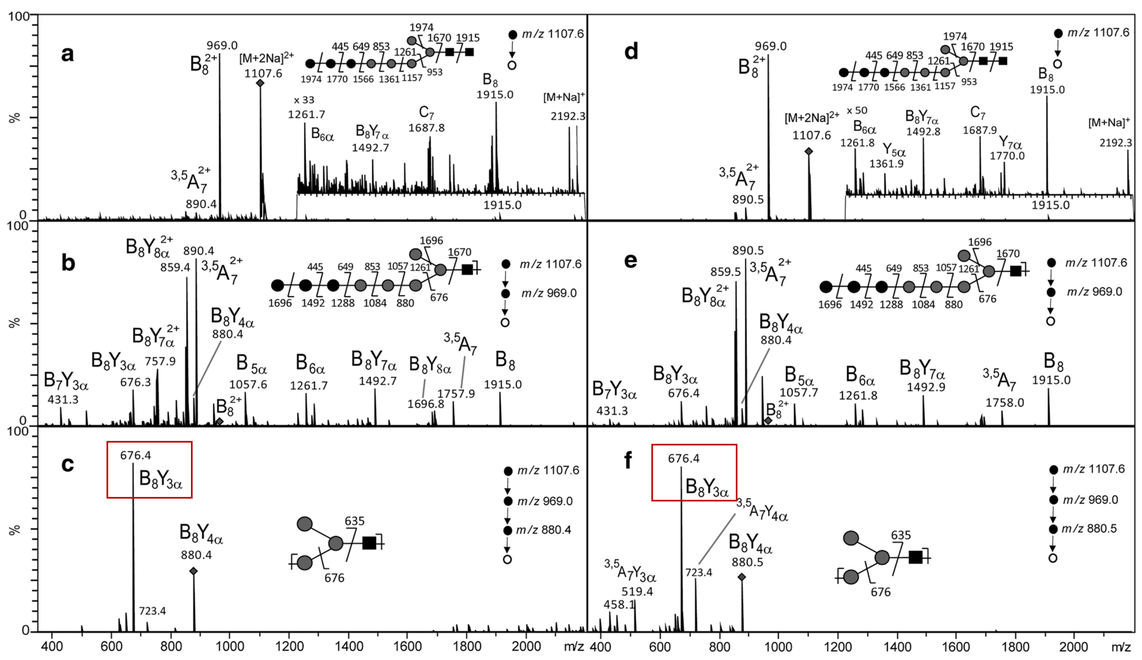


Fig. 2 : Multistage tandem mass spectrometry analysis of the permethylated LLO-released oligosaccharide isolated from *C. reinhardtii* xylosyltransferase mutant (a–c) and from wild-type (d–f).

ESI-MS^n 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 $Hex_8HexNAc_2$ 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 $Glc_3Man_9GlcNAc_2$ synthetized in most of the eukaryotes [1].**

P. tricornutum

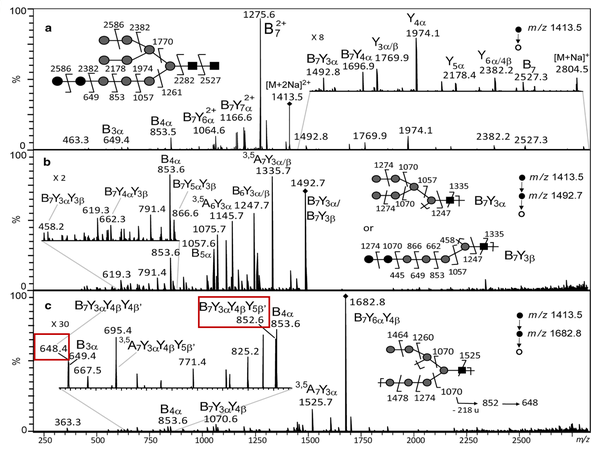


Fig. 3 : Multistage tandem mass spectrometry analysis allowing the determination of the structure of the permethylated LLO-released oligosaccharide isolated from the diatom *P. tricornutum*.

ESI-MS^n 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 $Hex_{11}HexNAc_2$ 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].

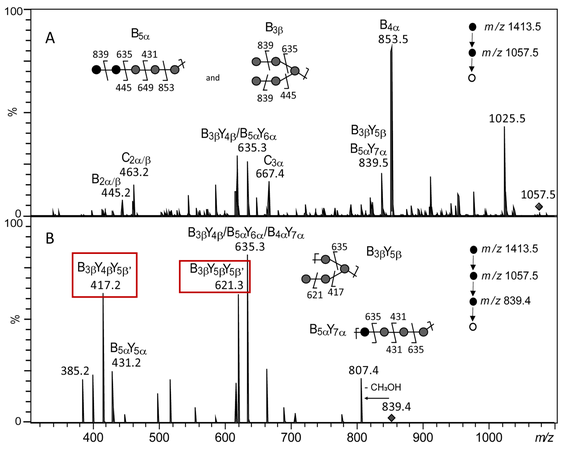


Fig. 4 : Multistage tandem mass spectrometry analysis allowing the determination of the structure of the permethylated LLO-released oligosaccharide isolated from the diatom *P. tricornutum*.

ESI-MS^n 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 $Hex_{11}HexNAc_2$ 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].

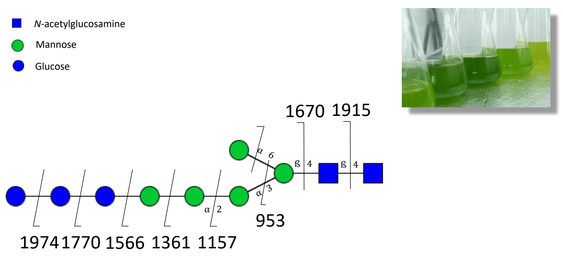
The ESI-MS^3 spectrum selecting m/z 1682.5 as the intermediate fragment ion revealed the presence of discriminant fragment ions at m/z 852.6 and m/z 648.4 corresponding respectively to the $Hex_3HexNAc$ and $Hex_2HexNAc$ structures arising from the fragmentation of a branched LLO (Fig. 3).

The presence of a tri-antenna LLO-released oligosaccharide structure was also evidenced by the ESI-MS^4 spectrum showing the fragmentation pattern m/z 1 413.5 → 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).

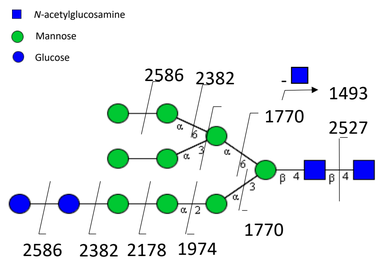
***P. tricornutum* accumulates a LLO-released oligosaccharide containing 11 hexose and 2 *N*-acetylglucosamine residues which differs from one hexose compared to the LLO structure synthetized in most of the eukaryotes [1].**

CONCLUSIONS

1) **Detailed structural analysis confirms the presence of a linear precursor type $Glc_3Man_5GlcNAc_2$ in the green microalgae *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 $Glc_2Man_9GlcNAc_2$ 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].**



References

[1] Aebi, M., 2013. N-linked protein glycosylation in the ER. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1833, 2430–2437.

[2] Prien JM, Ashline DJ, Lapadula AJ, Zhang H, Reinhold VN. The High Mannose Glycans from Bovine Ribonuclease B Isomer Characterization by Ion Trap MS. J Am Soc Mass Spectrom. 2009;20: 539–56.

[3] Domon, Bruno, and Catherine E. Costello, 1988 A Systematic Nomenclature for Carbohydrate Fragmentations in FAB-MS/MS Spectra of Glycoconjugates. Glycoconjugate Journal 5(4): 397–409.

[4] Vanier G, Lucas P-L, Loutelier-Bourhis C, Vanier J, Plasson C, Walet-Balieu M-L, et al. Heterologous expression of the *N*-acetylglucosaminyltransferase I dictates a reinvestigation of the *N*-glycosylation pathway in *Chlamydomonas reinhardtii*. Scientific Reports, 2017;7: 10156.

[5] Baïet B, Burel C, Saint-Jean B, Louvet R, Menu-Bouaouiche L, Kiefer-Meyer M-C, et al. *N*-Glycans of *Phaeodactylum tricornutum* Diatom and Functional Characterization of Its *N*-Acetylglucosaminyltransferase I Enzyme. J Biol Chem. 2011;286: 6152–64.

[6] Lucas, P.-L., Dumontier, R., Loutelier-Bourhis, C., Mareck, A., Afonso, C., Lerouge, P., Mati-Baouche, N., Bardor, M., 2018. User-friendly extraction and multistage tandem mass spectrometry based analysis of lipid-linked oligosaccharides in microalgae. Plant Methods 14, 107.

Acknowledgments

- The authors thank the European Regional Development Fund (ERDF, N°31708), the region Normandie (Crunch Network, N°20–13), the Labex SynOrg (ANR-11-LABX-0029), the Institut Universitaire of France, the University of Rouen Normandie for financial support.
- The authors would like to thank Dr Elodie Mathieu-Rivet for her help in screening the *C. reinhardtii* mutant clones.