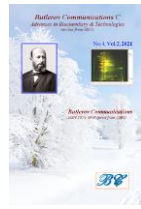
BUTLEROV HERITAGE

Butlerov Communications C
Advances in Biochemistry & Technologies
ISSN 2074-0948 (print)

2021. Vol.2, No.4, Id.18.
Journal Homepage: https://c-journal.butlerov.com/



Subsection: Bioorganic Chemistry.

The Reference Object Identifier – ROI-jbc-C/21-2-4-18
The Digital Object Identifier – DOI: 10.37952/ROI-jbc-C/21-2-4-18
Received 23 November 2021; Accepted 24 November 2021

The role of N-glycosylation of proteins in the cell wall formation processes of *Linum usitatissimum* L

Nadezhda N. Ibragimova, **Irina A. Larskaya**, and **Evgenia O. Fedina***[+]
Laboratory of Plant Glycobiology. Kazan Institute of Biochemistry – Subdivision of FRC Kazan SC of RAS. Lobachevsky St., 2/31. Kazan, 420111. Tatarstan Republic. Russia. Phone:+7 (843) 231-90-69. E-mail: solo_nika@mail.ru

*Supervising author; [+]Corresponding author

Keywords: N-glycosylation of proteins, *Linum usitatissimum* L., 2D-electrophoresis, immunoblotting, fucosylation, xylosylation, plant growth.

Abstract

The level of N-glycosylation proteins at different stages of cell wall formation was studied in flax stems. The xylem and phloem parts of the stem containing fibers enriched with secondary cell wall (XYL), tertiary cell wall at early (MID) and late (BOT) thickening stages were used as samples. To detect glycoproteins from MID, BOT, XYL samples the protein fractions were obtained by treating tissue homogenates with lithium salts (2 M LiCl) and calcium salts (0.2 M CaCl2) followed by the use of standard kits for obtaining glycoproteins from complex protein mixtures with concavalin A lectin (CONA) as an affinity matrix. The obtained glycoproteins were isofocused on gel strips with an immobilized pH gradient (pH 3-10. 7 cm long). The separation of proteins in the second direction (by molecular weight) were carried out using an electrode buffer containing 25 mM Tris-HCl, 192 mM glycine, 0.1% DSD-Na. To assess the contribution of N-glycosylation of proteins to the formation of the flax cell wall, immunoblotting was performed using anti-β1,2-xylose and anti-α1,3-fucose monoclonal antibodies. β1,2-xylose and cow α1,3-fucose are specific residues of N-glycans of plant proteins. The glycosylated proteins of the phloem and xylem parts of the stem separated by 2D electrophoresis had the molecular weights mainly in the range from 11 to 35 kDa and their isoelectric points are in the alkaline region. The results of our studies also showed that glycans detected on N-glycoprotein blots mainly contain α1,3-fucose residues than β1,2-xylose. The differences we obtained in the binding to α1,3-fucose and β1,2-xylose by N-glycans indicate the possibility of the existence of two independent pathways of N-glycosylation of proteins in flax plants. For the first time we characterized the profiles of N-glycosylated proteins of flax plants and found that the phloem part of the stem contained fibers with a mature thickened tertiary cell wall (BOT) was distinguished by the highest content of N-glycoproteins.

For citation: Nadezhda N. Ibragimova, Irina A. Larskaya, Evgenia O. Fedina. The role of N-glycosylation of proteins in the cell wall formation processes of *Linum usitatissimum* L. *Butlerov Communications C*. **2021**. Vol.2. No.4. Id.18. DOI: 10.37952/ROI-jbc-C/21-2-4-18

References

[1] S. Silberstein, R. Gilmore. Biochemistry, molecular biology, and genetics of the oligosaccharyltransferase. *FASEB J.* **1996**. Vol.10. P.849-858. DOI: 10.1096/fasebj.10.8.8666161

[2] A. Helenius, M. Aebi Intracellular functions of N-linked glycans. *Science.* **2001**. Vol.291. P.2364-2369. DOI: 10.1126/science.291.5512.2364

[3] H. Koiwa, F. Li, M.G. McCully et al. The STT3a subunit isoform of the Arabidopsis oligosaccharyltransferase controls adaptive responses to salt/osmotic stress. *Plant Cell*. **2003**. Vol.15. P.2273-2284. DOI: 10.1105/tpc.013862

[4] J.S. Kang, J. Frank, C.H. Kang et al. Salt tolerance of *Arabidopsis thaliana* requires maturation of N-glycosylated proteins in the Golgi apparatus. *Proc. Natl. Acad. Sci. USA*. **2008**. Vol.105. P.5933-5938. DOI: 10.1073/pnas.0800237105

[5] T. Bickel, L. Lehle, M. Schwarz *et al.*: Biosynthesis of lipid-linked oligosaccharides in Saccharomyces cerevisiae: Alg13p and Alg14p form a complex required for the formation of GlcNAc(2)-PP-dolichol. *J. Biol. Chem*. **2005**. Vol.280. P.34500-34506. DOI: 10.1074/jbc.M506358200

[6] J. Schoberer, Y.J. Shin, U. Vavra *et al.* Analysis of protein glycosylation in the ER. In: Hawes C., Kriechbaumer V. (ed.): The Plant Endoplasmic Reticulum. Methods in *Molecular Biology.* **2018**. Vol.1691. P.205-222. *New York: Humana Press.* DOI: 10.1007/978-1-4939-7389-716

[7] R. Strasser Plant protein glycosylation. *Glycobiology*. **2016**. Vol.26. P.926-939. DOI: 10.1093/glycob/cww023

[8] E. Ruiz-May, S.-J. Kim, F. Brandizzi, J.K.C. Rose. The secreted plant N-glycoproteome and ssociated secretory pathways. *Front. Plant Sci*. **2012**. Vol.3. P.1-15. DOI: 10.3389/fpls.2012.00117

[9] T.A. Gorshkova, V.V. Salnikov, S.B. Chemikosova, M.V. Ageeva, N.V. Pavlencheva The snap point: a transition point in *Linum usitatissimum* bast fiber development. *Ind. Crops Prod*. **2003**. Vol.18. Art.213. DOI: 10.1016/S0926-6690(03)00043-8

[10] S.M. Rozov, N.V. Permyakova, E.V. Deineko. Basic strategies for glycoengineering plant expression systems for the production of humanized recombinant pharmaceutical proteins. *Biochemistry.* **2018**. Vol.83. No.3. P.328-348. (Russian) DOI: 10.1134/S0006297918030033

[11] R. Strasser, F. Altmann, L. Mach, J. Glössl, H. Steinkellner. Generation of *Arabidopsis thaliana* plants with complex *N*-glycans lacking β-1,2-linked xylose and core α-1,3-linked fucose. *FEBS Lett.* **2004**. Vol.561. P.132-136. DOI: 10.1016/S0014-5793(04)00150-4

[12] V. Gomord, A-C. Fitchette, L. Laurence Menu-Bouaouiche, C. Saint-Jore Dupas, C. Plasson, D. Michaud, L. Faye. Plant-specific glycosylation patterns in the context of therapeutic protein production. *Plant Biotechnology Journal*. **2010**. Vol.8. P.564-587. DOI: 10.1111/j.1467-7652.2009.00497.x

[13] Y. Liu, L. Ma, D. Cao, Z. Gong, J. Fan, H. Hu, X. Jin. Investigation of cell wall proteins of C. sinensis leaves by combining cell wall proteomics and N-glycoproteomics. *BMC Plant Biology.* **2021**. Vol.21. Art.384. DOI: 10.1186/s12870-021-03166-4

[14] X-L. Chen, C. Liu, B. Tang, Z. Ren, G.L. Wang, W. Liu. Quantitative proteomics analysis reveals important roles of N-glycosylation on ER quality control system for

development and pathogenesis in *Magnaporthe oryzae*. *PLoS Pathog*. **2020**. Vol.16. Art. e1008355. DOI: 10.1371/journal.ppat.1008355

[15] Nadezhda N. Ibragimova, Irina A. Larskaya, Evgenia O. Fedina. The role of N-glycosylation of proteins in the cell wall formation processes of *Linum usitatissimum* L. *Butlerov Communications*. **2021**. Vol.68. No.12. P.155-160. DOI: 10.37952/ROI-jbc-01/21-68-12-155