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N-Glycosylation in the Green Microalgae Chlamydomonas reinhardtii

Authors : Pierre-Louis Lucas, Corinne Loutelier-Bourhis, Narimane Mati-Baouche, Philippe Chan Tchi-Song, Patrice Lerouge, Elodie Mathieu-Rivet, Muriel Bardor

Abstract : N-glycosylation is a post-translational modification taking place in the Endoplasmic Reticulum and the Golgi apparatus where defined glycan features are added on protein in a very specific sequence Asn-X-Thr/Ser/Cys were X can be any amino acid except proline. Because it is well-established that those N-glycans play a critical role in protein biological activity, protein half-life and that a different N-glycan structure may induce an immune response, they are very important in Biopharmaceuticals which are mainly glycoproteins bearing N-glycans. From now, most of the biopharmaceuticals are produced by mammalian cells like Chinese Hamster Ovary cells (CHO) for their N-glycosylation similar to the human, but due to the high production costs, several other species are investigated as the possible alternative system. In this purpose, the green microalgae Chlamydomonas reinhardtii was investigated as the potential production system for Biopharmaceuticals. This choice was influenced by the facts that C. reinhardtii is a well-study microalgae which is growing fast with a lot of molecular biology tools available. This organism is also producing N-glycan on its endogenous proteins. However, the analysis of the N-glycan structure of this microalgae has revealed some differences as compared to the human. Rather than in Human where the glycans are processed by key enzymes called N-acetylglucosaminyltransferase I and II (GnTI and GnTII) adding GlcNAc residue to form a $GlcNAc_2Man_3GlcNAc_2$ core N-glycan, C. reinhardtii lacks those two enzymes and possess a GnTI independent glycosylation pathway. Moreover, some enzymes like xylosyltransferases and methyltransferases not present in human are supposed to act on the glycans of C. reinhardtii. Furthermore, the recent structural study by mass spectrometry shows that the N-glycosylation precursor supposed to be conserved in almost all eukaryotic cells results in a linear $Man_5GlcNAc_2$ rather than a branched one in C. reinhardtii. In this work, we will discuss the new released MS information upon C. reinhardtii N-glycan structure and their impact on our attempt to modify the glycan in a Human manner. Two strategies will be discussed. The first one consisted in the study of Xylosyltransferase insertional mutants from the CLIP library in order to remove xyloses from the N-glycans. The second will go further in the humanization by transforming the microalgae with the exogenous gene from Toxoplasma gondii having an activity similar to GnTI and GnTII with the aim to synthesize $GlcNAc_2Man_3GlcNAc_2$ in C. reinhardtii.

Keywords : Chlamydomonas reinhardtii, N-glycosylation, glycosyltransferase, mass spectrometry, humanization
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