Tracing glycosylation events in *Chlamydomonas reinhardtii* using reporter glycoproteins targeted to different compartments of the secretory pathway

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Microalgae are eukaryotic photosynthetic and unicellular organisms that are intended as a cost-effective expression system for producing recombinant glycoproteins, such as glycosylated therapeutics. In this context post-translational modifications such as *N*-glycosylation, which are essential for the function of drugs, need to be well-understood[1]. In this study, we aim at deciphering the step by step *N*-glycosylation events in the green microalga *Chlamydomonas reinhardtii*. To do so, we developed an original approach based on the glycosylation analysis of a reporter protein expressed in specific compartments of the secretory system. Human erythropoietin (hEPO) or phytohemagglutinin (PHA) fused to a fluorescent protein (FP) were used as reporter glycoproteins. Various constructions were developed using the Golden Gate cloning technology allowing the expression of these reporter glycoproteins fused to either a C-terminal HDEL sequence for its retrieval in the endoplasmic reticulum (ER), or a 'cytoplasmic, transmembrane, stem' (CTS) domain for its targeting in specific Golgi cisternae of the microalgae. *C. reinhardtii* was transformed with these constructions and reporter glycoproteins were purified from positive clones. They were therefore submitted to a glycoproteomic analysis by Liquid-Chromatography coupled to Mass Spectrometry (LC-MS/MS) to characterize the micro- and macroheterogeneity of the glycosylation of reporter glycoproteins. We present here the first results obtained for hEPO expressed either in the ER, or in specific Golgi compartments. This original approach allows us to determine the step by step events of the *N*-glycosylation pathway in *C. reinhardtii* leading to the specific glycosylation profile in this microalga.

[1]*van Bockstaele-Fuentes J, Mati-Baouche N, Lupette J, Gargouch N, Rivet E, Lerouge P and Bardor M (2025) An overview of protein N-glycosylation diversity in microalgae. Front. Plant Sci. 16:1669918.*