Evolution and dynamics of the N-glycosylation pathway through oligosaccharyltransferases

Pedebos, C. [1]; Ligabue-Braun, R.[1]; Arantes, P. R.[1]; Verli, H.[1]
[1]Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Rio Grande do Sul, Brazil

INTRODUCTION: N-glycosylation is one of the most prevalent co/post-translational modifications. The addition of the glycan chain to a nascent polypeptide is fulfilled by the oligosaccharyltransferases (OSTs) PglB (Bacteria), AglB (Archaea), and Stt3 (Eukarya). Previous crystallographic data for these enzymes identified structural units with distinct functions, such as catalysis (CC) and structural stability (IS, P1, and P2). However, the atomic level mechanism of how glycosylation occurs, and the regulation of its accuracy, remain elusive. **OBJECTIVES:** Here, we examine different OSTs, assessing their evolution and the biological role of each structural unit. **MATERIAL AND METHODS:** We evaluated the dynamics of OSTs by molecular dynamics simulations, employing the GROMACS package with GROMOS53a6$_{GLYC}$ and GROMOS54a7 forcefields, in the presence of aqueous solvent, catalytic ions, and membrane bilayers. For the evolutionary inferences, we employed the Maximum Likelihood (MEGA software) and Bayesian Inference (MrBayes software) methods. **DISCUSSION AND RESULTS**: We described, with statistical significance, the phylogenetic diversity of OSTs structural units through all domains of life, which indicated a possible horizontal gene transfer of the N-glycosylation system from Archaea to Bacteria. Using PglB as an OST model for bacteria, we identified motions that could facilitate both binding and exit of substrates (peptide and glycolipid), as well as a detailed description of the catalytic cleft dynamics. From the Archaea side, we employed AglB to verify the previously hypothesized influence of P1 in the enzyme thermostability, which we did not observe. Nevertheless, P1 appears to affect the binding interface with its substrates, in a new putative role for this domain. **CONCLUSIONS:** For the first time, a robust evolutionary relationship of OSTs across all domains of life was obtained. In addition to new putative roles of OSTs units, the obtained data offer new basis for experimentation and biotechnological applications, such as development of vaccines and glycoprotein engineering.

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