INVITED LECTURE

Molecular mechanisms of retaining glycosyltransferases investigated by QM/MM metadynamics.

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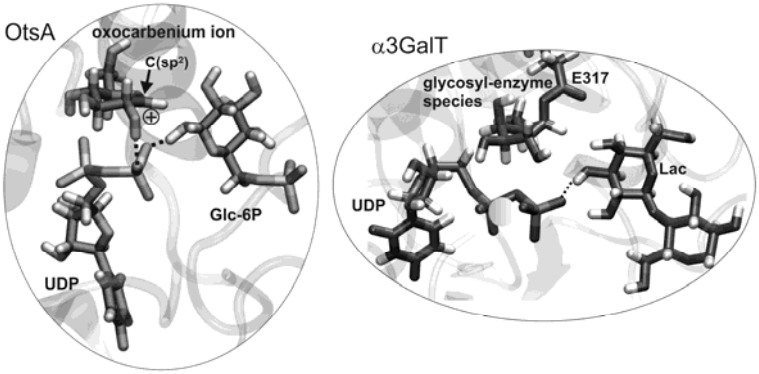
The catalytic mechanism of nucleotide-sugar dependent glycosyltransferases (GTs), especially those that act with retention of anomeric configuration, remains one an intriguing unanswered questions in glycobiology. In contrast to the well-characterized mechanistic strategies used by glycoside hydrolases (GHs) to catalyze the cleavage of glycosidic bonds, the mechanisms of retaining GTs remain unclear. Double displacement mechanisms have been proposed (but not fully proved) by analogy to retaining GHs. In addition, many GTs do not have a putative nucleophile protein residue. This prompted some authors to suggest an unusual mechanism, in which the reaction proceeds via a front side single displacement. This mechanism, usually named as S_Ni-like in the literature, has been surrounded by a strong controversy, since in principle it implies that two covalent bonds are forming and breaking, respectively, in the same region of space.



By means of QM/MM metadynamics simulations, based on Density Functional Theory (DFT), we demonstrate that the "front-face" mechanism is feasible in a GT lacking a putative nucleophile residue (trehalose-6-phosphate synthase, OtsA), thanks to the formation of a short-lived oxocarbenium-ion-like species (Figure) [1]. In contrast, a GT with a putative nucleophile residue, such as α3-galactosyltransferase (α3GalT, right panel), operates via a double-displacement mechanism, with the formation of a glycosyl-enzyme covalent intermediate [2]. A detailed picture of the atomic rearrangement during the complete reaction pathway will be provided and differences between both mechanisms will be analyzed.

References

[1] Ardèvol, A.; Rovira, C. *Angew. Chem. Int. Ed.* **2011**, 50, 10897-10901.
[2] Rojas-Cervellera, V.; Ardèvol, A.; Boero, M.; Planas, A.; Rovira, C. *Chem. Eur. J.* **2013**, 42, 14018–14023.