UNIVERSIDAD DE LA RIOJA

XI JORNADAS DE CARBOHIDRATOS

Conformational transitions as key features of PimA-mediated glycosyl transfer

Marcelo E. Guerin[1,2,3]

[1]Unidad de Biofísica, Consejo Superior de Investigaciones Científicas-Universidad del País Vasco/Euskal Herriko Unibertsitatea, Barrio Sarriena s/n, Leioa, Bizkaia 48940, Spain, [2]Departamento de Bioquímica, Universidad del País Vasco, Leioa, Bizkaia 48940, Spain, [3]IKERBASQUE, Basque Foundation for Science, Bilbao 48011, Spain; e-mail: mrcguerin@gmail.com

Remarkable progress has been made in recent years in our understanding of the catalytic mechanism and structural basis of glycosyl transfer. However, the study of the conformational changes and dynamics that govern substrate recognition and catalysis remains a major challenge in the field of glycosyltransferases (GTs).[2] Here we focus in PimA, an essential enzyme involved in the biosynthesis of phosphatidyl-*myo*-inositol mannosides (PIMs), which are key glycolipids of the mycobacterial cell envelope1. PimA is a paradigm of this family of GTs, which the molecular mechanism of substrate/membrane recognition and catalysis is still unknown. We have solved the crystal structure of PimA from *M. smegmatis* in complex with its donor substrate GDP-Man. The notion of a membrane-associated protein via electrostatic interactions is consistent with the finding of an amphipathic α-helix in the N-terminal domain of PimA. Based on structural, biophysics and biochemical studies, we proposed a model of interfacial catalysis in which PimA recognizes the fully acylated acceptor substrate, phosphatidyl-myo-inositol (PI), with its polar head within the catalytic cleft and the fatty acid moieties only partially sequestered from the bulk solvent. In addition, we provided strong evidence showing that PimA undergoes significant conformational changes upon substrate binding.[3] Single-molecule force spectroscopy revealed that the mannosyltransferase PimA exhibits weak mechanical stability albeit displaying -sheet topology expected to unfold at much higher forces. Notably, PimA unfolds following heterogeneous multiple step mechanical unfolding pathways at low force akin to molten globule states.[1] Interestingly, the ab initio low resolution envelopes obtained from small angle x-ray scattering of the unliganded PimA and the PimA-GDP complexed forms clearly demonstrate that not only the “open” and “closed” conformations of the GT-B enzyme are largely present in solution, but in addition, PimA experiences remarkable flexibility that undoubtedly corresponds to the N-terminal “Rossmann fold” domain, which has been proved to participate in protein-membrane interactions. Altogether, our experimental data support a model wherein the flexibility and conformational transitions confer adaptability of PimA to the substrates/membrane, which seems to be of importance during catalysis. The proposed mechanism has fundamental implications for the comprehension of membrane-associated GTs at the molecular level and the development of GT inhibitors.

[1] Giganti et al., *J. Biol. Chem.* 288, 29797-29808 (2013).
[2] Guerin et al., *J. Biol. Chem.* 285, 33577-33583 (2010).
[3] Guerin et al., *J. Biol. Chem.* 284, 21613-21625 (2009).