Modeling the Interaction of Oleic Acid with two α-Lactalbumin Folding Variants: in Route towards Deciphering the Molecular Basis of HAMLET's Antitumoral Activity

Florencia Klein, Florencia Ferraro, Alicia Merlino, E. Laura Coitiño

University of the Republic (UdelaR), School of Sciences, Institute of Biological Chemistry, Theoretical & Computational Chemistry Lab., Montevideo 11400, URUGUAY

HAMLET (*Human Alpha-lactalbumin Made Lethal to Tumor cells*) is a protein-lipid complex formed by α-Lactalbumin (*α-La*) and oleic acid (*OA*, an unsaturated C18:1 9-cis fatty acid) that displays cytotoxic activity with high selectivity against tumor cells. After more than a decade of research driven by its potential as chemotherapeutic agent, the underlying mechanism of action still remains unclear. Nevertheless, evidence surrounded by controversy showed that at a comparable concentration none of the components is an effective antitumor agent by itself, being the activity assigned to the entire complex. The interaction of different fatty acids (FA) with *α-La* was also studied, establishing that a complex rarely forms with saturated FAs (*e.g.* saturated C18:0 stearic acid, *SA*) and that when it does, cytotoxicity is very low. Gaining a deeper knowledge on the nature of *FA:α-La* interactions can be thus crucial to decipher the molecular basis of HAMLET's anticancer activity.

As previous experiments showed an open conformation of *α-La* is required for HAMLET to form and to enter the target cell, we applied *ligand-protein docking* and *atomistic MD simulations* to characterize the structural and dynamical behavior of FA(*OA/EA*)*:α-La* 1:1 complexes formed in solution with both "*closed*"/"*open*" folding variants of apo *α-La* lacking a constitutive Ca^{2+} ion and a couple of disulfide bridges regarding to the native structure. The analysis of mutual changes induced by each FA-protein interaction into the corresponding components was complemented by s*ingle-point* electronic structure calculations conducted at the *DFT/PCM(water)* level on regions within a 3 Å radius from each FA atom. This allowed us to identify aminoacids involved and detailed nature of the specific interactions in each FA:*α-La* entity, as well as the driving forces of protein of conformational changes and variations in relevant physicochemical properties due to the protein/fatty acid interaction.