Molecular mechanisms underlying the activity regulation of the phospholipase PlaF from P. aeruginosa by free fatty acids

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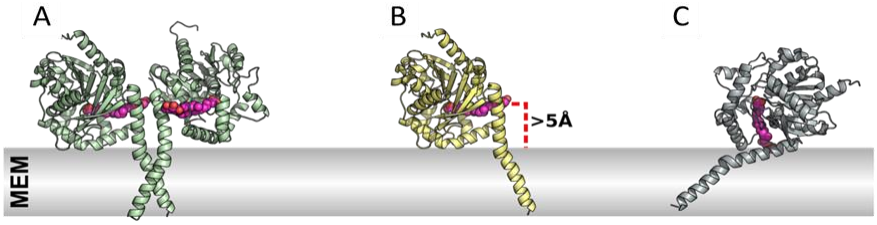
The Gram-negative Pseudomonas aeruginosa is an opportunistic pathogen that causes nosocomial infections by producing numerous virulence factors[1-3]. Among these factors, type A phospholipases (PLA) can contribute to host membrane damage and modulation of signaling networks in infected cells by modifying the membrane composition[2-3]. In this context, we focus on PlaF, a phospholipase A1 (PLA1).

This enzyme adopts a monomeric active and a dimeric inactive configuration[3]. A crystal structure of the dimeric PlaF (PDB_ID 6i8w) is available. Computational studies evaluated the dynamics and energetics of the dimerization process[3]. The results reveal that a single PlaF monomer can adopt a tilted configuration, which might facilitate phospholipid substrate access from the membrane. Furthermore, we elucidated the potential channeling mechanisms underlying substrate access and product egress in PlaF in accordance with the enzyme specificity and regioselectivity[4]. Additionally, we revealed that medium-sized free fatty acids (FFAs) can inhibit PlaF activity according to mixed inhibition kinetics[3]. However, the detailed molecular mechanism that governs the inhibition of PlaF by FFAs has remained elusive.

Here, we showed by molecular simulations that the presence of FFAs in the membrane affects the dynamics and the energetics of both PlaF dimer dissociation and monomer tilting. Moreover, free energy computations reveal an energetic stabilization of the dimeric inactive configuration, which was correlated to an increased FFA concentration in the membrane. Experimental studies also revealed that FFAs in the periplasmic space can inhibit PlaF activity. We propose a potential FFA-related mechanism of PlaF inhibition using free ligand diffusion simulations. Combined with experimental validations, the identification of FFA binding site(s) involved in the inhibition of PlaF can help design novel drugs against P. aeruginosa.



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