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Catalytic mechanism of conversion of ATP to cyclic-AMP catalyzed by the Edema Factor of Anthrax: a QM/MM study

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Abstract: The Edema Factor (EF) is one of three major toxins involved in the disruption of cellular functions resulting from infection of a host organism by anthrax. EF is an adenylyl cyclases and, as such, catalyzes the production of cyclic-AMP (cAMP) from ATP. In eukaryotes, cAMP is a key signaling molecules, and the infection by EF induces its overproduction, leading to cell death. Other pathogens, such as *Bordetella petrussis*, responsible for Pertussis, or *Yersina pestis*, which causes bubonic plague, also have similar edema factors [1,2]. On the other hand, mammalian adenylyl cyclases (mAC) catalyzes the same reaction. However, these proteins exhibit little structural homology with the anthrax EF. Several structures of the EF of anthrax were obtained, which allow a qualitative analysis of possible reaction pathways. Nevertheless, these same structures are not conclusive about fundamental aspects of the catalytic mechanism, as the reactive conformation of the ATP, the number and mode of coordination of the ions of the active site, and the amino-acids which are directly involved in the transfer of protons along the reaction. We have performed significant advances on the understanding of the reaction mechanism of EF and mAC.[3] In spite of the advances made at the moment, the causes of the catalytic power of the EF are not fully understood. A complete description of those causes could be obtained by studying EF and mAC mutants, specially, H577A in EF and S1028H in mAC. We present analysis of the mechanism of these two mutant and its free-energy profiles. The results are compared against the previous our previous results obtained for wild-type enzymes. The description of the mechanism of the reaction through the determination of the free energy surfaces determined by each plausible reaction coordinate. The study of the mechanism of the reaction were be performed using quantum mechanics/molecular mechanics simulations (QM/MM). The free energy profiles were calculated using non-equilibrium Quantum Mechanics-Molecular Mechanics/Steered Molecular Dynamics (QM-MM/SMD) simulations, by means of the Jarzynski equality. The QM subsystem is described at the Self-Charge Consistent Density Function Theory Tight-Binding (SCC-DFTB) level and the database that were parametrized to describe phosphate transfer reaction [4].
Besides, the deep analysis of the reaction in solution would add key elements to describe in detail the EF catalytic power. Here, we also present our results of the reaction in solution for identifying the catalytic factors that the enzyme introduces. The reaction has two step, chemical reaction (first step) and the separation of the products (second step). We present results for the two steps of the reaction. Different conditions were tested, e.g. different possible bases that accept the transferring proton. The methodology used in the first step were the similar to that used for mutants (QM/MM), while for the second step, classic molecular dynamics and umbrella sampling were used.

Key-words: QM/MM, Steered Molecular Dynamics, Free-energy profile, Jarzynski, Anthrax Edema Factor.
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