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Simulation of the AHAS-imazaquin system by Molecular Dynamics to design an enzyme-based AFM nanobiosensor

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Abstract: The monitoring of herbicides in the environment is extremely important to prevent and control contamination. The development of nanobiosensors is a powerful alternative because of the speed and accuracy of analysis [1-4]. In this study, our goal was develop and describe a molecular model of the enzyme-inhibiting interaction, which can be used for an optimized projection of an Atomic Force Microscope (AFM) nanobiosensor to detect pesticides molecules used in agriculture, in order to evaluate its accordance with limit levels stipulated in valid legislation for its use. Molecular Dynamics were performed to study the system Acetohydroxiacid Synthase (AHAS) and an herbicide inhibitor of its activity, imazaquin (IQ) – a typical member of imidazolinone family [5] (Figure 1).

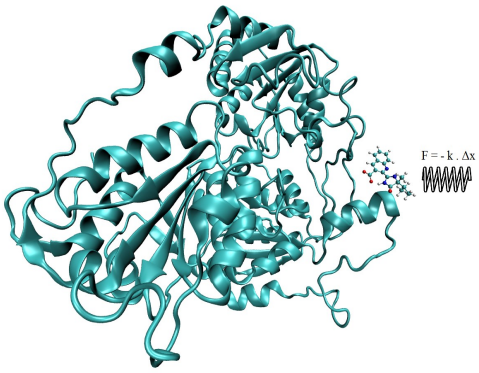

Figure 1: Possible unbinding pathway imazaquin from the AHAS active site.

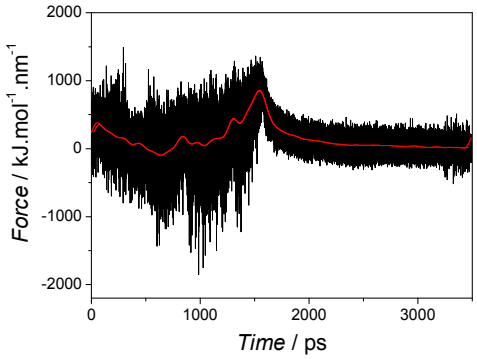

Figure 2: SMD force-extension profiles between IQ and AHAS active site.

In order to study the system AHAS-IQ (PDB-1Z8N) in the presence of FAD and TPP cofactors, a Molecular Dynamic was carried for 20 ns with 42169 *tip3p* water molecules. After that, a Steered Molecular Dynamics (SMD) was carried for 3.5 ns in vacuum applying to the distance constraints between the IQ and active site of AHAS-TPP with a force constant of 367 $kJ.mol^{-1}.nm^{-1}$ and a constant velocity of 0.001 $nm.ps^{-1}$. Molecular dynamics simulations were performed using GROMACS 4.6.5 program in an NVT ensemble (298K) with CHARMM27 force field [6].

The total energy for the enzymatic system (Figure 1) is almost constant along the 20 ns of simulation (- 1,413 x 10^6 kJ.mol^{-1}). Conformational stability of enzyme was showed calculating the Radius of Gyration (2,595 ± 0,029 nm) and RMSD (0,235 ± 0,006 nm), which confirm a small systematic fluctuation of protein in aqueous solution. The initial and final structures were compared using the Ramachandran plotting and results obtained shown no eligible differences φ and ψ dihedral angles from the initial and final structure after 20 ns of MD trajectory. The Figure 2 shows the force-extension profiles of the interaction between IQ and AHAS active site with the presence of FAD and TPP substrates. It is observed that the maximum of force necessary required to remove the herbicide from active site occurs at about 500 kJ.mol^{-1}.nm^{-1}, around 100 ps, from the start of the simulation, which can be attributed to the moment of rupture of the main interactions between residues of active site of the enzyme with the IQ. Another maximum peak can be observed around 1500 ps associated to the complete separation of the IQ molecule and the external amino acids residues of enzyme.

Experimental data of adhesion force using functionalized AFM tip with the enzyme ALS on three different substrates contaminated with the herbicide imazaquin (K = 0.20 N.m^{-1}) obtained an average of 40 (±4) nN [7]. The average force was calculated in the time interval from t_i = 0 to t_f = 65 ps and an average force of 0.39 (±0.2) nN per enzyme was obtained. Considering the standard deviation of the analysis of mean force obtained from SMD curve is a multiple of the experimental data. Therefore, the theoretical results validated the AHAS-IQ system proposed and are useful to predict optimized conditions for the fabrication of AFM nanobiosensors with high sensitivity.

Key-words: Nanobiosensor, Atomic Force Microscopy, Imazaquin, Molecular Dynamics.

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