Investigating the difffrent enviromrnts of active site glutamates of Glyoxalase I

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Abstract: a few studies in the last tow decades have shown that two glutamate residuses in the enzymes active site of Glyoxalase I (Glu-99 and Glu-172) are the most imporatant residuse in the catalytic mechanism of the enzyme. GlxI converts both enantiomers of the substrate into the same *S*-D-lactoylglutathione product. Interestingly the two glutamate residue are symmetrically coordinated to a Zn ion. However, experimental observations [1] and theoretical studies show that thaey must act differently inside the enzyme [2,3]. These suggest a hypothesis that environment and flexibility of the glutamates are different even they are symmetrically located in the active site.

Following this hypothesis and examaining it we performed molecular dynamics (MD) simulations. The MD simulations were based on a 2.00 Å crystal structure of GlxI (Protein Data Bank entry 1QIN) [4]. The entire enzyme was included in the calculations. The protonation states of all the residues were determined from a detailed study of the hydrogen-bond pattern and the solvent accessibility. The protein is a dimer and the two subunits were treated the same way. The enzyme was solvated in a periodic truncated octahedral box of TIP3P water molecules, extending at least 12 Å from the solute using the leap program in the Amber suite. The final system contained 38465 atoms. After the solvation, we performed 1000 cycles of minimization, with the heavy atoms of the protein restrained. This was followed by a 20 ps constant-volume and a 20 ps constant-pressure equilibration with the same restraints. Finally, the system was equilibrated for 1 ns without any restraints, followed by a 100 ns production simulation, during which coordinates were sampled every 10 ps. The root-mean-square deviation (RMSD) from the starting crystal structure was calculated with the AMBER cpptraj module, analyzing trajectories with coordinates saved every 10 ps. The reported values are averages over these 10000 sets of coordinates.

The results show that the RMSD values of Glu-172 in both active sites and with the both conformations of the substrate is 2–3 times larger than those of Glu-99, showing that Glu-172 is much more flexible than Glu-99. The MD results confirm our hypothesis of more flexible Glu-172.

Keywords: Glyoxalase I; Metalloenzyme; Mechanism; Glutamate; DFT.

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