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The Locally Enhanced Sampling Study of Large Ligands Diffusion inside Enzyme. Acrylonitrile and Acrylamide Journey in Nitrile Hydratase

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Nitrile Hydratese (NHase) is a metaloenzyme with non-standard active site containing noncorin Co^{3+}. In industry it is used for a large scale conversion of toxic nitriles into useful amides. In this research NHase from *Pseudonocardia Thermophila* JCM 3095 (1IRE) is investigated. In order to understand its excellent catalytic activity the possible transport routes of substrates and products have to be determined[1]. Transient states are not easily elucidated using experimental techniques, so computer modeling of molecular dynamics (MD) helps a lot. The main goals of finding cavities inside the protein and entry/exit pathways for a substrate (acrylonitrile) and the product (acrylamide) have been achieved. In our opinion a very convenient tool for that type of study is MD with the Locally Enhanced Sampling (LES) Hamiltonian[2]. The LES method, by multiplying non-interacting ligand copies, allows for better probing of the conformational space than the standard MD method, despite known problems with the energy equipartition[3].

1 Introduction

Nitrile hydratase (NHase) is microbial metaloenzyme containing non-corrin Co^{3+} or non-heme Fe^{3+} metal ion in the nonstandard active site. NHases are widely used in biotechnology for catalysis of the conversion of toxic nitriles to amides[4]. Products of this enzyme are used in pharmacy (nicotinamide is known as vitamin B3), as soil conditioners, components of diapers, paints and in paper industry[5].

Several crystallographic articles reported high sequence and structure similarity between NHases[4,6]. All native structures solved with high resolution confirmed that the protein is composed of two subunits - α(23 kDa) and β(26 kDa). It have nonstandard active cite composed of four residues: αCys109, αCys112, αSer113, αCys114. The αCys112 and αCys114 residues were found to be post-translationally oxidized to cysteine-sulfinic acid CysSO2H (CSD) and cysteine-sulfenic acid CysSOH (CEA), respectively. Metal ion is coordinated by three sulfur atoms from αCys109, CEA and CSD, and the two amide nitrogen atoms from αSer113 and CEA[4,6].

Although there are 14 structures of NHase known none of them contains a substrate or a product of the catalysis. Only two theoretical reports are known about substrate/product interaction inside protein matrix[7,8] and only one paper presents a comparison of the whole series of substrates and corresponding products[1]. Substrate and products entry/exit paths are not known either. In the x-ray structure of NHase a wide channel is visible[9]. Proper understanding of NHase activity requires new data on structural determinants of large ligands transport within this complex molecular system.

In this paper, for the first time, we describe newtonian molecular dynamics of substrate (acrylonitrile, **ACN**) and product (acryloamide, **ACA**) inside nitrile hydratase protein on 50 ns timescale.

2 Methods

Molecular dynamics simulations on a complex of *Pseudonocardia Thermophila* JCM 3095 Co-NHase (pdb code 1IRE)[6] and a docked **ACA** and **ACN** ligands[8] were performed using NAMD 2.6[10] with Locally Enhanced Sampling (LES) method[2]. Thus, starting structures have ligands located in the active site pocket. CHARMM27 force field was applied and three 6 ns long simulations with LES factor 10 (**ACN** Les10) or 10 and 15 (**ACA** Les10, **ACA** Les15). Periodic boundary conditions and a TIP3 water box with at least 7Å distance from protein atoms to the border were employed. The cutoffs for electrostatics and van der Waals interactions have been set on 12Å. The main simulations were preceded by 100 ps of water equilibration at 300K with a frozen protein, 1000 steps of minimization and 50 ps of heating from 0K to 300K. During the 6 ns production phase the Langevin dynamics protocol with temperature held at 300K has been used.

The idea of the LES method seems to be quite simple[2]. The molecular system is divided into two subsystems a big one for example a protein with surrounding water and a small one in our case **ACN** (7 atoms) or **ACA** (10 atoms). The small subsystem may be cloned giving several non-interacting copies of ligands. These copies are subject to nonbonding potential from the protein. The big subsystem feels an average potential from a swarm of ligands. Since ligands don't interact they may occupy the same place in the space. Due to its large number, a better sampling of the conformational space than the standard one-copy MD is achieved. Thus our 10 copies LES 6ns simulation is equivalent to normal 60 ns MD. In visualization and analysis the VMD 1.8.6 package[11] and home made TCL scripts have been used.

3 Results and Discussion

In both systems studied only one major ligand diffusion route along the NHase channel was observed. However, the detailed paths for the nitrile and the amide are different. Close to the exit **ACN** uses for its motion the upper part of channel, while **ACA** occupies mainly the lower part (see Fig. 1, the lower part is closer lying to the metal ion). Interactions of substrates and ligands with the NHase interior are different. **ACN** came out to the solvent after 1.5 ns but **ACA** remained buried in both 6 ns Les10 and Les15 simulations. Differences in ligand-protein interactions are also seen in statistics of collisions (atom-atom distance closer than 2.5Å) occurring during simulations. Data are presented in Fig. 2. **ACN** very often interacts with αLeu88, βPhe41 and quite often with βPhe118. It seems that these three residues stabilize nitrile on the NHase surface in the neighborhood of the channel entrance.

In **ACA** simulations αGln89, βLeu48, βPhe51 and αTrp52 most often collide with catalytic product. These residues compose the lower part of entry to the channel. αGln89 is particularly important because this residue stabilize a network of hydrogen bonds near active site[12]. Our calculation also shows that this residue very often forms H-bonds with the **ACA** amide group. In our opinion this is the reason why **ACA** did not leave the channel during the simulation despite the presence of the 7Å wide passage.

The space exploited by LES copies inside the NHase may be divided into two subcavities. The deeper one corresponds to the active site pocket and ligands stay there for about 1 ns. In the so called *rez-de-chaussée* more shallow cavity **ACN** remains only for 0.5 ns,

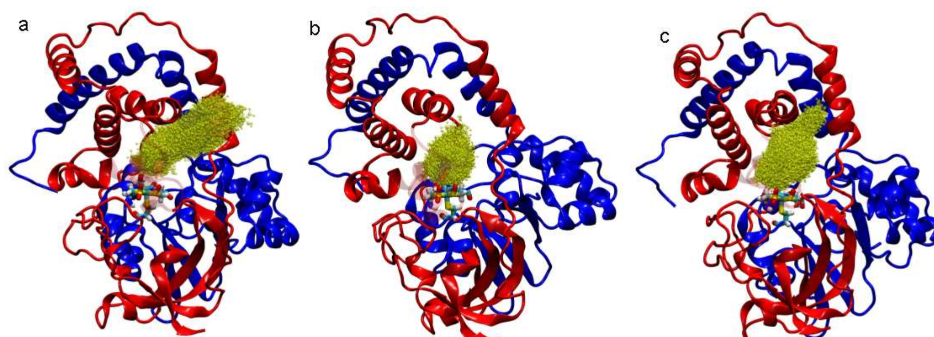

Figure 1. Diffusion paths of aliphatic ligands: (a) substrate **ACN** Les10, (b) product **ACA** Les10, (c) **ACA** Les15.

then it goes on the proteins surface and stays there by 4.5 ns interacting with αLeu88 and βPhe41. In both cavities this small aliphatic substrate has a very large conformational freedom and 180 deg. rotations are sometimes observed. It is worth to note that in **ACN** simulations we observe only weak interactions with CSD and CEA but no collisions with the other parts of the active are noticed. This may suggest that the substrate interacts with the active center via water molecules.

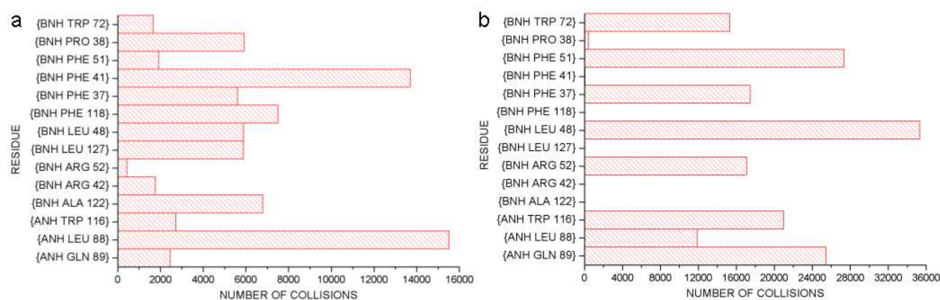

Figure 2. Statistics of the collisions for the **ACN** Les10 (a) and **ACA** Les10 (b).

In **ACA** Les10 simulation the product stays only in the deeper subcavity. Since more copies in the LES method lead to lowering of energy barriers, in Les15 trajectories **ACA** visits also *rez-de-chaussée* subcavity, but instantly goes back to deeper one (compare Fig. 1b and 1c).

4 Conclusions

LES simulations of the NHase from *Pseudonocardia Thermophila* JCM 3095 and its natural ligand reveals differences in the substrate and the product interactions with enzyme. Our calculations show that the **ACN** substrate prefers the upper part of the entrance and is

strongly stabilized by surface residues αLeu88, βPhe41 and βPhe118. The amide product stays at the other region of the entrance. Strong H-bond interactiona of **ACA** with αGln89 are clearly observed. Mutational studies show as well that this residue is critical for enzymatic activity of the NHase[12]. The LES method is a useful computational tool in qualitative studies of enzyme function.

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