Application of Molecular Dynamics for Study of Substrate Binding and Reaction Mechanism of Haloalkane Dehalogenase DhlA

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Motivation:
Haloalkane dehalogenases are microbial enzymes that catalyze dehalogenation reactions important for the degradation of environmental pollutants. Unfortunately, wild type enzymes often do not acquire sufficiently high activity or specificity for degradation of environmental pollutants. An attempt to explore reaction mechanism of hydrolytic dehalogenation involving binding and two subsequent reaction steps was performed in terms of structural changes and dynamic behavior using molecular dynamic simulations.

Methods:
Preparation of Structures. Molecular dynamics simulations were performed with the structure of haloalkane dehalogenase from *Xanthobacter autotrophicus* (DhlA). SANDER module of AMBER 5.0 [1] and force field of Cornell *et al.* [2] were used for calculations. The X-ray structure was obtained from the Brookhaven Protein Database (PDB-ID 2DHC) and prepared for molecular dynamic simulations as follows. Polar hydrogen atoms were added using the program WHATIF 5.0. His289 was singly protonated on N_{δ} in accordance with its catalytic function. Non-polar hydrogen atoms were added using AMBER 5.0. The script *q.kollua* was used for addition of partial charges on all atoms of the enzyme [3]. Two different enzyme–substrate complexes were built using the crystal orientation of DCE [4] in *trans* (180°) conformation and the docked DCE orientation in *+anticlinal* (+120°) conformation [5]. The catalytic water molecule was added to the both enzyme–substrate complexes and hydrated using cap of waters centered to C_{α} atom of Glu56 with 30 Å diameter.

Molecular Dynamic Simulations. DhlA is a globular protein that can be structurally divided into two domains: a main domain forming the core of the structure and a cap domain covering the main domain. The cap domain is connected with the main domain by flexible amino acid residues. Specific restraints were applied to the protein structure in order to prevent disruption of secondary elements based on the regions with different flexibility [6]. The main domain starts from residue 1 to residue 147 and continues from residue 231 to residue 310. Positional restraints for C_{α} atoms were applied to all residues of the main domain. Torsional restraints were used for residues of the cap domain located between the residues 159 and 227. Two regions that connect both preceding domains were kept fully flexible. The enzyme–solvent system was optimized before running the simulation. Several cycles of minimization and short simulations were performed to allow relaxation of the system. First, minimization of hydrogen atoms and water molecules was performed and followed by simulation of water molecules only. Second, minimization of the substrate and water molecules was done and followed by simulation of the substrate and the water molecules. Third, the whole system was minimized and dynamics of water molecules only was simulated. Finally, 200 ps long (100 steps) production phase of dynamic simulation was performed for the whole system.

The transition state structure of the nucleophilic substitution (S_N2) and the structure of covalently bound ethyl–enzyme ester with cleaved chlorine anion were parametrized and included into AMBER force field. The transition state structure was obtained by semiempirical quantum mechanic calculation (AM1 method) using program MOPAC. The transition state was subsequently confirmed by frequency calculation (AM1 method and RHF/3-21G* calculation using GAUSSIAN) and refined using *ab initio* optimization (RHF/6-31G*) in GAUSSIAN. The partial atomic charges were calculated using the RESP module of AMBER 4.1 program package. Two different approaches were used for simulation of transition state of nucleophilic substitution. The first considered transition state oxygen and chlorine atom to have the same radius and non-bonded parameters as standard chlorine and oxygen atoms. The second estimated the radius and non-bonded parameters for transition state oxygen and chlorine atom as linear interpolation between standard chlorine atom and chlorine anion, standard oxygen atom and ester type of oxygen atom, respectively. Partial atomic charges were derived also for the structure of ethyl–enzyme ester. Currently, two 400 ps simulations for S_N2 transition state structure and ethyl–enzyme ester with cleaved chlorine anion were calculated and analyzed in detail.

Results:

Three different ligands appearing in the DhlA active site during dehalogenation reaction were structurally compared: (i) enzyme–substrate complex E.R-X, (ii) nucleophilic substitution transition state complex $E.TS_1$ and (iii) alkyl–enzyme intermediate complex E-R.X. The average enzyme-ligand structures were extracted from three trajectories and subsequently minimized. Two different approaches used for simulation of transition state of nucleophilic substitution provided very similar results. Therefore, only the trajectory calculated using the estimated radius and non-bonded parameters is discussed. Several structurally interesting distances were monitored and analyzed. The most obvious structural change in all three simulations is the movement of cleaved chlorine atom towards halide-stabilizing residues (Table 6). The largest positional deviances are displayed by following amino acid residues: Trp175, Phe172 and His289 (Figure 3). Phe172 and Trp175 displacement follows movement of cleaved chlorine atom during the E.R-X to $E.TS_1$ change. The expected stabilization of cleaved anion by Trp125, Phe172 and Trp175 is increased during E.R-X –> $E.TS_1$ –> E-R.X transition (Table 6). Both tryptophan residues exchange chloride anion mutually. The catalytic water molecule shows two hydrogen bonds to Asp124 and Glu56 in E.R-X structure while in $E.TS_1$ structure the hydrogen bond to Asp124 is destroyed and is substituted by hydrogen bond with His289 due to Trp175 displacement.

Table 6. Average distances [Å].

	E.R-X	$E.TS_1$	E-R.X
Trp125 ... Cl	2.71	2.55	2.46
Phe172 ... Cl^-	3.40	3.14	3.08
Trp175 ... Cl^-	2.72	2.59	2.52

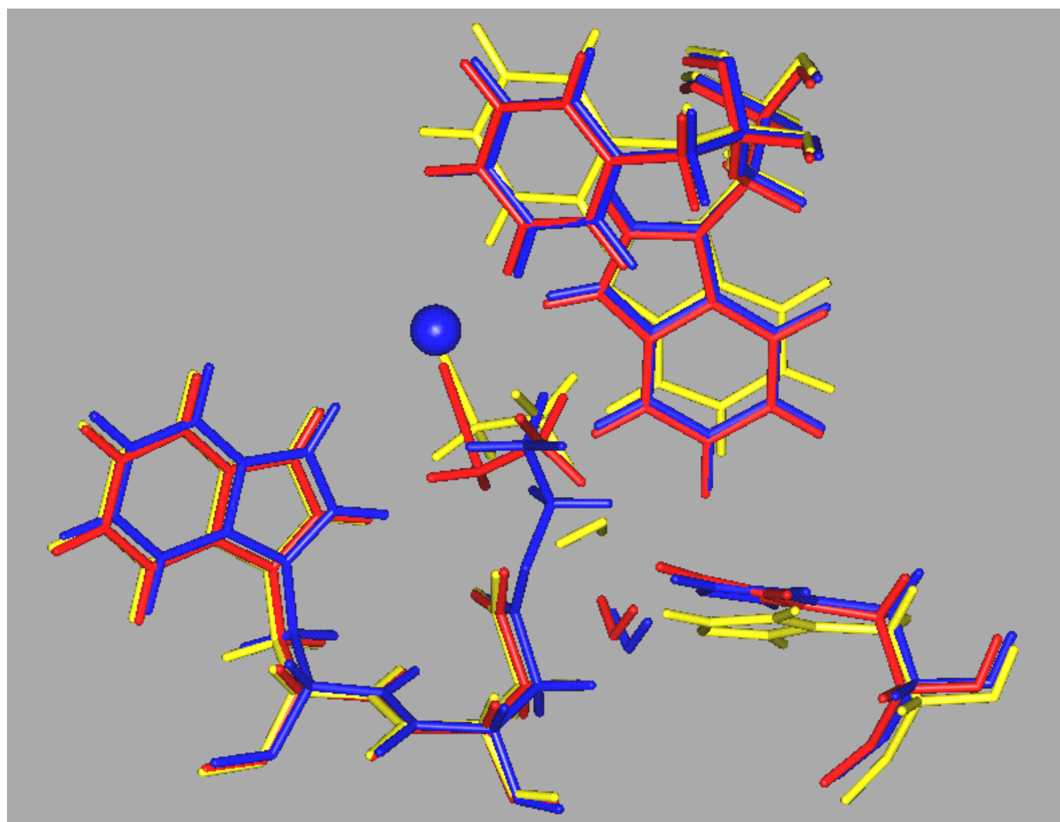

Figure 3. Amino acid active site residues together with bound ligands: E.R-X complex in yellow, E.TS$_1$ complex in red and E-R.X complex in blue.

Conclusions:
Several structurally significant movements of the amino acid residues were identified: (i) movement of catalytic base His289, (ii) displacement of halide-stabilizing residues Phe172 and Trp175, (iii) rotation of nucleophile Asp124 and (iv) repositioning of the catalytic water. The motion of the catalytic water seems to be the most interesting and important observation. Another water molecule that is located below the catalytic one was identified in the crystallographic structure of DCE complexed with DhlA enzyme that probably play a crucial role by stabilizing all reactive components of a system: a nucleophile, a base and a catalytic water. Unfortunately, this second structural water was not explicitly included in the enzyme-ligand complexes and some of identified structural changes could be an artifact.

References:

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