Application of Locally Enhanced Sampling for Study of 1,2-Dichloroethane Binding to the Haloalkane Dehalogenase DhlA

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Motivation:
Haloalkane dehalogenases are microbial enzymes that offer capability to convert haloalkanes to corresponding alcohols and halide ions. The enzymes with hydrolytic type of dehalogenation reaction mechanism are currently targets of intensive research due to their potential application for bioremediation purposes. Recently two COMBINE models of haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10 (DhlA) enzyme and a set of substrates were calculated and revealed that there are two distinct 1,2-dichloroethane (DCE) orientations that have to be taken into account. The key issue is to find out which one is more biologically relevant. Molecular dynamics (MD) with locally enhanced sampling (LES) was selected as suitable computational tool that could provide answer to this question.

Methods:
Preparation of Structures. Molecular dynamics simulations were performed with the structure of haloalkane dehalogenase from *Xanthobacter autotrophicus* (DhlA) using SANDER module of AMBER 5.0 [1] and force field of Cornell *et al.* [2]. 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: (i) the crystal orientation of DCE [4] in *trans* (180^o) conformation and (ii) DCE in +*anticlinal* ($+120^o$) conformation obtained from previous docking calculation [5]. The catalytic water molecule was added to the both enzyme–substrate complexes and the complexes were hydrated using a 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.

Locally Enhanced Sampling (LES). The multicopy dynamics simulation was carried out as follows. The 1,2-dichloroethane molecule was multiplied ten times and the topology information was recalculated using *addles* binary. LES protocol was divided into a heating phase (from 300 K to 400 K) that was 105 ps long and a cooling phase (from 400 K to 0 K) that was 160 ps long. All substrate copies were extracted from the trajectories allowing visual comparison and analysis.

Results:

Two LES analyses were performed for DhlA in complex with crystal (*trans*) or docked (+*anticlinal*) DCE conformation. Starting from crystal DCE conformation two distinct clusters of DCE orientations were found. One is composed of six DCE copies in the *–gauche* (-60^o) conformation while the rest (four copies) form the second cluster with the +*gauche* ($+60^o$) conformation. In total three clusters were found in a run starting from docked DCE conformation: (i) five copies in the +*gauche* conformation, (ii) two copies in the *–gauche* conformation suitably oriented for nucleophilic attack and (iii) three copies in the *–gauche* conformation structurally blocked for nucleophilic attack. Orientations are summarized in Table 1 and visualized in Figure 1 and Figure 2. LES analysis revealed that *trans* conformation of DCE was not found even when starting from a crystal structure (*trans*) or from a docked structure (+*anticlinal*).

Starting docked (+*anticlinal*) conformation corresponds positionally to +*gauche* orientations found by LES. The monitoring of dihedral angle Cl–C–C–Cl from DCE along 200 ps long production phase preceding LES runs was carried out (Figure 2 and Figure 3). It is apparent from its values that the structure starting from docked (+*anticlinal*) conformation moves entirely in +*gauche* conformation while the calculation starting from the crystal (*trans*) conformation immediately changes to *–gauche* and from time to time visits *trans* conformation. The production phase of the calculation starting from crystal DCE conformation was extended up to 400 ps to confirm/disprove the ability to change conformation from *trans* to +*gauche* and the rare visit to *trans* conformation. Three critical distances referring to compactness of the active site and level of stabilization of a halogen were monitored for 200 ps production trajectories that preceded LES runs (Figure 4 and Figure 5). These include distances between cleaved halogen atom and hydrogen atoms from stabilizing residues (HE1-Trp125 ... HE1-Trp175) and distance between the nucleophile and electrophile (OD2-Asp124 ... C2-DCE).

Table 1. Summary of LES results.

starting angle	number of LES copies		
Cl-C-C-Cl	+*gauche*	-*gauche*	*trans*
+*anticlinal*	5	2[a], 3[b]	0
trans	4	6	0

[a] reactive -*gauche* orientations

[a] non-reactive -*gauche* orientations

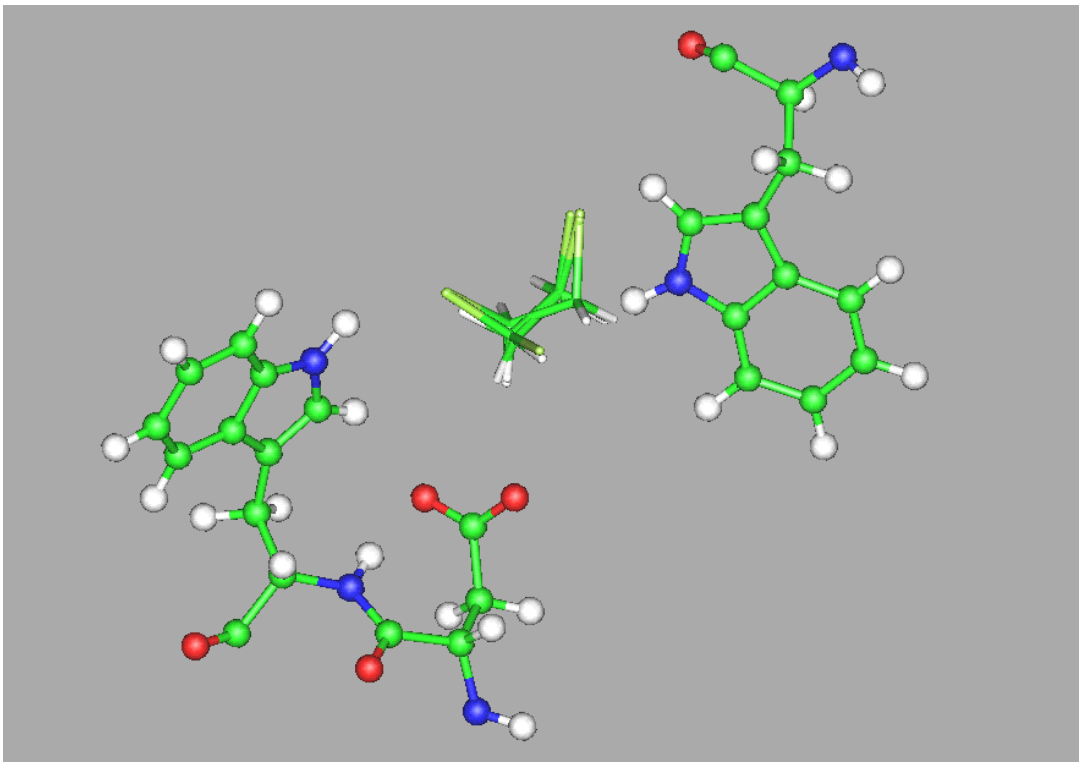

Figure 1. LES clusters for docked DCE system

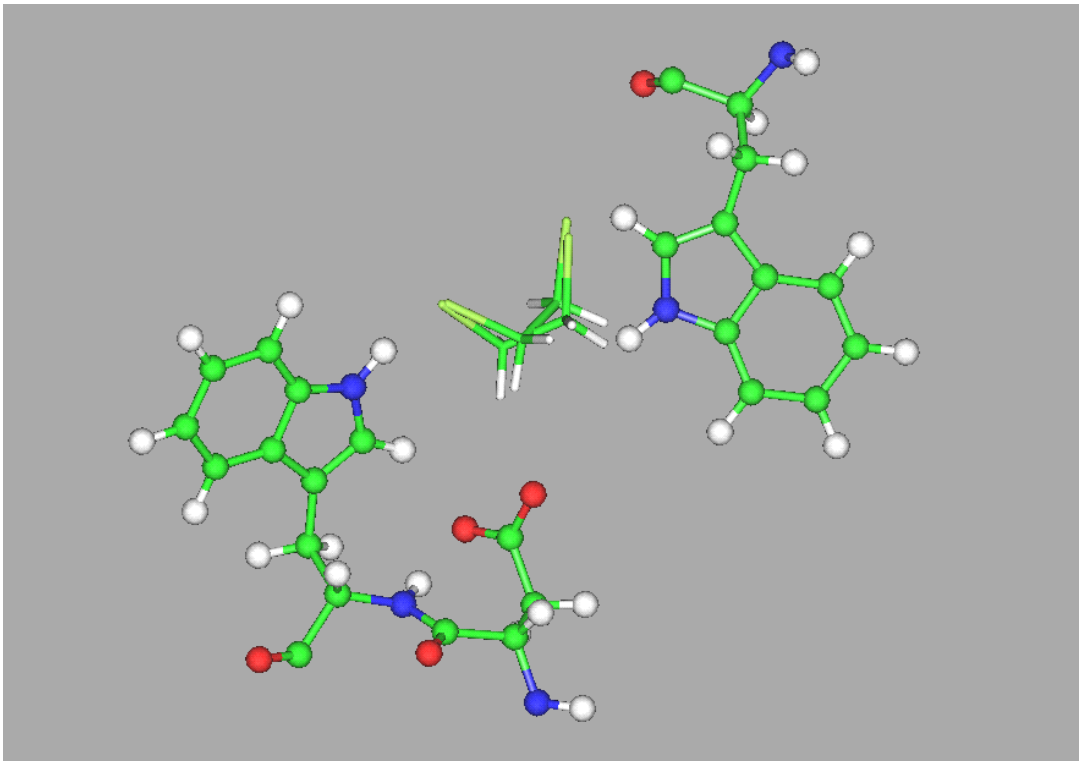

Figure 2. LES clusters for crystal DCE system

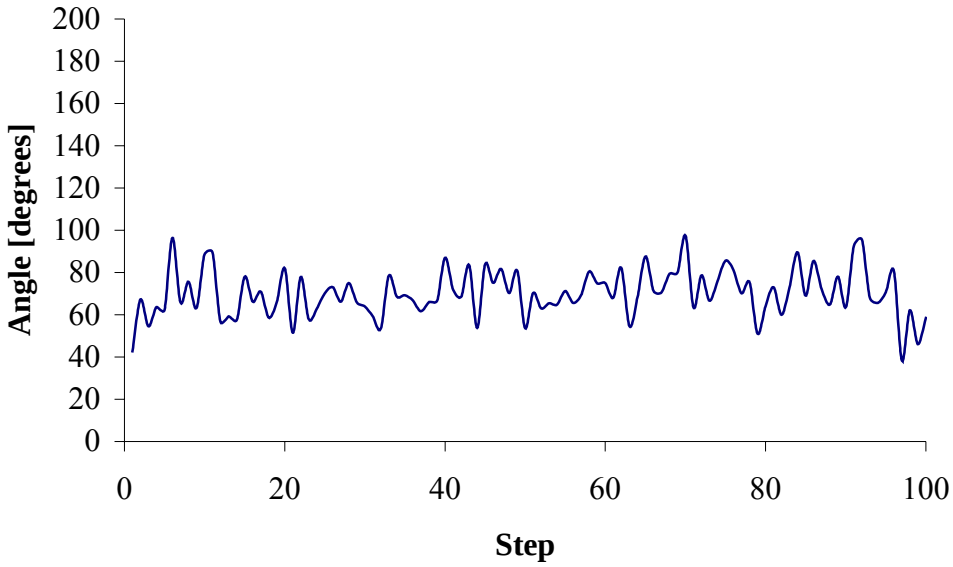

Figure 2 - Dihedral angle monitoring for docked DCE

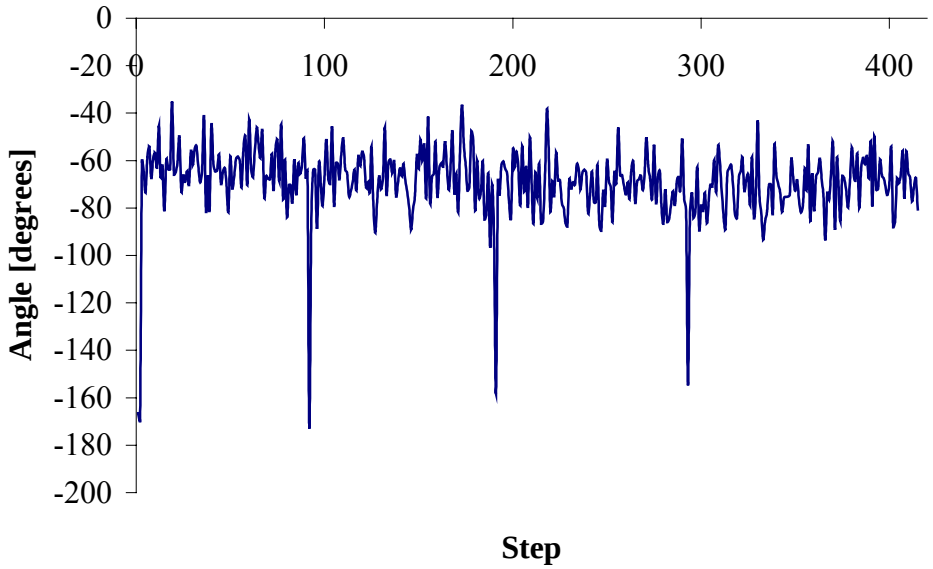

Figure 3 - Dihedral angle monitoring for crystal DCE

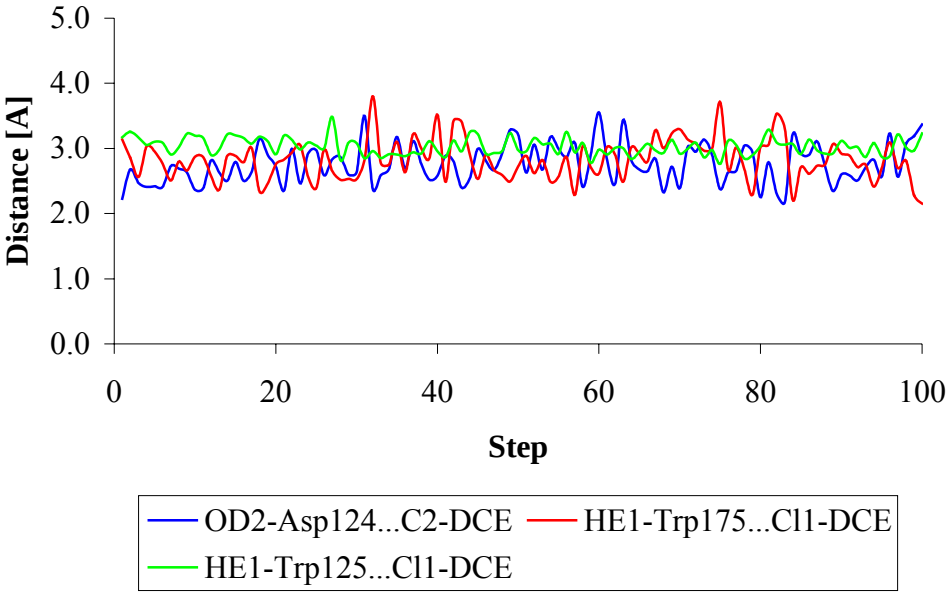

Figure 4 - Distance monitoring for docked DCE

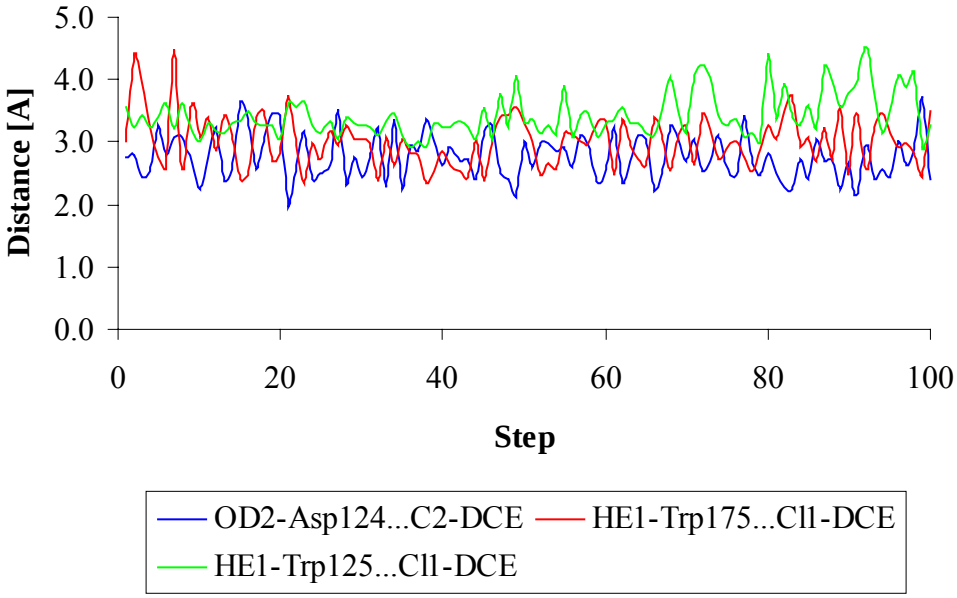

Figure 5 - Distance monitoring for crystal DCE

Conclusions:
We applied locally enhanced sampling methodology to investigate binding of 1,2-dichloroethane to the active site of DhlA enzyme. The study revealed two distinct conformations: +*gauche* and –*gauche*. The ratio between +*gauche* and –*gauche* was 4/6 in the run starting from crystal DCE and 5/5 in the run starting from docked DCE. Crystalographically determined *trans* conformation is not favorable and 1,2-dichloroethane preferably occupies *gauche* conformation. In the simulation starting from docked DCE conformation (+*anticlinal*) the molecule moves entirely in +*gauche* conformation, in the calculation starting from crystal DCE conformation (*trans*) the molecules changes conformation d to –*gauche*. We also observed that DCE can switch back to *trans* with very low frequency in the simulation starting from crystal DCE (*trans*) conformation. Change from *trans* to +*gauche* does not occur even in extended simulation.

References:

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