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Research Article

Deciphering the catalytic amino acid residues of L-2-haloacid dehalogenase (DehL) from *Rhizobium* sp. RC1: An *in silico* analysis

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ABSTRACT

The L-2-haloacid dehalogenases (EC 3.8.1.2) specifically cleave carbon-halogen bonds in the L-isomers of halogenated organic acids. These enzymes have potential applications for the bioremediation and synthesis of various industrial products. One such enzyme is DehL, the L-2-haloacid dehalogenase from *Rhizobium* sp. RC1, which converts the L-isomers of 2-halocarboxylic acids into the corresponding D-hydroxycarboxylic acids. However, its catalytic mechanism has not been delineated, and to enhance its efficiency and utility for environmental and industrial applications, knowledge of its catalytic mechanism, which includes identification of its catalytic residues, is required. Using *ab initio* fragment molecular orbital calculations, molecular mechanics Poisson-Boltzmann surface area calculations, and classical molecular dynamic simulation of a three-dimensional model of DehL-L-2-chloropropionic acid complex, we predicted the catalytic residues of DehL and propose its catalytic mechanism. We found that when Asp13, Thr17, Met48, Arg51, and His184 were individually replaced with an alanine *in silico*, a significant decrease in the free energy of binding for the DehL-L-2-chloropropionic acid model complex was seen, indicating the involvement of these residues in catalysis and/or structural integrity of the active site. Furthermore, strong inter-fragment interaction energies calculated for Asp13 and L-2-chloropropionic acid, and for a water molecule and His184, and maintenance of the distances between atoms in the aforementioned pairs during the molecular dynamics run suggest that Asp13 acts as the nucleophile and His184 activates the water involved in DehL catalysis. The results of this study should be important for the rational design of a DehL mutant with improved catalytic efficiency.

1. Introduction

Halogenated organic compounds occur in huge numbers in the biosphere as a consequence of their use during the production of various industrially important products, e.g., solvents, agrochemicals, and pharmaceuticals (Gribble, 2003). The toxic nature of halogenated compounds and their potential long-term persistence in the environment pose a serious threat to both the environment and human and animal health. Fortunately, certain microorganisms produce dehalogenases that catalyze the cleavage of carbon-halogen bonds in halogenated compounds, thereby minimizing the effects of environmental halogen-associated pollution (Gribble, 2012; Liu et al., 1994; Wong et al., 1992).

Many types of bacteria produce L-2-haloacid dehalogenases, but only a few have an experimentally solved three-dimensional structure and a well characterized catalytic mechanism. The L-2-halohacid dehalogenases for which structures exist include L-DEX from *Pseudomonas* sp. YL (Hisano et al., 1996a, 1996b), DhlB of *Xanthobacter autotrophicus* GJ10 (Ridder et al., 1997, 1995), DehIVa of *Burkholderia cepacia* MBA4 (Schmidberger et al., 2007), L-HAD of *Sulfolobus tokodaii* 7 (Rye et al., 2007), and DehRhb from a marine *Rhodobacteraceae* (Novak et al., 2013). The L-2-halohacid dehalogenase from *Rhizobium* sp. RC1, namely DehL (EC 3.8.1.2), which is the focus of our current study, specifically cleaves halogen atoms from L-isomers of 2-halocarboxylic acids to produce the corresponding D-hydroxycarboxylic acids and halide anions

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(Huyop et al., 2008). However, an experimentally determined structure for DehL is unavailable, although a model has been built *in silico* (Adamu et al., 2016a).

Previous studies have found that mutations of certain residues substantially affect the catalytic abilities of L-2-haloacid dehalogenases. For instance, a comprehensive site-directed mutagenesis study, the effects of which were monitored by a dehalogenase assay (Kurihara et al., 1995), revealed that Asp10, Thr14, Arg41, Ser118, Lys151, Tyr157, Ser175, and Asp180 are important for L-DEX catalysis. These residues are conserved among most L-2-haloacid dehalogenases (Kurihara et al., 1995; Murdiyatmo et al., 1992; Nardi-Dei et al., 1994).

As noted above, a three-dimensional model of DehL was built *in silico* using the crystal structures of other L-2-haloacid dehalogenases as templates (Adamu et al., 2016a). Given the similar folds of DehL and other L-2-haloacid dehalogenases, the catalytic residues identified in other L-2-haloacid dehalogenases might be expected to be conserved in DehL, resulting in its dehalogenation mechanism also being conserved. However, of the nine residues that, when mutated, negatively affect catalysis of L-DEX (Kurihara et al., 1995) and are conserved in almost all other L-2-haloacid dehalogenases, only Asp10 is conserved in DehL (Adamu et al., 2016a). Therefore, to fully elucidate the catalytic mechanism of DehL and potential residues that, upon mutagenesis, would improve its catalytic efficiency, its catalytically important residues must be identified. For the study reported herein, we used various computational methods to predict the catalytically important DehL residues and then proposed a possible catalytic mechanism.

2. Materials and methods

2.1. Fragment molecular orbital (FMO) calculations

The optimized structure of the DehL-L-2CP (Adamu et al., 2016a) was used as the starting structure in this study. To identify residues in DehL that interact with L-2CP in the complex, the complex was subjected to an *ab initio* FMO calculation procedure, which first divided the complex into a collection of small fragments and then performed *ab initio* molecular orbital calculations for each fragment and fragment pair to compute the total energy of the complex (Tanaka et al., 2014). These calculations diminish the computational cost and scaling problems associated with conventional molecular orbital methods (Tanaka et al., 2014). The accuracy of the FMO calculations relative to those of conventional molecular orbital methods has been shown to be within a standard error of ~2 kcal/mol for small proteins (Nakano et al., 2000). Inter-fragment interaction energy (IFIE) analyses of FMO calculations have been used by others to investigate atomic interactions in protein-ligand complexes such as in the cyclic-AMP receptor protein-DNA (Fukuzawa et al., 2006a), the vitamin D-vitamin D receptor (Yamagishi et al., 2006), and the estrogen–estrogen receptor (Fukuzawa et al., 2006b) complexes. Additionally, in combination with a molecular dynamics (MD) simulation, an *ab initio* FMO calculation was performed to determine the roles of certain active-site residues in L-DEX (Nakamura et al., 2009).

All solvent water molecules except for the two labeled WT1 and WT2 were removed from the DehL-L-2CP model. Because the protonation states of polar and charged active-site residues and ligands markedly influence enzymes catalytic reaction mechanisms (Moenig et al., 1985), the carboxyl of L-2CP was treated as being protonated (neutral) given that the optimum pH for DehL activity is 7.5 (Cairns et al., 1996). The side chains of Arg41, Asp13, and His184 were treated as if they were cationic, anionic, and neutral, respectively.

Using the BioStation viewer, version 13.00 (available from the RSS21 project website), the structure of the complex was manually fragmented into individual amino acids, and L-2CP fragments. The *ab initio* FMO calculations were carried out under gas-phase conditions at the MP2 level with a 6–31G basis set in ABINIT-MP, version 7.0 (Nakano et al., 2006) on an Intel Core i7-2600 CPU 3.40, GHz × 8. A BioStation viewer was used to examine the IFIEs.

2.2. Force field for the L-2CP and MD simulation of the dehL-L-2CP complex

Current Programs used MD simulations does not recognizes small molecules like L-2CP. Therefore, before performing the MD simulation for the DehL-L-2CP complex, force-field parameters for L-2CP were calculated using Topolbuild version 1.3. (available from the GROMAC website).

The MD simulation of the complex was then carried out on an Intel Core i7-2600 CPU 3.40 GHz × 8 using the GROMACS 4.6.5 software suite (Van Der Spoel et al., 2005) and the OPLS/AA force field. The simulation system consisted of a 407.781 nm^3 dodecahedron box containing the DehL-L-2CP complex solvated in 12115 SPC/E water molecules and a catalytic water molecule. For the Edwald equation to accurately describe the simulated long-range interactions, three chloride ions were added to neutralize the three positive charges in the system. The entire system was minimized using the steepest descent method with 884 steps. The system was first isothermally and isochorically equilibrated and then isothermally and isobarically equilibrated using NVT and NPT ensembles, respectively to stabilize its temperature and pressure. Both ensembles were implemented using a coupling time of 0.1 ps, and equilibration was achieved within 100 ps.

The MD simulation was run at 303 K and 1 atm for 3 ns with an integration time step of 2 fs. Using the grid option, the neighbor list was updated every five steps with a cut-off distance for the short-range neighbor list of 0.9 nm. The linear constraint solver algorithm (LINCS) (Hess et al., 1997) was used to constrain the bond lengths, electrostatic interactions were described by the particle mesh Ewald (PME) method (Darden et al., 1993) with an rcoulomb of 0.9 nm, and van der Waals integrations were treated with a van der Waals radius cut-off of 1.4 nm. Structural frames were generated every 2 ps to update the trajectory. The validity of the calculated inter-atomic interactions was investigated by a distance plot versus time generated from the MD simulation trajectories in GROMACS.

2.3. In silico alanine scanning

Catalytic residues are part of the active sites of enzymes and contribute to the non-covalent interaction free energy in enzyme-substrate complexes. *In silico* alanine scanning and molecular mechanics Poisson-Boltzmann surface areas (MM-PBSA) calculations have been used to estimate the contribution of each residue to the binding of a substrate (Massova and Kollman, 1999). To identify the catalytic residues in DehL, with the exception of the putative active-site residues Gly16 and Ala185–Asp13, Thr17, Pro47, Met48, Arg51, Hsi184, and Ile186 were individually mutated to an alanine. These residues the putative active-site residues Gly16 and Ala185–Asp13, Thr17, Pro47, Met48, Arg51, His184, and Ile186 were individually mutated to an alanine. These residues were mutated to alanine because it is small in size and chemically inert, and alanine often conforms to the secondary structure(s) of its neighboring residues. The mutant structures were generated from the optimized wild-type DehL-L-2-CP structure by truncating the mutated residue at Cγ and then replacing that atom with a hydrogen atom and shortening the Cb-Cγ bond to 1.09Å using the editing tool in UCSF Chimera version 1.10.2 (Pettersen et al., 2004).