In silico assessment of dehalogenase from Bacillus thuringiensis H2 in relation to its salinity-stability and pollutants degradation

Habeebat Adekilekun Oyewusi\textsuperscript{a,b,c}, Fahrul Huyop\textsuperscript{a,b} \textsuperscript{d}, Roswanira Abdul Wahab\textsuperscript{b,d} \textsuperscript{e} and Azzmer Azzar Abdul Hamid\textsuperscript{a} \textsuperscript{d}

\textsuperscript{a}Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, Johor Bahru, Malaysia; \textsuperscript{b}Enzyme Technology and Green Synthesis Research Group, Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, Johor Bahru, Malaysia; \textsuperscript{c}Department of Biochemistry, School of Science and Computer Studies, Federal Polytechnic Ado Ekiti, Ado Ekiti, Ekiti State, Nigeria; \textsuperscript{d}Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, Johor Bahru, Malaysia; \textsuperscript{e}Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia (IIUM), Kuantan Pahang, Malaysia

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\textbf{ABSTRACT}

Increased scientific interest has led to the rise in biotechnological uses of halophilic and halotolerant microbes for hypersaline wastewater bioremediation. Hence, this study performed molecular docking, molecular dynamic (MD) simulations, and validation by Molecular Mechanic Poisson-Boltzmann Surface Area (MM-PBSA) calculations on the DehH2 from \textit{Bacillus thuringiensis} H2. We aimed to identify the interactions of DehH2 with substrates haloacids, haloacetates, and chlorpyrifos under extreme salinity (35% NaCl). MD simulations revealed that DehH2 preferentially degraded haloacids and haloacetates (−6.3 to −4.7 kcal/mol) by forming three or four hydrogen bonds to the catalytic triad, Asp125, Arg201, and Lys202. Conversely, chlorpyrifos was the least preferred substrate in both MD simulations and MM-PBSA calculations. MD simulation results ranked the DehH2-L-2CP complex (RMSD 0.125–0.23 nm) as the most stable while the least was the DehH2-chlorpyrifos complex (RMSD 0.32 nm; RMSF 0.0−0.29). The order of stability was as follows: DehH2-L-2CP > DehH2-MCA > DehH2-D-2CP > DehH2-2,2-DCP > DehH2-2,3-DCP > DehH2-3CP > DehH2-TCA > DehH2-chlorpyrifos. The MM-PBSA calculations further affirmed the DehH2-L-2CP complex’s highest stability with the lowest binding energy of −45.14 kcal/mol, followed closely by DehH2-MCA (−41.21 kcal/mol), DehH2-D-2CP (−31.59 kcal/mol), DehH2-3CP (−30.75 kcal/mol), DehH2-2,2-DCP (−29.72 kcal/mol), DehH2-2,3-DCP (−22.20 kcal/mol) and DehH2-TCA (−18.46 kcal/mol). The positive binding energy of the DehH2-chlorpyrifos complex (+180.57 kcal/mol) proved the enzyme’s non-preference for the substrate. The results ultimately illustrated the unique specificity of the DehH2 to degrade the above-said pollutants under a hypersaline condition.

\textbf{1. Introduction}

Hypersaline environments reaching saturation salinities (~350 g L\textsuperscript{−1} of NaCl) occur either by natural or anthropic actions that favor salt deposition and accumulation (Le Borgne et al., 2008). Agricultural run-off creates similar conditions because of the high concentrations of herbicides, insecticides, and other pesticides they carry. Hence, pesticide and herbicide-rich environments pose severe threats to human and aquatic biota (Ewere et al., 2021). As a matter of fact, organochlorides and organophosphorus pesticides such as Dalapon\textsuperscript{®} and chlorpyrifos are the most common pollutants found in brine samples (Fetter et al., 2017; Mondal & Kole, 2021; Serrano et al., 2012) collected in different countries. These toxic substances are commonplace in surface waters (Smith et al., 2021), with the capability to bioconcentrate in a variety of aquatic organisms (Smith et al., 2021).

The organisms inhabiting such a challenging environment are called halophiles (Oyewusi, Wahab, & Huyop, 2020). Simultaneously, microorganisms that grow optimally in this environment and non-saline ones are deemed halotolerant (Ben-Laouane et al., 2020; Madigan et al., 2009; Remonsellez et al., 2018; Satari Faghihi et al., 2019). Polluted environments are often hypersaline, and microorganisms found in them would habitually have adapted and thrived in the presence of such pollutants because of specific enzymes they produced. These enzyme-producing halophiles and halotolerant microorganisms can degrade an array of hydrocarbons, viz. aliphatic, aromatic, halogenated- and nitrated compounds (Akçay & Kaya, 2019; Giovanelia et al., 2020; Oyewusi, Wahab, Kaya, et al., 2020).

Studies discovered that several enzymes function best at moderate temperature, pH, and salinity range, beyond which their proteins become quickly denatured. The extreme...
salinity of hypersaline habitats could render non-halophilic enzymes non-active through aggregation. Salt-sensitive enzymes undergo a more significant hydrophobic effect, structurally collapsing their proteins as the high ion concentration inhibits electrostatic interactions between amino acid residues (Zaccar & Eisenberg, 1990). Conversely, surfaces of the more negatively charged halophilic enzymes are less destabilizing under a highly saline condition because of fewer lysine and cysteine residues. Other unique characteristics include a greater number of random coils at the expense of fewer lysine and cysteine residues (Siglioccolo et al., 2011; Edbeib et al., 2020). The general decrease in hydrophobic residues’ size in halophilic enzymes was thought to elevate the hydrophobic effect, created by a rise in the dielectric nature at extreme salt concentrations. However, stable and effective enzymes capable of degrading halogenated pollutants under hypersaline environments remain scarce.

The Bacillus thuringiensis H2 isolated from a hypersaline Lake Tuz in Turkey is among the microorganisms that could render different haloacids, haloacetates, and chlorpyrifos less harmful. The bacterium functions optimally at 30 °C and pH 8.0 and is reasonably stable over a broad range of salinity (0 – 35%) with a maximum tolerance of 35% (w/v) (Oyewusi, Wahab, Kaya, et al., 2020). Although our earlier in-silico analysis proved the DehH2 dehalogenase could degrade several of the mentioned compounds (pollutants) (Oyewusi, Wahab, Kaya, et al., 2020), its ability to do the same under a hypersaline condition remains untested. Therefore, this study aimed to computationally compare the specificity of the DehH2 interactions with several types of halogenated substrates at different salinities. The comparison was based on the calculated binding affinities of the enzyme-substrate complexes, which will also validate our observation in an earlier empirical investigation (Oyewusi, Huyop, et al., 2020).

In this work, several computational tools were used to provide structural insights into the molecular binding process of the DehH2 with the substrates under a simulated hypersaline condition. The stability of the DehH2 protein was assessed by molecular dynamics (MD) simulation at various salinities (0 – 50% NaCl) to bring to light the different interactions between the complexes. Next, the stabilized DehH2 generated pdb file of the identified best salinity in the simulation was subjected to substrate docking analysis and further MD simulations of the enzyme-ligand complex. We then compared the calculated binding free energy via the g_mmpbsa tool to compute complex trajectories, which information is germane for predicting the probable conformational shifts, alongside the complexes’ dynamical behavior that dictates the substrate specificity of DehH2 under a hypersaline environment. Comprehension of the structure-function and the DehH2 structural adaptation could better explain its varying ability to degrade the different halogenated compounds under highly saline conditions. This study also provides specifics on the DehH2 protein fold quality and the possible mechanism by which the enzyme could hydrolyze the tested halogenated substrates with a particular preference.

**2. Materials and methods**

**2.1. Sequence retrieval and molecular modeling**

The DehH2 sequence was obtained from the National Centre for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/protein) with a gene identification number MT920427. The sequence was searched against the Protein Data Bank (PDB) (http://www.pdb.org/) using BLASTp (Basic Local Alignment Search Tool) and the SWISS-MODEL (https://swissmodel.expasy.org/) modeled the three-dimensional (3D) structure of the enzyme, as reported in previous study (Oyewusi, Huyop, et al., 2020). The BRENDA database showed that the homodimeric DehH2 structure is a member of the L-2 haloacid dehalogenase family (EC 3.8.1.2) (Oyewusi, Huyop, et al., 2020).

**2.2. Model refinement of DehH2 at various salinities**

MD simulations by the GROMACS 5.1.2 and Gromos96 53a7 forcefield (https://www.gromacs.org/About_Gromacs) examined the structural stability of the DehH2 at salinities between 0 and 50% NaCl. The simulation was done in a defined cubic box with a 1.0 nm minimum distance between the solutes and the box’s edge. The simple point charge (SPC) water model solvated the solutes by adding seven sodium ions to neutralize the system electrically. The tripled production simulation was set to neutral for the following NaCl concentrations; 0, 1.75, 3.5, 5.25, 6.125, 7.0 and 8.75 M which corresponded to salinity levels 0, 10, 20, 30, 35, 40 and 50% (w/v) of NaCl, respectively. For brevity, at 0%, the DehH2 model was neutralized by adding 7 Na+, while 0.2, 0.41, 0.61, 0.71, 0.81, and 1.02 each of Na+ and Cl− ions were further added into the solvent box to mimic the condition of a hypersaline environments that corresponded to 1.75 M (10% w/v NaCl), 3.5 M (20% w/v NaCl), 5.25 M (30% w/v NaCl), 6.125 M (35% w/v NaCl), 7.0 M (40% w/v NaCl) and 8.75 M (50% w/v NaCl), respectively.

The simulation was done at 300 K and 1 atm, respectively, and energy minimization used the steepest descent and conjugate gradient methods at 515 steps. The equilibrated structures were further added into the solvent box to mimic the condition of a hypersaline environment that corresponded to 1.75 M (10% w/v NaCl), 3.5 M (20% w/v NaCl), 5.25 M (30% w/v NaCl), 6.125 M (35% w/v NaCl), 7.0 M (40% w/v NaCl) and 8.75 M (50% w/v NaCl), respectively.

The simulations were performed at 100 ns with 2 fs integration time steps. The outputs were plotted as finished Xmgrace graphs to analyze the simulation trajectories using the step-by-step configuration (Lemkul, 2018; Lindahl et al., 2001). The dynamic behavior and structural changes of the DehH2 protein model were gauged by root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF). Stabilized proteins pdb under specific salinities at the end of the simulation were generated and then validated.

**2.3. Structural validation of the refined model**

Refined 3D structures of the DehH2 modeled at different salinities were evaluated for chemical structure stability by the UCLA-DOE server. Verify_3D assessed the compatibility of the 3D model with its amino acid sequence (ID) categorized by structural class to alpha, beta, loop, polar, and nonpolar, and compared the output with suitable structures (Lüthy et al., 2020).
2.4. Molecular docking

Next, molecular docking analysis was performed on selected best MD simulation structures based on the lowest RMSD and RMSF values at different salinities. The AutoDock version 4.2.6 and AutoTools 1.5.6 software docked the following ligands {2,2-dichloropropionic acid, 2,2-DCP, 2,3-dichloropropionic acid, L-2-chloropropionic acid (L-2-CP), D-2-chloropropionic acid (D-2-CP), 3-chloropropionic acid (3CP), monochloroacetate (MCA), trichloroacetate (TCA), and organophosphate (chlorpyrifos)} into the DehH2 active site under varying salinities, to observe the binding orientations of the protein-ligand complexes. Water molecules were first excluded from the DehH2 protein, followed by the addition of polar hydrogens and nonpolar hydrogen and the total Kollman and Gasteiger charges. Similar treatments were done on the ligands to ensure the correct adoption of torsions for rotation during docking. Pertinently, the information gained here could predict the complex’s binding affinity based on an empirical free energy scoring function. The binding region demarcated by the 191 Autogrid tools in Autodock, for the DehH2 was fixed at ±1.000 Å from the binding region demarcated by the 191 Autogrid tools in SWISS-MODEL. The resultant number of PostScript plots then analyzes the overall and residue-by-residue geometry.

2.5. MD Simulations of the enzyme-ligand complex at best salinity (35% NaCl)

The GROMACS 2018.6 software simulated the DehH2 protein using the 3a37 Gromos as the force field (http://www.gromacs.org/About_Gromacs), and the coordinate and topology files of the DehH2 protein and ligands were prepared separately as inputs. The ATB server optimized the ligands, and the pdb2gmx program prepared the DehH2 protein (Koziara et al., 2014; Malde et al., 2011). The protein and the ligands were merged before the production run to obtain a complex structure. In a 1.0 nm x 1.0 nm x 1.0 nm cubic simulation box, the solutes were solvated in 180,000 SPC/E water molecules under a constant system of 300 K and 1.0 atm for 100 ns. The enzyme was electrically neutralized by adding seven Na⁺ ions before the system was energy minimized using the steepest descent algorithm to a maximum of 10,000 steps or until the maximum force (Fmax) was no longer greater than 1000 kJ mol⁻¹nm⁻¹ (the default threshold). The Visual Molecular Dynamics visualized the simulation trajectories, and the final outputs were plotted as Xmgrace graphs (Lindahl et al., 2001). Interactions between the DehH2 protein and ligands were observed and compared using values of RMSD, RMSF, radius of gyration (Rg), and hydrogen bonds trajectory. Other observations include structural changes and the dynamic behavior of the enzyme-substrate complex.

2.6. Binding free energy calculations

In this study, the g_mmpbsa package estimated the molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) interaction. The g_mmpbsa tool, together with the GROMACS package coupled with an Adaptive Poisson-Boltzmann Solver (APBS), calculated the binding free energy of the DehH2-substrate complexes (Kumari et al., 2014), as shown in the following equations (Adamu et al., 2017; Kumari et al., 2014; Singh et al., 2020) (Equation (1)).

$$\Delta G_{binding} = G_{complex} - (G_{protein} + G_{ligand})$$

where \( \Delta G_{binding} \) is the binding free energy of DehH2 with each substrate, G_{complex} is the total free energy of the DehH2 with each substrate, and G_{DehH2} and G_{substrate} are the total free energies of DehH2 and each substrate in the solvent, respectively. The following Equation (2) was used to estimate the free energy of each molecule:

$$G_{molecule} = (E_{MM}) - TS + (G_{solvation})$$

where G_{molecule} is defined as the protein or ligand or protein-ligand complex. E_{MM} is the average molecular mechanics’ potential energy in a vacuum. TS denotes the entropic contribution to the free energy, while T and S represent the temperature and entropy. The entropic term was ignored when calculating the G_{molecule} as the study only observed the relative binding energies of the DehH2-substrate complexes (Adamu et al., 2017). G_{solvation} is the free energy solvation to transfer a solute from a vacuum to the solvent. Next, the molecular mechanics (MM) potential energy was expressed as:

$$E_{MM} = E_{bonded} + E_{nonbonded} = E_{bonded} + (E_{vdw} + E_{elec})$$

where, E_{bonded} are interactions that contributed to the bond, angle, dihedral, and improper interactions. E_{nonbonded} is the non-bonded interaction, including the van der Waals (E_{vdw}) and electrostatic (E_{elec}) energy terms, while \( \Delta E_{bonded} \) is always taken as zero. The MM-PBSA approach calculated the free energy of solvation in an implicit solvent as represented in the following term:

$$G_{solvation} = G_{polar} + G_{nonpolar}$$

The term G_{polar} refers to the solvation-free energy of electrostatic interactions calculated by solving the Poisson-Boltzmann (PB) equation. At the same time, G_{nonpolar} signifies...
The program of PH0459 as a template for the homology modeling study. Pyrococcus horikoshii hyperthermophilic structure of the PH0459 haloacid dehalogenase from a sequence similarity of the DehH2 (63.71%) to the crystal and the model built by SWISS-MODEL revealed a high not available in the PDB. The BLASTp search against the PDB MT920427) as the enzyme sequence obtained from NCBI (Accession number: 3.1. Homology modeling, refinement, and structure validation of DehH2

The 3D structure of DehH2 was determined through the sequence obtained from NCBI (Accession number: MT920427) as the enzyme’s experimental 3D structure was not available in the PDB. The BLASTp search against the PDB and the model built by SWISS-MODEL revealed a high sequence similarity of the DehH2 (63.71%) to the crystal structure of the PH0459 haloacid dehalogenase from a hyperthermophilic Pyrococcus horikoshii OT3 (PDB ID: 1x42). The high similarity seen here affirmed (>30%) the suitability of PH0459 as a template for the homology modeling study. The program’s accuracy was verified by retrieving the actual complex structure, i.e., l-haloacid Deh bound with L-substrate. From here, we separated the two molecules and performed docking at the specific binding region or active site. Here, the best-docked conformation was compared with the actual ones (supporting information Figure S1), which showed the RMSD for the superimposed structures to be less than 1 Å. The low value indicated the high accuracy and predictive capability of the program used by this study, where the acquired binding conformation was similar to the actual bound ligand in the PDB.

Next, the refined output structure from MD simulation showed good conformation with protein folding akin to a native protein; the refinement effectively reduced side chain clashes and steric hindrances. The same treatment also minimized the protein’s potential energy by the steepest gradient method. Hence, the study’s final Deh protein structure with the lowest global conformation (supporting information Figure S2) is the most stabilized 3D structure arrangement. Quality of the DehH2 structure modeled at various salinities assessed by the Ramachandran plot, Verify-3D, and ERRAT revealed that 94.6% of residues occupied the most favorable region, with 5.4% in the additional allowed region, and none are in the disallowed region. As over 90% of the residues resided in the most favorable region, this signified an outstanding and acceptable quality of the DehH2 homology model, similar to those described in earlier studies (Anuar et al., 2020; Oyewusi, Huyop, et al., 2020).

3.2. Model Simulation at different salinity

This study performed MD simulations under different salinities (0%, 10%, 20%, 30%, 35%, 40%, and 50%) to observe the atomic-level and the dynamical properties of the DehH2. Furthermore, the conformational sampling during simulation could establish the protein’s most stable state concerning its function (Batumalaie et al., 2018). The RMSD and RMSF of the DehH2 model are depicted in Figures 1a,b, which illustrates the root mean square fluctuation (RMSF), representing the motions of amino acid residues around their mean position after 100 ns MD simulation. Notably, a low RMSD value (RMSD ~0.2 – 0.3 nm) implies the high stability of the DehH2 structure, while less stable ones have higher values (Anuar et al., 2020; Oyewusi, Huyop, et al., 2020). The general convergence from the DehH2 initial structure at different salinities (0 – 50% NaCl) (Figure 1) from the onset of MD simulation (at 300K, 1 atm) implied its good stability at the predicted maximum stability of 35% salinity.

It was apparent that the RMSD of the DehH2 simulated at 30% NaCl (Figure 1a) stabilized more rapidly at 15 ns (average RMSD = 0.15 nm) compared to others. Nevertheless, the study could see that the most stable trajectory of the DehH2 protein occurred in 35% NaCl (Figure 1). Conversely, the DehH2 trajectories at other salinities were either erratic and slower to stabilize. MD simulations at 0% (unstable), 10% (55 ns), 20% (40 ns), 40 (erratic) and 50% (erratic) of NaCl registered average RMSDs amounting to 0.28 nm, 0.16 nm, 0.19 nm, 0.2 nm, and 0.3 nm, respectively (Figure 1a). While the results demonstrated that the DehH2 began to denature at salinities above 35%, it also proved that the enzyme could tolerate a reasonable salinity range.

Results of the corresponding RMSF are shown in Figure 1b. Notably, RMSF of > 0.05 nm (0.5 Å) is the threshold where a significant change in residue-specific flexibility occurred (Dong et al., 2018; Kovacic et al., 2016). MD simulations for the DehH2 for salinities between 0 and 40% NaCl...
revealed that the average RMSF values for all residues were < 0.05 nm. The low RMSF values in all regions (helix, sheet, and loop) of the DehH2 (Figure 1b) was the likely result of the residues’ flexibility and stability. The exception occurred at 50% NaCl that exhibited markedly high RMSF values, with fluctuations at lysine-11 (0.2 nm), glutamate-70 (0.24 nm), arginine-75 (0.24 nm), aspartate-183 (0.25 nm), and valine-230 (0.3 nm). Major fluctuations were also noted for residues sited on exposed loops on the DehH2, namely the valine-32 (0.28 nm) and threonine-41 (0.26 nm) at 30% and lysine-205 (0.29 nm) at 0% NaCl (Figure 1b).

The study somewhat expected this as residues located on exposed loops typically score higher RMSF values. Characteristically, the lower RMSF values seen in the DehH2 simulated at 0 – 40% (w/v) NaCl, except at 50%, were proof of the enzyme’s ability to remain adequately flexible under dehydrating high-salt conditions. Conversely, the fluctuating RMSD and RMSF values at 50% (w/v) NaCl revealed the onset of protein structure destabilization. Typically, an unadapted enzyme structure to a high-salt environment could be easily distorted through water loss, thus exerting a considerable impact on protein stability, solubility, and function. Dehydration of cellular proteins becomes pronounced because of decreased water content due to water-molecule entrapment within external ionic lattices. The same denaturing mechanism also inhibits a non-halophilic bacterium’s growth in a hypersaline condition (Edbeib et al., 2017).

The results seen here corroborate our previous empirically proven stability of the B. thuringiensis over a broad range of salinity (0 – 35%), with maximum tolerance to salt at 35% (w/v). The results also support the high number of acidic residues (62% Asp and Glu) in the DehH2 structure that could electrostatically interact with the cations in the salt solution (Oyewusi, Huyop, et al., 2020). Having said that, it is unlikely

Figure 1. MD simulation of DehH2 at various salinities. Average RMSD and RMSF of the backbone conformation are shown as function of simulation time (100 ns) at 300 K (a) the overlayed RMSD plots and (b) the overlayed RMSF plots of DehH2_0% NaCl in black, DehH2_10% NaCl in green, DehH2_20% NaCl in magenta, DehH2_30% NaCl in purple, DehH2_35% NaCl in brown, DehH2_40% NaCl in blue and DehH2_50% NaCl in red.
Figure 2. LigPlot analysis for DeH2-ligand interaction shows hydrogen bonds with equivalent distance and non-ligand residues involved in the hydrophobic interaction. (a) Chlorpyrifos (b) L-2CP (c) 2,2-DCP (d) D-CP (e) 3-CP (f) MCA (g) 2,3-DCP and (h) TCA.
the negatively charged amino acids in the DehH2 is ‘un-specific’ as a random distribution of acidic amino acids is not always able to ionically stabilize in a high-salt condition (Liang et al., 2011). Edbeib et al. (2020) also described a similar observation on dehalogenase DehHX isolated from a marine bacterium that showed salinity tolerance up to 25% NaCl.

3.3. Structural validation of DehH2 protein at various salinities

A stereochemical quality check on the modeled DEhH2 structures was performed (Ramachandran plot, $\Phi$-$\Psi$ plot), alongside other factors using Verify-3D and ERRAT. The Ramachandran plot for the DehH2 model at 0—50% NaCl showed residues occupying the most favorable region at 90.1—94.6%, between 0.5 and 12.9% of the residues are in the additional favored region, 0—1.0% are in the generously allowed, and 0—0.5% in the disallowed region, simulated at 40—50% NaCl concentration (supporting information Figure S3). The results implied an excellent and acceptable quality of the homology model DehH2 protein as over 90% of residues resided in the most favorable region (supporting information Figure S3) (Anuar et al., 2020; Batumalaie et al., 2018; Oyewusi, Huyop, et al., 2020). The ERRAT histogram shows two lines drawn to represent the confidence to reject regions beyond the 99% error value. Here, the correct regions are colored in grey, and the incorrect regions appear as black. One of the ERRAT scores for the non-energy minimized DehH2 model fell below the rejection limit of 95% and scored between 90.991 and 98.994%, before improving to 97.297—99.550% after energy minimization (Table 1). It should be noted that the accepted range for a good protein model has an ERRAT score of >50% (Rosdi et al., 2018).

Lastly, the Verify-3D scores for all models were higher than 0, which implied good side-chain environments (Table 1). Literature has shown that a satisfactory protein model should score >80% (Rosdi et al., 2018). Hence, the subsequent modeling of the DehH2 protein was done at the optimal 35% (w/v) NaCl based on the attained stable RMSD and RMSF values.

3.4. Substrate docking

Ligands 2,2-DCP, 2,3-DCP, L-2CP, D-2CP, and 3-CP, haloacetate (MCA and TCA), and chlorpyrifos were docked into the active site of the DehH2, using the Autodock version 4.2.6 and AutoTools 1.5.6. Three catalytic residues (Asp125, Arg201, and Lys202) of the DehH2 were identified in previous study to form hydrogen bonds with the carbonyl oxygen atom of the substrates (Oyewusi, Huyop, et al., 2020). In this study, the molecular docking results illustrated that the DehH2 and the ligands were linked by inter-atomic interactions such as hydrogen bonding interactions and hydrophobic interactions. The observed minimum binding energies were between $-6.5$ and $-4.7$ kcal/mol, stabilized by at least two hydrogen bonds (Table 2). The DehH2 docked with chlorpyrifos ($-6.5$ kcal mol$^{-1}$) showed the most favorable binding energy followed by L-2CP ($-6.3$ kcal mol$^{-1}$), 2,2-DCP ($-5.4$ kcal mol$^{-1}$), 3-CP ($-5.3$ kcal mol$^{-1}$), MCA ($-5.1$ kcal mol$^{-1}$), 2,3-DCP and TCA ($-4.7$ kcal mol$^{-1}$) (Table 2).

Two hydrogen bonds with distances of 3.1 Å and 3.4 Å linked the DehH2-chlorpyrifos to Thr122 and Ser124, respectively (Figure 2a). Interestingly, the results seen here contradicted the earlier predicted catalytic triad (Table 3) (Oyewusi, Huyop, et al., 2020). The L-2CP showed marginally shorter hydrogen bonds (2.3 Å and 2.7 Å) than 2,2-DCP (2.6 Å and
2.5 Å) despite their comparable binding energies (Figure 2b) to (Figure 2c). A shorter hydrogen bond distance between two atoms usually increases the probability of forming a bond, given that one atom acts as an electron donor. At the same time, the other is an electron acceptor (Abidin et al., 2019). Low binding energies were estimated for DehH2 complexes with D-2DP, DehH2-3CP, and MCA (Table 2). All three complexes only formed a single hydrogen bond (2.6 Å, 2.7 Å, and 2.8 Å, respectively) to the catalytic Asp125 (Figure 2d,e,f), while 2,3-DCP and TCA formed distinctly weaker bonds (Table 2).

Crucially, the hydrogen bond distances seen in all DehH2-ligand complexes were within the acceptable cut-off limit to form intermolecular hydrogen bonds (< 3.5 Å) (Fu et al., 2018). Contrariwise, a longer hydrogen bond distance (> 3.5 Å) conveys a lower affinity of an enzyme towards a substrate and is less likely to catalyze the compound. A notable outlier seen in this investigation was the unusual low binding energy for the DehH2-chlorpyrifos complex (−6.5 kcal/mol), which does not correlate with the predicted absence of hydrogen bonds catalytic trial. The outcome was ascribable to the chloro-organophosphate group in chlorpyrifos, which is not present in other tested halogenated substrates. The difference in chemical environments agreed with a previous study that mentioned its effect on changing the overall surface charge for the docking prediction (Anwar et al., 2020).

Pertinently, the docking results point to L-2CP being the favored substrate of DehH2, which supported our earlier docking observation in a non-hypersaline condition (Oyewusi, Huyop, et al., 2020). However, molecular docking alone cannot accurately represent an enzyme substrate-ligand interaction as the simulation was in the absence of water. MD simulation is a more reliable investigation as it accounts for the contribution of water to the system (Anwar et al., 2020; Bahaman et al., 2020).

### 3.5. MD Simulations of DehH2-substrate complexes at a high salt concentration

Structural and conformational stability is influenced by the ionization state of a protein (Adcock & McCammon, 2006), both observable in MD simulations. The established optimized salinity at 35% (w/v) NaCl was employed in the MD simulations. The 100 ns production simulations were triplicated for each of the DehH2-substrate complexes, and average values of the RMSD, RMSF and gyration radius alongside hydrogen bonds assessment results were determined.

#### 3.5.1. Root-Mean square deviation (RMSD)

The study determined the RMSD of the DehH2 protein backbone and substrates to assess the resultant complex’s

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**Table 1.** Results of structural validation of DehH2 protein model with the SAVEs Server (at different salinity).

| Validation  | Parameter Scheme       | Over quality factor | 0%  | 10%  | 20%  | 30%  | 35%  | 40%  | 50%  | Range of scores (%) |
|-------------|------------------------|---------------------|-----|-----|-----|-----|-----|-----|-----|--------------------|
| ERRAT       | Amino acid compatibility | 90.991              | 97.297 | 97.297 | 98.649 | 99.550 | 97.307 | 98.994 | > 50               |
| Verify-3D   |                        | 100                 | 100  | 100  | 100  | 100  | 99.57 | 95.65 | > 80               |
| Procheck    |                        | 94.6                | 90.2 | 90.1 | 91.1 | 87.6 | 86.1  | > 90  |                    |
|             |                        | 3.5 Å               | 4.7  | 9.4  | 9.4  | 9.4  | 10.9  | 12.9  |                    |
|             |                        | 0.0                 | 0.0  | 0.5  | 0.0  | 0.0  | 1.0   | 0.5   |                    |
|             |                        | 0.0                 | 0.5  | 0.0  | 0.0  | 0.5  | 0.5   |        |                    |

**Table 2.** Summary of DehH2 docking analysis with various halogenated substrates at 35% salinity based on AutoDock Vina scores.

| Protein-ligand complexes | Score (kcal/mol) | Interacting residues | Distance (Å) |
|--------------------------|------------------|----------------------|--------------|
| DehH2-2,2-DCP            | −5.9             | Asp125               | 2.6 Å        |
| DehH2-2,3-DCP            | −4.7             | Asp125               | 2.9 Å        |
| DehH2-3CP                | −5.3             | Asp125               | 2.6 Å        |
| DehH2-L-2CP              | −6.3             | Asp125               | 2.3 Å        |
| DehH2-D-2CP              | −5.4             | Asp125               | 2.6 Å        |
| DehH2-MCA                | −5.1             | Asp125               | 2.7 Å        |
| DehH2-TCA                | −4.7             | Asp125               | 3.0 Å        |
| DehH2-chlorpyrifos       | −6.5             | Thr122               | 3.1 Å        |

**Table 3.** The MD simulations result on the eight DehH2-substrate complexes at low-salt conditions (Oyewusi et al., 2020c).

| Substrate      | Time (ns) | RMSD (nm) | RMSF (nm) | Average Rg (nm) | Number of hydrogen bonds | Binding affinity (kcal mol$^{-1}$) |
|----------------|-----------|-----------|-----------|-----------------|--------------------------|----------------------------------|
| DehH2-L-2CP    | 12        | ~ 0.155   | 0.05 – 0.20 | 1.82            | 2/3                      | −4.6                             |
| DehH2-D-2CP    | 75        | ~ 0.15    | 0.05 – 0.20 | 1.83            | 2/3                      | −3.7                             |
| DehH2-2,2-DCP  | 50        | 0.187     | 0.05 – 0.20 | 1.85            | 2                        | −4.1                             |
| DehH2-2,3-DCP  | 85        | 0.23      | 0.05 – 0.19 | 1.83            | 2                        | −3.3                             |
| DehH2-TCA      | 80        | 0.189     | 0.05 – 0.25 | 1.85            | 4                        | −3.4                             |
| DehH2-3CP      | 80        | 0.189     | 0.05 – 0.20 | 1.82            | 2                        | −3.7                             |
| DehH2-MCA      | 21        | ~ 0.162   | 0.05 – 0.19 | 1.83            | 2/3                      | −3.6                             |
| DehH2-chlorpyrifos | unstable | ~ 0.295  | 0.05 – 0.59 | 1.85            | 3                        | −6.5                             |
conformational stability and dynamic behavior concerning the optimal salt concentration condition (35% NaCl). The study noted that RMSD fluctuations ranged from low to moderate throughout 100 ns MD simulation runs, which implied an adequate equilibration duration of the DehH2-substrate complexes (Hamid, Hamid, et al., 2015a, Hamid, Tengku Abdul Hamid, et al., 2015). Figure 3 illustrates that all DehH2-substrate complexes achieved equilibrium as early as 10 ns with the DehH2-L-2CP complex, and the DehH2-MCA complex was the slowest at 64 ns, while the DehH2-chlorpyrifos complex remained unstable till the end of the simulation. The averaged RMSD values of the DehH2-substrate complexes were equally similar in trend and ranged between 0.13 and 0.21 nm, with the DehH2-L-2CP complex being the most stable. In contrast, the DehH2-chlorpyrifos complex fluctuated continually until the simulation (Figure 3). The order of the DehH2-substrate complexes stability based on achieved equilibrium durations and calculated averaged RMSD values are as follows:

DehH2-L-2CP = DehH2-3CP > DehH2-D-2CP = DehH2-TCA > DehH2-2,2-DCP > DehH2-MCA > DehH2-2,3-DCP > DehH2-chlorpyrifos

The rapid stabilization of the DehH2-L-2CP complex (10 ns) over the DehH2-3CP (23 ns) supported the higher stability of the former. Likewise, the DehH2-3CP complex was more stable than the DehH2-TCA because of the former’s shorter equilibrium duration during the MD simulations. Thus, the findings seen here confirmed the results of earlier molecular docking study (refer to section 3.4), where L-2CP (−6.3 kcal mol⁻¹) showed the highest binding affinity for DehH2 protein compared to 2,2-DCP (−5.9 kcal mol⁻¹), D-2CP (−5.4 kcal mol⁻¹), 3-CP (−5.3 kcal mol⁻¹), MCA (−5.1 kcal mol⁻¹), 2,3-DCP and TCA (−4.7 kcal mol⁻¹), respectively. The exception was chlorpyrifos (−6.5 kcal/mol). Despite the extreme salinity, the L-2CP remained the preferred substrate for DehH2. Table 4 tabulates the RSMD values of the eight DehH2-substrate complexes.

The above results were then compared with the previous DehH2 dehalogenase enzyme simulated at a low-salt concentration (0% NaCl) (Oyewusi, Huyop, et al., 2020), and Table 3 tabulates the average RMSD values of the eight DehH2-substrate complexes. L-2CP was the most preferred substrate of DehH2, followed by MCA, 2,2-DCP, D-2CP, 3CP, TCA, 2,3-DCP, and chlorpyrifos was the least preferred. In short, the comparable MD analysis of the halotolerant DehH2 enzyme in both high- and low-salt environments showed that nearly all ligands were within the favorable range for interaction with the enzyme. The exception was chlorpyrifos which stabilized at the upper end of the spectrum (Tables 3 and 4). We had expected this outcome for chlorpyrifos, which is a non-natural

| Substrate       | Time (ns) | RMSD (nm) | RMSF (nm) | Average Rg (nm) | Number of hydrogen bonds | Binding affinity (kcal mol⁻¹) |
|-----------------|-----------|-----------|-----------|-----------------|--------------------------|-----------------------------|
| DehH2-L-2CP     | 10        | ~ 0.13    | 0.05–0.18 | 1.79            | 2/3                      | −6.3                        |
| DehH2-D-2CP     | 25        | ~ 0.14    | 0.05–0.23 | 1.79            | 2                        | −5.4                        |
| DehH2-2,2-DCP   | 30        | 0.16      | 0.05–0.20 | 1.81            | 3/4                      | −5.9                        |
| DehH2-2,3-DCP   | 15        | 0.20      | 0.05–0.18 | 1.81            | 3                        | −4.7                        |
| DehH2-TCA       | 35        | 0.14      | 0.05–0.20 | 1.80            | 3/4                      | −4.7                        |
| DehH2-3CP       | 23        | 0.13      | 0.05–0.20 | 1.80            | 3                        | −5.3                        |
| DehH2-MCA       | 64        | ~ 0.18    | 0.05–0.23 | 1.80            | 2                        | −5.1                        |
| DehH2-chlorpyrifos | unstable | ~ 0.21    | 0.05–0.32 | 1.79            | 2/3                      | −6.5                        |

Figure 4. Average RMSF plots of Cα atoms for the DehH2 under extreme salinity (35 % NaCl), showing overlapped RMSF plots throughout 100 ns production simulations with 2,3-DCP (green), 3-CP (yellow), L-2CP (purple), 2,2-DCP (red), D-2CP (magenta), MCA (maroon), TCA (turbine) and chlorpyrifos (orange).
substrate of DehH2, although the enzyme was more efficient in degrading the tested substrates in high-salt environments. In brief, the MD simulation data demonstrated that almost all the substrates were within the acceptable distance (<0.3 nm) (Hamid, Hamid, et al., 2015; Hamid, Tengku Abdul Hamid, et al., 2015) to form hydrogen bonds with the DehH2.

3.5.2. Root-Mean square fluctuation (RMSF)

The RMSF of each residual amino acid contributing to protein or complex structural deviations under various salinity can be determined (Dong et al., 2018). Figure 4 describes the results of this study which showed a residue with a notably low RMSF value due to reduced movement during simulations, while a ΔRMSF of >0.05 nm (0.5 Å) threshold value stipulates a major residue-specific flexibility change (Dong et al., 2018; Kovacic et al., 2016). In this study, the highest stability estimated for the test complexes based on the lowest RMSF values as follows:

DehH2-L-2CP = DehH2-2,3-DCP > DehH2-2,2-DCP = DehH2-3CP = DehH2-TCA > DehH2-D-2CP, DehH2-MCA > DehH2-chlorpyrifos

The results thus proved that under an optimal salt concentration (35% (w/w) NaCl), substrates L-2CP and 2,3-DCP were the most tightly bonded to the DehH2 in relation to their average positions (Nemaysh & Luthra, 2017). The data also indicated the high structural stability of the two complexes and the dehalogenase’s preferential binding to 2CP and 2,3-DCP (Anuar et al., 2020; Hamid, Hamid, et al., 2015; Oyewusi, Huyop, et al., 2020). Table 4 tabulates the RMSF values estimated for the eight DehH2-substrate complexes.

The DehH2-D-2CP complex was very stable, and only serine-212 that fluctuated excessively. Conversely, the DehH2-chlorpyrifos complex’s instability can be explained by highly fluctuating aspartate, asparagine, and serine at positions 183, 190, and 121, respectively. These residues demonstrated greater flexibility from their average positions because of their locations in the α-helix region. Hence, our observation agreed with previous observation as reported by Nemaysh and Luthra (2017). Residues sited on exposed loops also exhibit greater RMSF values (Fuentes et al., 2018). Pertinently, the low RMSF values of the DehH2 catalytic triad (Asp125-Arg201-Lys202) verify their strong bonding with the substrates. Their behavior is also consistent with the fluctuating residues being in the core protein regions.

Comparatively, the RMSF values of the present study corroborated our previous observation on DehH2 simulated in a low-salt environment, and the results were summarized in Table 3. Low average RMSF values were observed for complexes DehH2 — 2,3-DCP, DehH2 — 3CP (0.05 – 0.19 nm), followed by DehH2 — L-2CP, DehH2 — 2,2-DCP, DehH2 — D-2CP, DehH2 — MCA complexes (0.05 – 2.0 nm) and the DehH2 TCA complex (0.05 – 0.25 nm) (Table 3). The exceptionally high RMSF value for DehH2-chlorpyrifos (0.05 – 0.59 nm) signified its low structural stability in both salt conditions. The results also indicated that the DehH2 bonded tightly to all ligands in high- and low-salt conditions, except for chlorpyrifos in relation to their average positions.

The results seen here supported the halotolerant nature of the DehH2 and its ability to bind to the tested substrates at a hypersaline condition (35% NaCl) than in a non-hypersaline one. Still, minor differences in binding energy might not categorically substantiate the substrate preference of DehH2, as previously indicated by Anbarasu and Jayanthi (2018). The study then assessed the parameter of the radius of gyration for further clarification.

3.5.3. Radius of gyration (Rg)

The gyrate radius (Rg) could estimate changes in the DehH2-substrate complexes compaction level and measure the enzyme’s overall dimensions during the MD simulation. Rg is defined as the mean square mass-weighted root distance of a set of atoms from their common mass center (Kumar et al., 2014). A reasonably constant Rg value depicts a stable folded structure, in which any changes reflect the unfolding/refolding of the structure throughout the simulation (Liao et al., 2014). A looser packing of residues has a high Rg value and vice versa (Lobanov et al., 2008). Table 4 enlists the average Rg for the MD simulations of eight DehH2-substrate complexes performed at 35% (w/v).

We found that the average Rg values for the eight simulated DehH2-L-substrate complexes ranged between 1.7 and 1.81 nm (Figure 5). The DehH2-L-2CP complex showed the lowest and least fluctuating average Rg value and, complexes DehH2-D-2CP and DehH2-chlorpyrifos showing similar average Rg values, except they were increasingly erratic. The eight DehH2-substrate complexes folded structures appeared stable and bonded rather well. The order of the average Rg values identified by this study according to ascending order are as follows:

DehH2-L-2CP = DehH2-D-2CP = DehH2-chlorpyrifos > DehH2-TCA = DehH2-MCA > DehH2-2,2-DCP = DehH2-2,3-DCP = DehH2-3CP

A quick survey of the Rg plots revealed that DehH2-L-2CP, DehH2-D-2CP, DehH2-MCA, and DehH2-2,2-DCP, DehH2-2,3-DCP, DehH2-3CP, and DehH2-TCA fluctuated considerably less than the DehH2-chlorpyrifos complex, despite the latter having the lowest average Rg value. The results thus supported the DehH2 being better at binding the halogenated substrates than the organophosphorus-containing chlorpyrifos. Theoretically, the additional four oxygen-, one nitrogen- atoms and phosphorus and sulfur atoms each would generate a larger dipole moment in the former. The feature probably contributed to its lower average Rg value.

The average Rg values seen in this present study was compared with the average Rg of DehH2 simulated at low-salt condition. At low-salt conditions, the average Rg was low for DehH2-L-2CP, DehH2-3CP, DehH2-MCA, DehH2-D-2CP, DehH2-2,3-DCP complexes (1.83 – 1.78 nm) (Table 3), followed by DehH2-2,2-DCP, DehH2-TCA, and lastly DehH2-chlorpyrifos (1.85 – 1.75 nm) (Table 3). The notable lowest Rg observed for DehH2-L-2CP in both conditions indicated a highly compact complex, which implied an excellent binding.
3.5.4. Hydrogen bonds (H-bonds)

The number of hydrogen bonds and their relative strength in an aqueous environment are germane in influencing protein-ligand binding affinity (Chen et al., 2016). A hydrogen bond occurs when an electronegative atom of a hydrogen bond acceptor interacts with a hydrogen bond donor directly bonded to an electronegative atom (Gao et al., 2015). In this study, the intermolecular hydrogen bonds formed between the DehH2 protein and substrates were investigated, and the results are depicted in Figure 6. The DehH2-MCA (Figure 6a) and DehH2-D-2CP (Figure 6b) complexes showed fewer H-bond numbers with a maximum of two H-bonds compared to three bonds in complexes, DehH2-L-2CP (Figure 6c), DehH2-2,2DCP (Figure 6d), DehH2-2,3DCP (Figure 6e), DehH2-3CP (Figure 6f) and DehH2-chlorpyrifos (Figure 6g). Remarkably, the DehH2-TCA complex (Figure 6h) occasionally formed three to four hydrogen bonds with the DehH2. Contrariwise, the DehH2-L-2CP complex exhibited a fluctuating number of hydrogen bonds between two to three bonds, although only two hydrogen bonds formed between 3 and 70 ns of simulation.

Meanwhile, the calculated binding energy for each complex was established as L-2CP (−6.3 kcal mol⁻¹), 2,2-DCP (−5.9 kcal mol⁻¹), D-2CP (−5.4 kcal mol⁻¹), 3-CP (−5.3 kcal mol⁻¹), MCA (−5.1 kcal mol⁻¹), 2,3-DCP and TCA (−4.7 kcal mol⁻¹). Thus far, the DehH2-chlorpyrifos complex gave the highest binding affinity (−6.5 Kcal mol⁻¹), which contradicted an earlier result (refer to subsection 3.4) that showed the DehH2's non-preference to bind with the compound. The discrepancy for chlorpyrifos can be correlated to the organophosphate group in its structure that appreciably altered the chemical environment. The two functional groups could have contributed to the observed calculation, possibly due to the DehH2's substrate sensitivity to chlorpyrifos (Oyewusi, Huyop, et al., 2020). Hence, the number of H-bonds formed in the DehH2-substrates complexes seen here generally agreed with previous RMSD data (refer to subsection 3.5.1), which showed the DehH2 substrate preference.

3.6. MM-PBSA binding free energy calculations

To calculate the binding strength between the DehH2 and substrates, binding free energy calculation through MMPBSA was used to account for the MD trajectories (Kalathiyi et al., 2019), based on van der Waals, electrostatics, polar solvation, and nonpolar solvation energies (Kumari et al., 2014). MM-PBSA can better predict the DehH2-substrate interaction and preference compared to molecular docking analysis. Furthermore, energies extracted from MD trajectories are more sensitive and flexible when the protein-substrate complex interchanges during 100 ns MD simulation. The binding energies (kcal mol⁻¹) for eight DehH2-substrate complexes simulated at 35% (w/v) NaCl from the MM-PBSA calculations are tabulated in Table 5. The stability of the DehH2-substrate complexes estimated in this study are of the following order;

DehH2-L-2CP > DehH2-MCA > DehH2-D-2CP > DehH2-3CP > DehH2-2,2-DCP > DehH2-2,3-DCP > DehH2-TCA > DehH2-chlorpyrifos

The calculated values for ΔEvdw, ΔEele, and ΔGpolar were between −17.13 and −155.62, −1.15 and +131.27 kcal mol⁻¹, and 16.28 and 189.84 kcal mol⁻¹, respectively. The ΔEnonpolar and ΔGbinding were between −2.55 and −31.62 kcal mol⁻¹ and −18.46 and +180.57 kcal mol⁻¹. Of which the lowest ΔGbinding was observed for DehH2-L-2CP (−45.14 kcal mol⁻¹) followed by DehH2-MCA (−41.21 kcal mol⁻¹), DehH2-D-2CP (−31.59 kcal mol⁻¹), DehH2-3CP (−30.75 kcal mol⁻¹), DehH2-2,2-DCP (−29.72 kcal mol⁻¹) DehH2-2,3-DCP (−22.20 kcal mol⁻¹) DehH2-TCA (−18.46 kcal mol⁻¹) and DehH2-chlorpyrifos (+180.57 kcal mol⁻¹).

The MM-PBSA calculations crucially highlighted a major difference in the DehH2-L-2CP, which showed a relatively low binding free energy (−45.14 kcal mol⁻¹) and exhibited the lowest van der Waals energy (−51.68 kcal mol⁻¹). The MM-PBSA calculations dispelled the favorable observations for the DehH2-chlorpyrifos complex in both the molecular docking and MD simulation. The calculations confirmed that chlorpyrifos does not spontaneously interact with DehH2. The DehH2-chlorpyrifos stability in molecular docking and MD simulations was due to the chlorpyrifos’ binding with non-catalytic residues, namely, Thr122 and Ser124. Hence, the results fitted well with chlorpyrifos’ non-degradability by the DehH2 in our earlier empirical study (Oyewusi, Huyop, et al., 2020).

The data revealed that the binding free energy of DehH2 to halogenated substrates was somewhat affected by DehH2 simulation in extreme saline conditions. Compared to the previous DehH2-substrate complexes simulated at a low-salt environment, the DehH2-substrate complexes for all halogenated compounds exhibited the most favorable binding energy in the order of DehH2-L-2CP (−24.27 kcal mol⁻¹), followed by DehH2-MCA (−22.78 kcal mol⁻¹), DehH2-D-2CP (−21.82 kcal mol⁻¹), DehH2-3CP (−21.11 kcal mol⁻¹), DehH2-2,2-DCP (−18.34 kcal mol⁻¹), DehH2-2,3-DCP (−8.34 kcal mol⁻¹), DehH2-TCA (−7.62 kcal mol⁻¹). The binding of DehH2 was the least favorable for chlorpyrifos (+127.16 kcal mol⁻¹) (Oyewusi, Huyop, et al., 2020). The obtained MM-
Figure 6. Intermolecular hydrogen bonds in DehH2 along the 100 ns simulation time when chemically bonded to (a) MCA (maroon), (b) L-2CP (purple), (c) D-2CP (magenta), (d) 2,2-DCP (red), (e) 2,3-DCP (green) (f) 3-CP (yellow) (g) chlorpyrifos (orange), and (h) TCA (turquoise).

Table 5. Binding free energies from MM-PBSA in kcal mol$^{-1}$ for the DehH2-substrates complexes.

| Energy components | DehH2 with various halogenated substrates |
|-------------------|------------------------------------------|
| (kcal/mol)        | L-2CP | MCA  | 2,2-DCP | D-2CP | 3-CP  | TCA  | 2,3-DCP | Chlorpyrifos |
| $\Delta E_{vdw}$  | −51.68 | −48.20 | −35.34 | −30.27 | −35.23 | −17.13 | −25.62 | −155.62 |
| $\Delta E_{ele}$  | −1.15  | −2.71  | −5.34  | −3.99  | −9.47  | −14.78 | −13.71 | 131.27  |
| $\Delta G_{polar}$| 22.91  | 16.28  | 19.68  | 24.35  | 22.05  | 17.39  | 26.82  | 189.84  |
| $\Delta G_{nonpolar}$| −2.55  | −10.96 | −10.19 | −9.74  | −3.10  | −15.67 | −3.57  | −31.62  |
| $\Delta G_{binding}$| −43.14 | −41.21 | −29.72 | −31.59 | −30.75 | −18.46 | −22.20 | 180.57  |
PBSA outcomes substantiated the improved stability of DehH2-substrate complexes in the previous MD simulation results as a contribution of correctly oriented and stable substrate-enzyme bindings.

4. Conclusions

Extreme environmental conditions may act as a natural barrier to pollutant degradation, making bioremediation difficult in this situation. Biomolecular simulations are usually carried out in an aqueous environment with a fixed number of ions for the simulated duration, using either a minimal number of neutralizing ions or several salt pairs designated for neutralizing the system. In contrast, actual biomolecules encounter local ion conditions where the salt concentration is dynamic and vary from the bulk. Thus, determining the empirical degradation potential of pollutants at high salt concentrations is difficult.

In this study, we demonstrated that the best activity of DehH2 was obtained at a high salt concentration (35% (w/v) NaCl). We also uncovered the molecular changes that occurred when the substrates bind to DehH2 under a simulated hypersaline condition (35% (w/v) NaCl). The molecular docking analyses showed that L-2CP and chlorpyrifos were the preferred and least preferred substrates; the L-2CP showed the highest affinity to DehH2 (−6.3 kcal mol$^{-1}$). The subsequent MD simulation under the molecular docking-established optimal salinity at 35% (w/v) found that the DehH2-L-2CP was the most stable, followed by DehH2-MCA, DehH2-D-2CP, DehH2-3CP, DehH2-2,2-DCP, DehH2-2,3-DCP, DehH2-TCA, and DehH2-chlorpyrifos. The DehH2 was predicted to show a unique structural adaptation and a broad substrate specificity to degrade organohalides under extreme salt conditions, with L-2CP as the preferred substrate. The study discovered that the DehH2 was more apt at degrading the different tested halogenated compounds under a high-salt condition than under a low salt environment. Hence, the findings could offer valuable insights to rationally design the DehH2 enzyme for improved salinity threshold and substrate specificity. The study’s data may also serve as a guideline to introduce halotolerant properties in other non-halotolerant dehalogenases, based on the key amino acids that impart exceptional halotolerant to DehH2. Consequently, better dehalogenases could be designed to clean up the environment of toxic pesticides or herbicides.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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ORCID

Fahrud Huyop [1] http://orcid.org/0000-0003-3978-4087
Roswanira Abdul Wahab [2] http://orcid.org/0000-0002-9982-6587
Azzer Azzar Abdul Hamid [3] http://orcid.org/0000-0003-2404-6890

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