Docking and Molecular Dynamics Simulation Studies for the Evaluation of Laccase Mediated Biodegradation of Triclosan

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Abstract. Triclosan (TCA) is an antibacterial and antimicrobial compound that is incorporated into toothpaste, soap, and liquid dishwasher. Continuous TCA exposure may contribute to the emergence of antibiotic-resistant bacteria in the microbiome. Triclosan also reacts to form dioxins, which bioaccumulate and are toxic to aquatic organisms, impedes the thyroid hormone metabolism of the human body. Laccases are multi copper-containing enzymes that can degrade the aromatic compounds and thus reduce their toxicity. To effectively degrade the compound, it is essential to understand the molecular function of the enzyme. Hence, a molecular docking study of laccase enzymes with Triclosan was done. The *Tramates versicolor* laccase structure was retrieved from PDB and ligand structure was taken from Pubchem. The binding mode and interaction of TCA and laccase were studied using Auto dock Vina software and the stability of the docked complex had been explored via Molecular Dynamics (MD) simulation study using Schrodinger Desmonde. The binding affinity score was found to be −6.5kcal/mol. The majority of the residues in RMSF were within the 2.5Å limit. The radius of gyration remained within the range from 21.7 to 22.1Å for Laccase – TCA complex throughout the 50 ns simulation. MD simulation results show that the enzyme complex remains stable all through the catalytic action.

Keywords: Triclosan · laccase · dioxin · stability · docking · simulation

1 Introduction

Triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol) (TCA) is an antibacterial chemical incorporated into the personal care products such as soaps, toothpaste, sanitizers, liquid detergents, toys, beddings, clothing etc. Triclosan is a chlorinated aromatic compound. The degraded product of TCA is methyl-Triclosan, which was found in one of the 22 drinking water samples in Barcelona [1]. Main sources of TCA to the environment are (a) sewage from houses (b) land application of pesticides containing residues of TCA.

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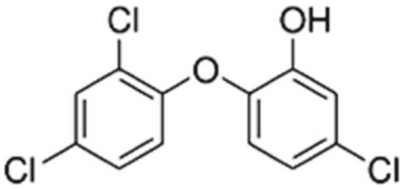

Fig. 1. Structure of Triclosan

TCA causes endocrine disruption and has potential adverse effects in humans. It has been detected in human blood, breast milk, nails, and urine samples [2]. TCA stores itself in fatty tissues which make it difficult to get rid of upon accumulation. The studies of the effect of TCA in human body are showing that it causes miscarriages, certain allergies, weakness of immune system and it is pro-oxidant hence cytotoxic in nature [3]. Numerous antimicrobial consumer products are washed out through drainage and are treated using municipal treatment plants [4]. Studies have proved that TCA persists in water even after the typical wastewater treatment processes. It also reacts with chlorine present in tap water and forms chlorophenol which is a possible human carcinogen [5]. Another study proved that it is converted into dioxins and accumulates in aquatic organisms and is highly toxic [6]. It doesn't possess any additional benefit to human health and on the contrary causes health risks. TCA regulation is not well restricted thus resulting in an increased concentration in aquatic and terrestrial environments [7]. Owing to these threats posed by TCA its removal is considered to be crucial in the long run (Fig. 1).

Laccases (EC 1.10.3.2) are multi copper oxidoreductase enzymes having 4 copper atoms and are distributed in three clusters T1, T2, T3. Laccase catalysis indicates the reduction of O_2. It catalyses one electron oxidation of a wide range of phenolic substrates and subsequently transfers four electrons to the Cu atom and is used to reduce oxygen to two water molecules [8]. Laccases have low substrate specificity and possess the capability to degrade phenolic compounds using oxygen. These are extracellular enzymes found in higher plants, fungi, bacteria and insects. In fungi it has been functionalized in lignin degradation. Most studied laccase are from white-rot fungi such as *Phlebia radiata*, *Pleurotus ostreatus*, and *Trametes versicolor*.

Bioremediation is an emerging technique which has been studied extensively for the removal of environmental micropollutants. Several microorganisms are being used to degrade the chemical pollutants. Treating the pollutants with enzymes also has attained attention. In this study we are analysing the capability of laccase enzymes to effectively associate with TCA via in silico studies which would provide a cue for developing effective degradation strategies. In this study the molecular docking of laccase enzyme and ligand was done to check the binding affinity of TCA to the active site of the enzyme. Molecular dynamics simulation study was then done to predict the stability of the complex.

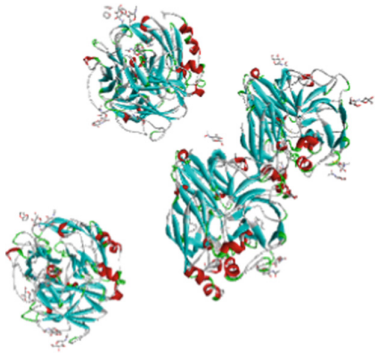

Fig. 2. Structure of 1 KYA protein

2 Materials and Methods

2.1 Protein Preparation

The structure of laccase from *Trametes versicolor* was downloaded from RCSB PDB with Protein ID 1KYA in PDB format [9]. The structure contains 4 chains, chain A, chain B, chain C, and chain D. The structure was visualized using BIOVIA Discovery studio. So the protein was prepared by removing the unwanted chains. Het atoms and water molecules were also removed from the A chain (Fig. 2).

2.2 Ligand Preparation

The structure of Triclosan in SDS file format was downloaded from Pubchem (pubchem ID-CID5564). This ligand molecule was imported into PyRx using the Open Babel tool. It was energy minimized and converted into pdbqt format.

2.3 Molecular Docking

Molecular docking is an In-silico method for the prediction of preferred orientation of ligand molecules to the protein when bound to form a protein ligand complex. In this study PyRx software is used for molecular docking study, it is a virtual screening tool facilitating the multiple ligand screening at a time. The lowest binding energy score was used to identify the optimal protein-ligand complex. Molecular docking was done using Vina Wizard and the bonds involved in the interactions were visualized and analysed using Discovery studio.

2.4 Molecular Dynamic Simulation

After the molecular docking study, the protein- ligand complex structure was examined by Molecular dynamics simulation Software Desmond V12 Schrodinger at 50 nano second time duration. This study was performed to check the confirmation changes

occurring at the binding site of the protein and to evaluate these changes over the complex [10]. This depicts the stability of laccase enzyme with TCA molecule during the run. For optimization analysis the Laccase-Triclosan complex was subjected to protein preparation wizard. Complex was solvated in an orthorhombic box with periodic boundary conditions by the addition of simple point charge (SPC) water molecules [11]. The entire system was neutralized by the addition of Na+ and Cl- for balancing the net charge [12]. During simulation the minimized system has been relaxed with an NPT (Number of atoms, pressure and temperature) ensemble without restraints. Whole system was composed of 49000 atoms. The temperature and pressure was maintained at 300 K and 1.01325 bar respectively. The RMSD and energy fluctuation have been used to assess the complex's dynamic response and structural shifts. The root mean square fluctuations (RMSF) for the backbone and side chain of each CT protein residue were evaluated.

3 Results and Discussion

3.1 Preparation of Protein

Protein was prepared by removing unwanted side chains, water molecules and Het atoms. The structural representation of the prepared protein is shown in Fig. 3.

3.2 Molecular Docking Study

The most reliable method for analysing and predicting the best fit protein-ligand confirmation is termed as Molecular Docking studies. A molecular docking analysis was carried out with Laccase and TCA molecules. TCA was found to have strong affinity in the binding site of laccase enzymes based on the confirmation, hydrogen bond interaction, and lowest binding energy score (ΔG_b, kcal/Mol) [13]. The binding affinity score of Triclosan was −6.5 kcal/mol. As a result, this molecule may be regarded as a good substrate for laccase enzyme Moreno et al., 2019 suggests benzidine as a potential substrate for laccase binding with a docking affinity of −6.5 kcal/mol [14] (Fig. 4). The hydrogen bond interaction between the enzyme and ligand is shown in Fig. 5. The diagrammatic representation of amino acids involved in the Laccase- TCA interaction is given in Fig. 6.

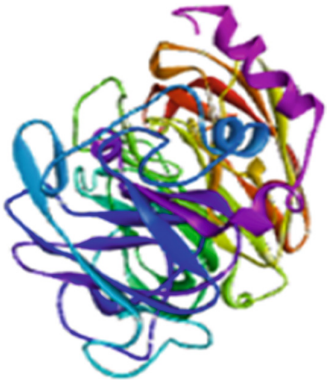

Fig. 3. Structure of prepared protein

Fig. 4. Laccase- Triclosan complex

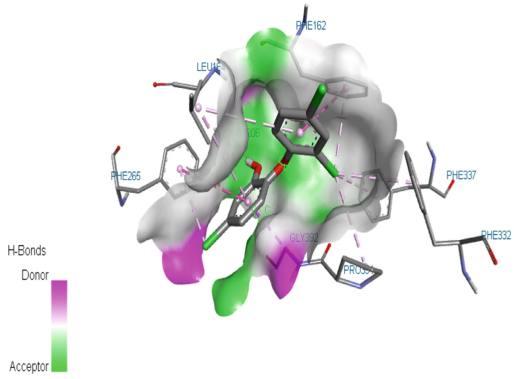

Fig. 5. Molecular interaction study of laccase enzyme receptors with TCA. The green dot indicates the H-bond interaction between the protein and ligand

3.3 Molecular Dynamic Simulation Study

Based on docking studies and an H-bond interpretation of the collected data, a molecular dynamic (MD) simulation study was done to test the stability of the laccase– TCA association using Desmond V12. The firm crystal structure of laccase enzyme was used in MD simulation studies. The laccase- TCA complex trajectory data for RMSD, RMSF and energy have been plotted. The dynamic parameters of the docked complex were analyzed with plotted data. The MD simulation plots confirmed that the docked complex remained stable throughout the 50 ns simulation. The RMSD was observed to be less than 3Å, confirming that the structure is stable. Earlier work by Arnittali et al., on the modelling of native state proteins reveals that an RMSD value less than 2.5 Å indicates that the protein structure under consideration is very close to reference structure, which is in line with the finding of the present study.

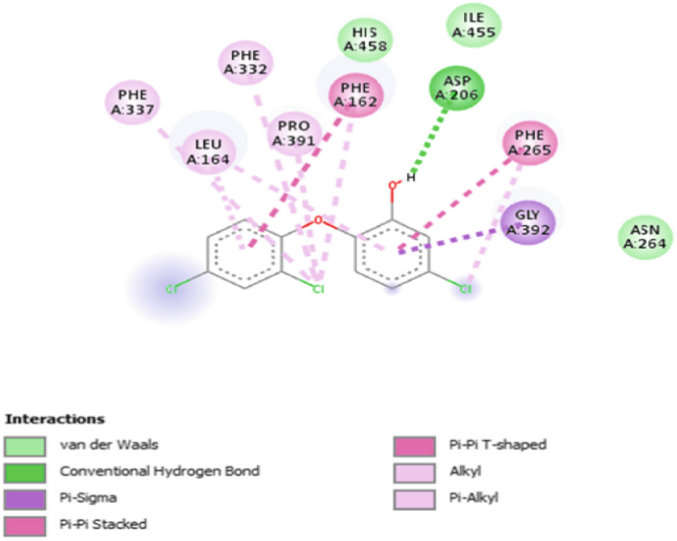

Fig. 6. Amino acids involved in the interaction

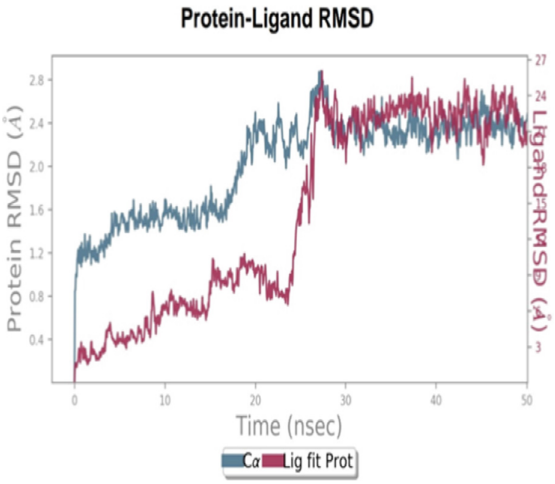

Fig. 7. Root mean square deviation (RMSD) plot for laccase—TCA complex during 50 ns of molecular dynamics simulation. Laccase is shown in the red colour and Triclosan in the blue colour.

The RMSD result for laccase-TCA complex is shown in Fig. 7 in which there is a minor rearrangement in the docking initial conformation, but it remains stable throughout the simulation time. Averaging all the atoms of the provided residues, the RMSF of the respective residues in the trajectories was determined. The majority of the residues in RMSF were within the 2.5 Å limit. The root mean square fluctuation (RMSF) plot was used to assess the variations in fluctuation between the apo and holo forms of the

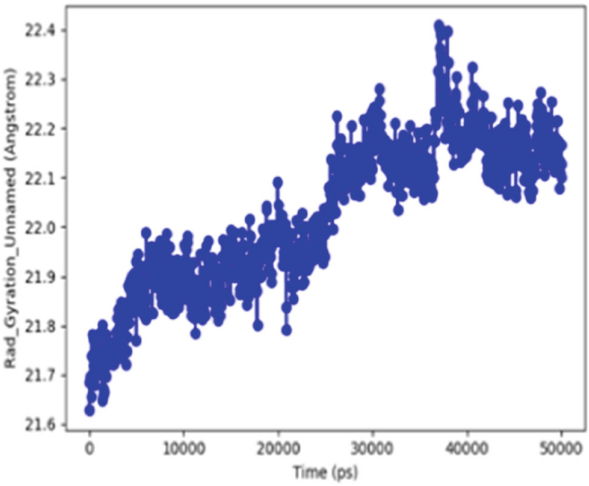

Fig. 8. Radius of gyration

residues. This value also indicates the protein ligand complex remained stable throughout the simulation. The radius of gyration (Rg) was found within the range of 22.4 Å is depicted in Fig. 8. Rg determines the compactness of protein structure. Previous reports by Ramakrishnan et al., shows that Rg value of 24 Å was obtained for the molecular dynamic simulation study carried out for bisphenol laccase complex. So the result indicates that the laccase enzyme could retain the structure even after binding with TCA.

4 Conclusion

The docking score obtained was −6.5 kcal/mol. This low value shows a strong binding affinity between the enzyme and TCA molecule. A RMSD value below 2.5 during the MD simulation indicates that the protein – ligand complex is more stable. The Triclosan had a ligand RMSD of 1–3 obtained after initial fluctuation, indicating that it attached firmly within the cavity of the laccase enzyme binding site. Triclosan formed hydrophobic interaction with PHE 239 of laccase enzyme and ionic interaction between TCA and GLU 302 residue. Throughout the 50ns simulation, the radius of gyration remained consistent ranging from 21.7 to 22.1 Å for Laccase – TCA complex. The result implies that the structural compounds have a strong binding affinity and are stable. This finding could be relevant in the future for a wet lab research of TCA degradation using laccase enzymes.

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