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Molecular Docking (MDP) and Molecular Dynamics Simulation (MDS) approaches to investigate the relationship and interactions of Berberin natural with class D β-lactamase OXA-10

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ABSTRACT

Introduction: Researchers are focusing on bacterial survival against antibiotics due to their hydrolyzing activity, which allows them to bypass the antibiotic's effect, leading to high fatalities due to their lack of effect. The study investigated the inhibitory effect of berberin, an isoquinoline alkaloid, on the β-lactamase enzyme, thereby enhancing the efficacy of clinical antimicrobial drugs.

Materials and Methods: The study utilized molecular docking to understand the binding pose and affinity of a new inhibitory ligand with the OXA-10 enzyme, using Autodock software version 4.2.2 and performing MD simulations in free and complexed forms under physiological conditions.

Results: The molecular docking result showed a suitable interaction between berberin and OXA-10 β-lactamase enzyme, with a binding energy of -6.29 kcal/mol. MD simulation confirmed constant hydrogen bonds between berberin and OXA-10, indicating well-equilibrated RMSD and RMSF.

Conclusion: The findings of this paper suggest that berberin, which is a natural compound with several medicinal effects, can be used as a potential inhibitor of class D β-lactamase OXA-10. Therefore, it is clear that this identified inhibitor will serve as a better initiating tip for further experimental studies of β-lactamase inhibitors in the drug design and discovery process.

Keywords: Molecular docking Molecular dynamics simulation, Berberin, Class D β-Lactamase.

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1. Introduction

β-lactams are effective antimicrobial substances (1) that deactivate the penicillin-binding protein (enzymes that play an essential role in the synthesis of the bacterial cell wall) and subsequently cause the death of bacteria (2 and 3). Based on their amino acid sequence, β-lactams are classified into four different groups. Class A, C, and D β-lactamases open the β-lactam ring through a serine-active site. This is despite the fact that class B enzymes include metallo-β-lactamase (4). Class D β-lactams, also known as oxacillinase or OXA enzymes (4 and 5), contain a heterogeneous group of enzymes. The genes encoding this enzyme are found in the chromosomes and plasmids of various bacteria, including Acinetobacter, Schwanella, Pseudomonas, and Burkeldria (6 and 7). But currently, many chromosomal class D to β-lactam ases have been transferred to plasmids, which is considered a clinical threat (8).

OXA-β-lactamases are distributed in a wide variety of Gram-negative species. They are most commonly found in Enterobacteriaceae, Pseudomonas, and Acinetobacter, while their chromosomal genes are detected in most Gram-negative bacteria, such as Aeromonas, Legionella, and Campylobacter species (9). OXA enzymes are a group of β-lactam ase enzymes that hydrolyze oxacillins. Recently, these enzymes have been widely distributed among different strains of Acinetobacter bacteria. They can also hydrolyze carbapenems. Since these antibiotics are among the most effective antibiotics, resistance to them has been identified as an important concern (10).

B-lactamases, which are synthesized by gram-negative pathogens, contribute to their becoming resistant to β-lactam antibiotics. Through hydrolyzing the to β-lactam ring, to β-lactam ases are enzymes that render antibiotics inactive (11, 12). Currently, more than 1000 to β-lactam ases have been identified, and their number is increasing. Class D to β-lactam ases are represented by more than 350 genetically diverse enzymes widely distributed in gram-negative bacteria (13–15). Of these, OXA-2 and OXA-10 to β-lactam ases are examples of enzymes that cause resistance to penicillins and some early cephalosporins (16). The efficacy of antibiotics increases through inhibiting the activity of the β-lactamases and reducing bacterial resistance, enabling physicians to administer antibiotics and helping patients avoid expensive and prolonged hospitalization (17). Consequently, the discovery of new β-lactamase inhibitors and their usage in addition to antibiotics is an intriguing method of treating infections brought on by β-lactamase-secreting bacteria. (18).

β-lactam ase inhibitors have been used in combination with antibiotics in numerous studies so far, including those involving the use of avibactam with ceftazidime, relbactam with imipenem and cilastatin, sulbactam with ampicillin, piperacillin with tazobactam, and vaborbactam with meropenem (17). Natural products have been embraced by pharmaceutical companies due to their affordability and low risk (19).

Berberin is an isoquinoline alkaloid (20). It has been demonstrated that this substance has a potent synergistic effect with antibiotics (21, 22). Synergism between Berberin and antimicrobial agents has shown therapeutic benefits against a wide range of pathogenic microorganisms, including methicillin-resistant Staphylococcus aureus (MRSA) (23). Furthermore, in addition to its ability to regulate host immunity, berberin also has a direct antibacterial impact. According to some experimental data, berberin has substantial antibacterial activity in vivo and in vitro, whether used alone or in combination with other substances (24). Berberin even has the potential to inhibit antibiotic-resistant bacteria, which gives it great potential in the development of new antibiotics. Studies show that berberin may interfere with the nucleic acid, cell wall, cell membrane transport, and

movement functions of Escherichia coli and inhibit its metabolism. Berberin can destroy the cell wall structure and cell membrane integrity of methicillin-resistant Staphylococcus aureus (MRSA) and play a synergistic antimicrobial role with clindamycin or rifamycin. In addition, several studies have suggested possible antimicrobial mechanisms of Berberin, including inhibition of biofilm formation, protein synthesis, and bacterial division (25).

Berberin presents an enormous quantity of potential for the creation of novel antibiotics because it may even be able to inhibit bacteria that are resistant to antibiotics. According to studies, berberin may hinder the metabolism of Escherichia coli and interfere with its ability to transport nucleic acids, cell walls, and cell membranes. Methicillin-resistant Staphylococcus aureus (MRSA) can have their cell walls and membranes destroyed by Berberin, which also functions as a synergistic antibacterial agent with clindamycin or rifamycin. Additionally, a number of studies have proposed other potential antibacterial actions of Berberin, such as suppression of protein synthesis, biofilm formation, and bacterial division (25). Considering the importance of identifying bitalactemise inhibitors in the treatment of inflammation, the aim of this study is to evaluate the inhibitory effects of Berberin inside the active site of type D bitalactemises of OXA-10 type by using computational methods of molecular docking and molecular dynamics simulation.

2. Material and methods

2-1. Computational methods

Computational methods lead to obtaining information that is very difficult to obtain experimentally, as computer simulations for any combination are cheaper than doing any type of laboratory test. In general, the drug design project is done with the efforts of modeling specialists if it is accompanied by high accuracy and sufficient knowledge, rather than the heavy burden on the shoulders of the experimental method, which is reduced to a great extent and leads to a huge saving in time and money. In recent years, computational methods for drug design have attracted the attention of large pharmaceutical companies around the world. Molecular docking is a valuable method for studying ligand-protein interactions and one of the main tools in computational drug design. Molecular docking is a type of computer calculation to predict the most suitable protein binding site to bond with a ligand and the best orientation of that ligand inside the protein binding site to create appropriate interactions between two compounds. In this way, by using the molecular docking method of binding free energy, hydrogen bonds between ligand and protein and functional groups that play a role in creating more effective interactions between ligand and protein are identified.

2-2. Molecular docking

Molecular docking was done using Autodock 4.2.2 software for class D β-lactamase OXA-10 (26). The RCSB protein data bank provided the x-ray crystallographic structures of the OXA-10 β-lactamases with pdb code (4S2O). Non-polar hydrogen atoms that are better for docking calculations were added to the PDB file in place of water molecules and the original ligands. The Pubchem server was used to download the SDF format of Berberin's 3D structure with CID 2353, which was then converted to PDB format using the OpenBabel program. The Gaussian 09W program was then used to optimize the structure, utilizing the B3LYP hybrid density functional theory approach at the 6-31G level (27).

The GROMACS 2019.6 package was used to minimize the energy of the enzyme using the AMBER99SB force field. The macromolecule was held rigid for docking calculations, whereas Berberin was allowed to rotate freely. With the use of co-crystallized ligands from the enzyme's pdb files, the active site of the macromolecule was identified. The grid map

with 50 points and a grid point spacing of 0.375 was chosen after locating the active site. The Lamarckian genetic algorithm (LGA) approach was used to run 200 docking calculations with 25 million energy evaluations. Finally, the optimal docking mode was chosen as the conformation in the cluster with the lowest binding energy.

2-3. Molecular dynamic simulation

The enzyme was subjected to molecular dynamic simulations for both their free forms and in complex with Berberin in a cubic box solvated by the tip3p water molecule model using the GROMACS 2019.6 program running on the Kubuntu 2020.4 Linux operating system (28). Berberin force field parameters were generated with the Python-based ACPYPE tool (AnteChamber Python Parser Interface) (29). Enough ions have been added in order to neutralize the system. The dissolved systems were initially reduced using the steepest descent approach in order to remove the enormous forces. The simulated systems were then stabilized at 310 K and 1 bar by running 1 ns simulations in nvt and npt ensembles. Following thorough system balancing, an MD run was conducted with a time step of 2 fs for a simulation time of 100 ns. The molecular structure of enzymes, ligands, and intermolecular interactions was finally studied using simulated trajectories.

3. Results and discussion

3-1. Molecular docking

The binding pose and interaction of Berberin with the key residues of AmpC β-lactamase in its active site are exhibited in Figure 1. This diagram shows the main amino acids in the active site of OXA-10 as follows: Ala66, Ser67, Met99, Trp102, Val114, Ser115, Leu155, Glu156, Gly207, Phe208, and Ser109. Some residues interact with Berberin through van der Waals interactions. It is apparent that the carbonyl group of Berberin formed two H-bond complexes with the carboxyl groups of Ser67 and Ser115, respectively. Binding energy and the inhibition constants of Berberin with the OXA-10 are displayed in Table 1. This table indicates that the OXA-10/berberin system has the lowest binding energy, with a mean ΔG binding of 6.29 Kcal/mol and an inhibition constant of 24.33 μM. Moreover, these findings indicate that Berberin has a high affinity for OXA-10, which can potentially be an inhibitor of class D β-lactamase.
MD simulation

3-2. Analyses of RMSD (root mean square deviation)

The root mean square deviation (RMSD) of the trajectory with respect to their initial conformation obtained in MD simulations provided us with the stability measure of the docked complexes. Figure 2 shows the OXA-10 RMSD of 100 ns for the simulated system. In the beginning, there was a sharp rise in RMSD for the protein-ligand complex, and after the initial rise, a subsequent gradual decrease occurred. As shown in this figure, the OXA-10 enzyme reached equilibrium at 75 ns for the free and bond systems, with a minor shift for the bond system at a 90 ns time period. As ligands bind the OXA-10, the deviation of the protein decreases, which shows the stabilization of the OXA-10 in the presence of berberin. The averages of the MD parameters for free and complex systems represented in Table 2 over the last 20 ns indicate that Berberin binding causes significant stabilization. As with the binding of berberin to OXA-10, the average number of RMSD shifts from the free form of 0.174±0.042 nm to 0.238±0.035 nm.

3-3. MD simulation

3-3-1. Analyses of RMSD (root mean square deviation)

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Table 1: The binding energies and inhibition constants of OXA-10/Berberin

System	ΔG binding (KCal/mol)	Ki (μM)
OXA-10/Berberin	-6.29	24.33

3-3-2. Analysis of RMSF (root mean square fluctuation)

In the RMSF profile, the conformational flexibility residues of the protein-ligand complexes were evaluated. Figure 3 depicts the protein RMSF and the ligand RMSF, respectively. It is clear from the figure that the fluctuations were very low, and the RMSF for the OXA-10 residues was observed to be a minimum of 0.07 Å for all the complexes and a maximum of 0.46 Å. For the ligand-protein RMSF, the atoms showed the minimum fluctuation for residues in the OXA-10 active site compared to the free form. As shown in Table 2, the mean RMSF value in the presence of berberin for the enzyme has decreased, indicating that the bound state of the OXA-10 enzyme has a relatively lower conformational fluctuation than the free form of the enzyme.

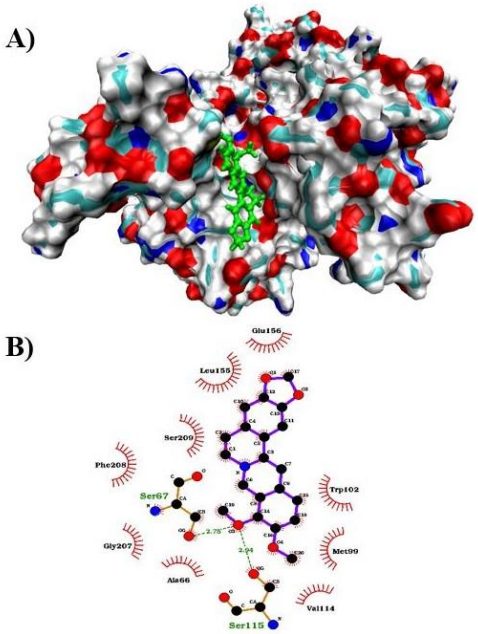

Figure 1. Binding mode of Berberin with docked OXA-10 (A) Ribbon model: Berberin, Surface model: Berberin, (B) Ligplot showing interactions between Berberin residues and OXA-10: the residues in green are involved in hydrogen bonding and the black in hydrophobic interactions. The atoms in contact are shown with spokes radiating back. Figures are provided by the VMD1.9.3 and Ligplot+ programs.

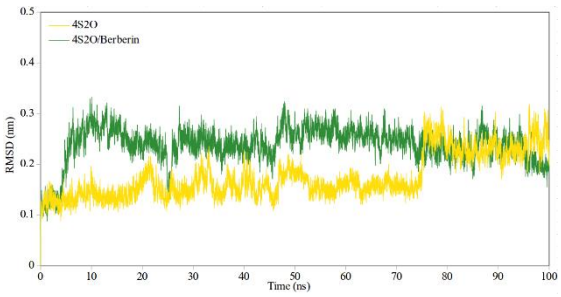

Figure 2. The time evolution of average RMSD during 100 ns MD simulations.

3-3-3. Analysis of RG (radius of gyration)

RG is an important parameter that yields information about structural compactness, the

third structure of the enzyme, and its deviation in the presence of ligand. The radius of gyration (Rg) of the OXA-10 and ligand complexes were found to be between 1.76 and 1.85 nm initially, as represented in figure 4. The Rg values were stabilized after 78 ns for both free and complex systems. RG for complexed systems shows less fluctuation during simulation time, which shows the 3rd structure of OXA-10 has been compacted as bound to berberin. Table 2 represents the average amount of Rg during the last 20 ns of simulation time. The average number of OXA-10 has decreased with the presence of berberin, which shows the compression of the enzyme's 3rd structure due to binding to berberin.

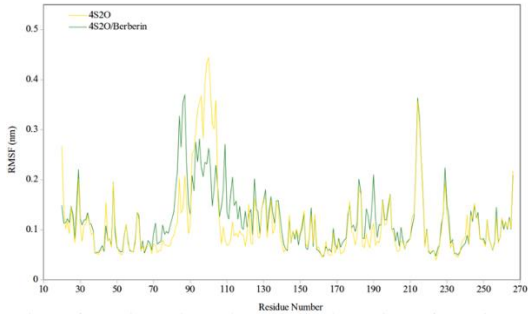

Figure 3. The average RMSF for 100 ns period of MD simulations.

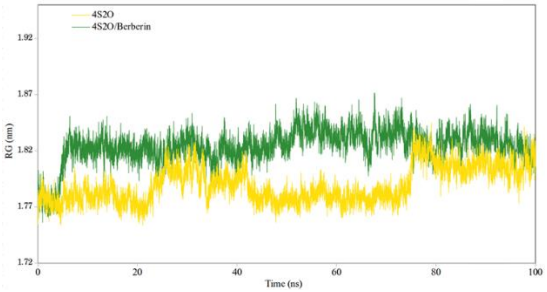

Figure 4. The average RG for 100 ns period of MD simulations.

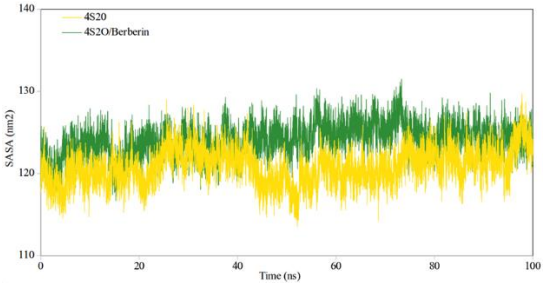

Figure 5. The average SASA for 100 ns period of MD simulations

3-3-4. Analysis of SASA (solvent-accessible surface area)

In the SASA profile, the reachable surface of the enzyme to its solvent in free and complex form has been studied during periodic simulation. Figure 5 exhibits the SASA diagram, as the results indicate the minimum 105 nm to maximum 130 nm SASA, and both systems have reached equilibrium in 75 ns. As Berberin binds to OXA-10, the SASA has less deviation than the free form in the diagram. According to Table 2, the average amount of SASA produced by the binding of berberin to OXA-10 increased, which shows the surface of the enzymes for water molecules was extended into complex form.

3-3-5. Analysis of the number of hydrogen bonds

Investigating the H-bond between enzyme-enzyme, enzyme-solvent, and enzyme-ligand provides more details about the binding affinity and interaction tendency of systems. The number of contacts formed by the Berberin with OXA-10 residues and the corresponding probability over the 100 ns simulation time are exhibited in Figure 6. The maximum number of H-bonds formed by the berberin with OXA-10 residues was 2 in the MD simulations study, which shows the stability of complexes and showed that berberin has a binding tendency to this OXA-10. Enzyme-solvent and enzyme-enzyme hydrogen bonds for free and bound are exhibited in Figures 7 and 8, respectively.

The interpretation of these figures shows no significant variation or alteration in enzyme-solvent for both systems, but in the case of enzyme-enzyme hydrogen bonds, the enzyme H-bond has slightly changed as complexed with berberin. The average number of enzyme-solvent and enzyme-enzyme hydrogen bonds is shown in Table 3 for systems during the last 20 ns. The findings in this table suggest that the average number of hydrogen bonds between enzyme atoms in the presence of Berberin has slightly decreased, while the hydrogen bonds

Table 2: The average and standard deviations of RMSD, Rg, RMSF, and SASA for free and complex enzymes during the last 20 years

System	Mean RMSD (nm)	Mean Rg (nm)	Mean RMSF (nm)	Mean SASA (nm)
Free OXA_10	0.174±0.042	1.789±0.0158	0.119±0.073	120.884±2.177
OXA-10/Berberin	0.238±0.035	1.823±0.015	0.111±0.060	124.024±1.999

Table 3: The average and standard deviations of intramolecular enzyme and enzyme-solvent hydrogen bonds during the last 20 ns

System	Enzyme-Enzyme	Enzyme-Solvent
Free OXA-10	195.650±6.915	486.769±13.281
OXA-10/Berberin	191.060±6.600	494.386±12.701

between the OXA-10 enzyme and the solvent molecules increased in the presence of Berberin. The binding affinity of the berberin with OXA-10 in the MD simulations study validated the docking results.

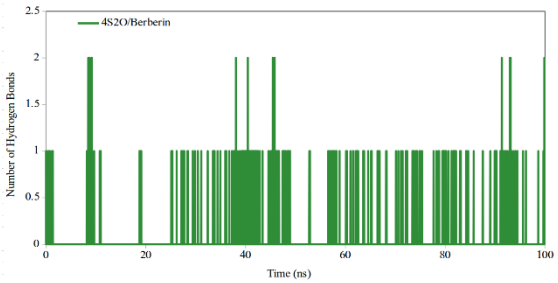

Figure 6. The number of hydrogen bonds between Berberin and OXA-10 for a 100-ns period of MD simulations.

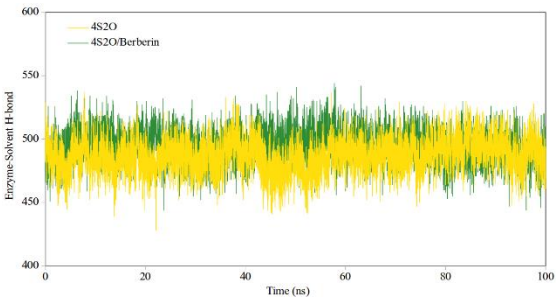

Figure 7. The enzyme-solvent H-bond for a 100-ns period of MD simulations.

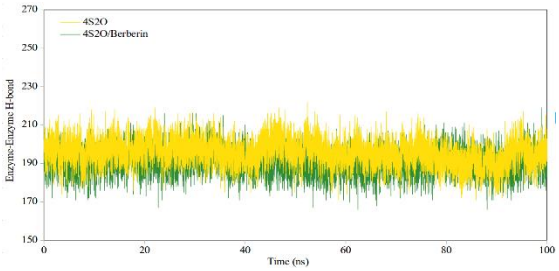

Figure 8. The enzyme-enzyme H-bond for a 100-ns period of MD simulations.s

5. Conclusion

The present study is based upon a new molecular technique to identify a natural compound inhibitor (berberin) for OXA-10, combined with the analysis to validate the findings by utilizing molecular docking and MD simulation. The docking study exhibits suitable binding energy and significant hydrogen bond interactions. A MD simulation study was performed to verify the overall stability of the berberin within the active site of the protein, as shown in different structural analyses of MD simulation, following RMSD,RMSF, RG, and SASA. Additionally, the interactional analysis indicated a constant hydrogen bond between berberin and OXA-10. The current study provides information for further in vitro and in vivo studies and is a good starting point for experimental studies.

Conflict of interest

We declare that we have no conflict of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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