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Integrating molecular docking and molecular dynamics simulation approaches for the investigation of the affinity and interactions of curcumin with Class D β-lactamase

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ABSTRACT

Introduction: Antimicrobial resistance has become a major concern in treating infectious diseases due to their extensive use, which has led to bacterial gene modification and the secretion of some enzymes by these microorganisms that make them survive despite the presence of antibiotics. Searching for inhibitors for these resistant pathogens was helpful to elevate the impact of antibiotics in curing diseases. Curcumin is a natural compound that has several medicinal effects and can be used to inhibit OXA-10 β-lactamase class D enzymes.

Materials and Methods: Molecular docking and molecular dynamic simulation were utilized to understand the binding pose, structural integrity, stability, and binding energy of class D beta-lactamase with curcumin using Autodock 4.2.2 software and the GROMACS 2019.6 program applying the AMBER99SB force field, respectively.

Results: Molecular docking results and interaction analysis of molecular dynamics simulations indicated stable hydrogen bonds and van der Waals interactions of curcumin with OXA-10 β-lactamase.

Conclusion: This paper indicates that curcumin, which is a natural ingredient, can be used as a potential inhibitor of class D β-lactamase OXA-10.

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

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1. Introduction

Class D β-lactamases, also called OXA-type β-lactamases, are bacterial enzymes that confer resistance to β-lactam antibiotics. β-Lactam antibiotics, which include penicillins, cephalosporins, and carbapenems, are commonly used to treat bacterial infections (1). The identification of class D β-lactamases dates back to the 1980s, when they were first identified in a bacterial strain called Acinetobacter baumannii. They are currently observed in other bacteria, including other species of the genus Acinetobacter and numerous species of the Enterobacteriaceae (2). These enzymes differ from other classes of β-lactamases, such as class A (TEM and SHV enzymes) and class B (metallo-β- Class D β-lactamases exhibit variations in their protein structure and mechanism of action, allowing them to catalyze the hydrolysis and inactivation of β-lactam antibiotics (3). One of the characteristic features of class D β-lactamases is their ability to confer resistance to carbapenems, which are highly potent broad-spectrum β-lactam antibiotics. Carbapenem-resistant bacterial strains, known as carbapenem-resistant Enterobacteriaceae (CRE) or carbapenem-resistant Acinetobacter baumannii (CRAB), pose a significant healthcare challenge because they can limit treatment options and are associated with increased morbidity and mortality (4). The proliferation of class D β-lactamases is greatly facilitated by mobile genetic elements such as plasmids that can transfer resistance genes between bacteria. This facilitates the rapid spread of resistance within and between bacterial species (5).

Antibiotic resistance is a significant global health problem. This involves the ability of bacteria and other microorganisms to survive and reproduce despite the presence of antibiotics that would normally eradicate or hinder their reproduction (6). Consequently, the effectiveness of antibiotics in treating diseases caused by resistant bacteria is decreasing. The emergence of antimicrobial resistance can occur spontaneously as a result of genetic changes in bacteria, but is primarily due to the misuse and overuse of antibiotics (7). Resistance to antibiotics is more likely to develop when they are used improperly or unnecessarily, for example, in viral infections or when treatment is not fully completed. In addition, the use of antibiotics in livestock and agriculture contributes to the spread of resistant microorganisms throughout the food chain and in the environment (8).

Curcumin, an inherent chemical compound found in the spice turmeric, has been the subject of research due to its potential effects as an antimicrobial agent (9). While curcumin itself does not possess direct antibiotic properties against a broad spectrum of bacteria, it has been observed to exhibit antibacterial effects against certain strains under certain circumstances (10). The inhibitory effect of curcumin on the proliferation of various types of bacteria, including those resistant to multiple drugs, has been scientifically proven. Additionally, curcumin has been found to increase the effectiveness of select antibiotics when used together (11). Research has shown that curcumin can enhance the effectiveness of antibiotics such as ciprofloxacin, gentamicin, and ampicillin against bacteria that have developed resistance to these drugs (12). This finding suggests that curcumin is suitable as an adjunctive therapy to enhance the effectiveness of antibiotics.

Bacterial biofilms are complex collections of bacteria enclosed in a protective matrix, making them extremely insensitive to the effects of antibiotics. Curcumin has demonstrated the ability to prevent biofilm formation and destroy existing biofilms in various bacterial strains, including those associated with persistent infections (13). Additionally, curcumin has been found to impair bacterial virulence factors, which are

molecules that allow bacteria to cause infections and evade the host's immune response. By specifically targeting these virulence factors, curcumin may help reduce the pathogenicity of bacteria and improve the effectiveness of antimicrobial treatment (14). In addition to its potential as an antimicrobial, curcumin is known for its powerful anti-inflammatory properties. Chronic inflammation can contribute to the progression and severity of various infections (15). By modulating the inflammatory response, curcumin may indirectly support the body's ability to fight infections (16). Given the importance of identifying β-lactamase inhibitors for the treatment of infections, as well as the importance of natural compounds and the comprehensive and valuable medicinal properties of curcumin, the aim of this research is to investigate the inhibitory behavior of curcumin within the active site of the β OXA-10 type group D lactamase using computational molecular docking and molecular dynamics simulation methods.

2. Material and methods

2-.1 Computational methods

Computational methodologies acquiesce for the accretion of advice that is backbreaking to access through beginning means. The use of computer simulations for assorted combinations proves to be more cost-effective compared to administering class tests of any kind. Generally, the biologic architecture endeavor is agitated out by clay experts, provided they acquire an aerial amount of accurateness and all-encompassing knowledge. This access alleviates the abundant accountability imposed by beginning methods, leading to cogent reductions in time and banking resources. In contemporary times, biologic giants beyond the apple accept apparent a agog absorption in the acceptance of computational methodologies for biologic design. Atomic advancing stands as an invaluable address for investigating the interactions among ligands and proteins, a basic axiological apparatus in computational biologic design. Atomic advancing entails employing computer calculations to determine the optimal protein-bound armpit for ligand attachment as well as the most favorable acclimatization of the ligand to the protein-bound armpit to facilitate acceptable interactions between the two compounds. Consequently, through the appliance of the atomic advancing method, one can analyze the bounden chargeless energy, hydrogen bonds, and anatomic groups that accord with the enactment of added almighty ligand-protein interactions.

2-2. Enzyme and Ligand Structure Selection

The enzymes were docked using the software Autodock 4.2.2 (17, 18). For this purpose, the structures of class D β-lactamase obtained from the RCSB protein database were used (19). The pdb file was modified by including non-polar hydrogen atoms optimized for docking calculations and by removing water molecules and original ligands. The SDF format of the curcumin 3D structures was obtained from the PubChem service and converted to PDF format using Open Babel, which was optimized by Gaussia (20, 21)S. Energy minimization for the three enzymes was performed using the AMBER99SB force field in the GROMACS 2019.6 package. The macromolecule remained rigid during the docking calculations while the pipeline was allowed to rotate freely. The active site of the macromolecule was determined using cocrystallized ligands present in the pdb files of the enzyme. A total of 200 docking calculations were performed using the Lamarckian Genetic Algorithm (LGA) method, which included 25 million energy

assessments. The active site was selected using a 50-point grid map with a grid point spacing of 0.375. The optimal docking mode was determined to be the conformation in the more populated cluster with the lowest binding energy.

2-3. Molecular dynamic simulation

The enzyme was subjected to molecular dynamics simulation both in its free form and in complex with curcumin in a cubic box solvated with tip3p water. The GROMACS 2019.6 program was used to perform the molecular modeling on the Linux operating system Kubuntu 2020.4 using the AMBER99SB force field. The force field parameters for curcumin (22). were generated using the program ACPYPE (AnteChamber Python Parser Interface). Sufficient ions were added to the system to maintain equilibrium. To initially weaken the influence of strong forces, the solvated systems were minimized using the steepest descent approach. To achieve stability at 310 K and 1 bar, 1 ns simulations were performed in NVT instead of ensembles. Once the system was sufficiently equilibrated, an MD run was performed with a time step of 2 fish for a total of 200 NS simulation times. The simulated trajectories were used to analyze the molecular structure of the enzymes and ligands as well as the interactions between molecules.

3. Results and discussion

Table 1 represents the binding energies and inhibition constants of curcumin with the OXA-10. As shown in this table, the 4S2O curcumin system has the lowest binding energy. The results indicate that curcumin has a high affinity for enzymes and can play an inhibitory role for OXA-10 β-lactamase. Figure 1 represents the binding position of curcumin in the active site of the OXA-10 β-lactamase enzyme and the interactions of this compound with the key residues. This figure exhibits the amino acids Ala66, Ser67, Ala98, Tpr102, Ser115, Val117, Leu155, Thr206, Gly207, Phe208, Ser209, Leu247, and Arg250 in the active site of the OXA-10 interact with the curcumin through van der Waals interactions. As shown in this figure, the carbonyl groups of curcumin were found to interact with the carboxyl group of Leu155 and form three H-bonds with amine and the carbonyl group of Arg250, respectively.

Table 1: The obtained docking results, binding energies and inhibition constants predicted by AutoDock program

System	ΔG binding (KCal/mol)	Ki (μM)
OXA-10-Curcumin	-6.56	15.6

3-1. Molecular dynamic simulation Analysis of the root mean square deviation (RMSD)

RMSD indicates the stability and structural variation of the free enzyme and enzyme-ligand systems. Figure 2 represents the RMSD of the free protein and its bond to the ligand. According to this figure, the OXA-10 enzyme has reached equilibrium in about 75 ns for both systems. Structural fluctuation of OXA-10 had declined as complexed with curcumin, which shows the stability of complexes. Table 2 shows the averages of the MD parameters for the system in the last 30 ns. According to Table 2, as with the binding of curcumin to OXA-10, the average number of RMSD shifts from the free form (0.156±0.020 nm) to the complexed form (0.150±0.010 nm).

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

RMSF analysis shows the fluctuations and flexibility of each residue in different regions of the enzyme system in the free and bound states. Figure 3 displays the RMSF of free protein and its bond to the ligand. According to this figure, the binding of curcumin to OXA-10 causes less fluctuation in the system. The RMSF for the OXA-10 residues was observed to be a minimum of 0.06 Å for all the

complexes and a maximum of 0.45 Å. According to 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 had less conformational fluctuation than the free form of the enzyme.

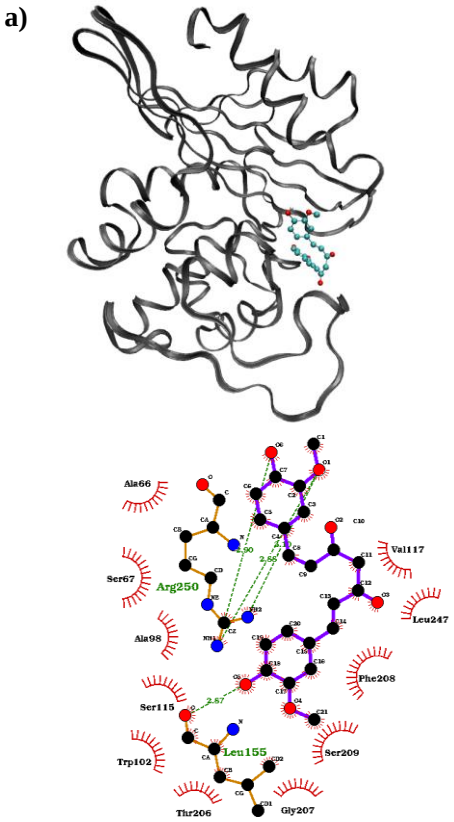

Fig. 1. Shows the best docking pose and molecular interactions of the curcumin and the residues of the enzyme. The C, N, and O atoms are indicated in black, blue, and red, respectively. Hydrogen bonds are identified by green drops, and hydrophobic interactions are shown by red curves with spokes radiating towards the ligand atoms with which they interact. The atoms in contact are shown with spokes radiating back. Figures are provided by the VMD1.9.3 and Ligplot+ programs.

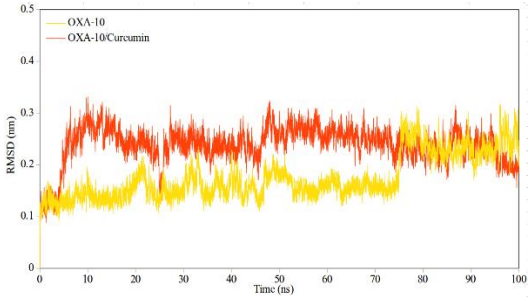

Fig. 2. RMSD plots of free and bound enzymes as a function of time.

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

RMSF analysis shows the fluctuations and flexibility of each residue in different regions of the enzyme system in the free and bound states. Figure 3 displays the RMSF of free protein and its bond to the ligand. According to this figure, the binding of curcumin to OXA-10 causes less fluctuation in the system. The RMSF for the OXA-10 residues was observed to be a minimum of 0.06 Å for all the complexes and a maximum of 0.45 Å. According to 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 had less conformational fluctuation than the free form of the enzyme.

3-4. Analysis of the radius of gyration (Rg)

The Rg calculation depicts the shape of the protein and its structural compactness during the simulation time when complexed with ligand. Figure 4 exhibits the Rg of the free enzyme and the enzyme-curcumin complexes. According to this figure, the OXA-10 enzyme has reached equilibrium in about 75 ns for free and complexed systems. The third structure of the system has been compressed as the curcumin complexed with OXA-10. Table 2 represents the average amount of Rg during the last 30 ns of simulation time. The average number of OXA-10 has increased with the presence of curcumin, which shows the compression of the enzyme due to binding to curcumin.

3-5. Analysis of the solvent-accessible surface area (SASA)

SASA analysis shows the surface space of enzymes that is reachable to solvent molecules in the system over the simulation time. Figure 5 shows the SASA diagrams. According to this figure, the average amount of SASA for the enzyme has increased due to the binding to curcumin, which is caused by contact between

curcumin and the residue of the enzyme in a cavity on the surface of OXA-10. According to Table 2, the average amount of SASA produced by the binding of curcumin to OXA-10 was mildly elevated, which shows the surface of the enzymes for water molecules was extended in complex form.

3-6. Hydrogen bond analysis

Investigating the number of hydrogen bonds between enzyme and ligand shows the stability of the complexes during simulation time. Figure 6 represents the number of hydrogen bonds between curcumin and the enzyme over the 100 ns simulation time. During the simulation time, the maximum number of hydrogen bonds formed between curcumin and OXA-10 was 3, which shows the stability of complexes. Curcumin has the highest binding tendency to this enzyme. Figures 7 and 8 represent the enzyme-enzyme and enzyme-solvent hydrogen bonds for free and bound enzymes during the simulation time, respectively. Accordingly, the average number of hydrogen bonds between enzyme atoms in the presence of curcumin has slightly decreased, and the hydrogen bonds between the OXA-10 enzyme and the solvent molecules have increased in the presence of curcumin.

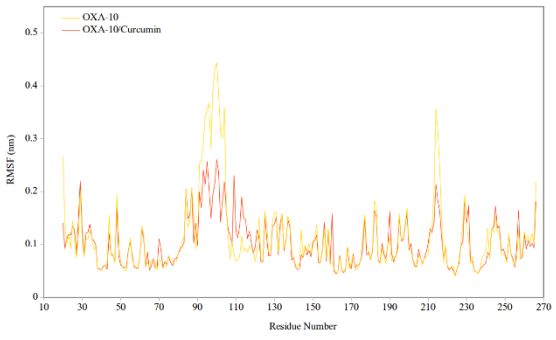

Fig. 3. RMSF plots of free and bound enzymes as a function of time.

4. Conclusions

In the present study, molecular docking and molecular dynamics simulation approaches were utilized to find the inhibitory effect of

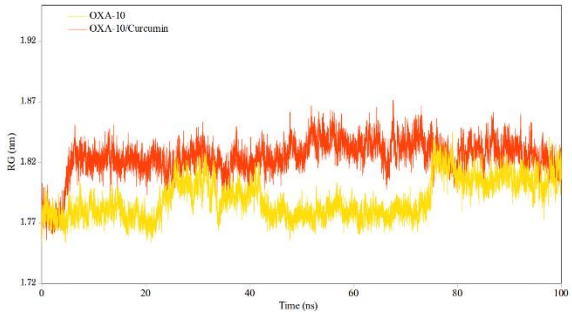

Fig. 4. RG plots of free and bound enzymes as a function of time

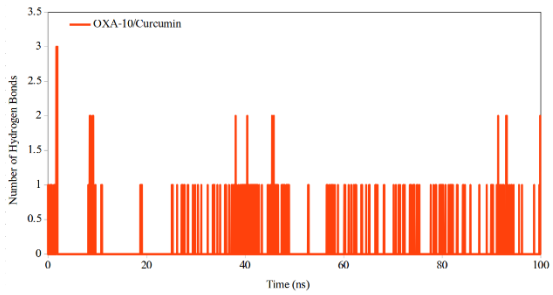

Fig. 6. Time dependence of the number of hydrogen bonds between curcumin and enzyme during the simulation time.

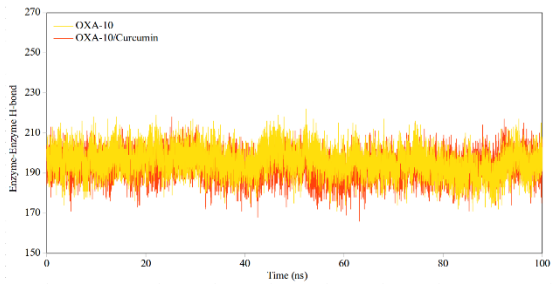

Fig. 7. Enzyme-enzyme hydrogen-bond plots of free and bound enzymes as a function of time.

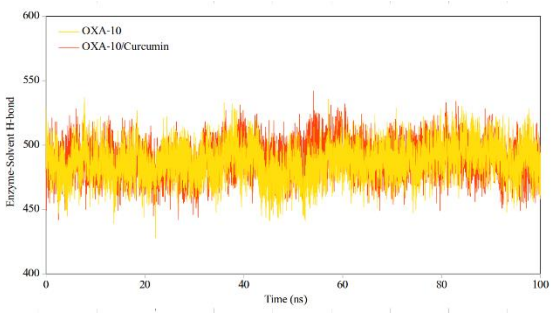

Fig. 8. Enzyme-solvent hydrogen bond plots of free and bound enzymes as a function of time.

curcumin on OXA-10 β-lactamase class D enzymes. Molecular docking studies showed curcumin's favorable interactions with the β-lactamase enzyme through hydrogen bonds

and van der Waals interactions. Analysis of the RMSD describes the stabilization of OXA-10 as curcumin binding. R_g plots indicated the compression of OXA-10 due to binding with curcumin to OXA-10.

Table 2: The average and standard deviations of RMSD, Rg, RMSF, and SASA for free and complex enzymes during the last 30 years

System	Mean RMSD (nm)	Mean Rg (nm)	Mean RMSF (nm)	Mean SASA (nm^2)
Free OXA-10	0.156±0.020	1.802±0.007	0.089±0.045	121.412±1.980
OXA-10-Curcumin	0.150±0.010	1.837±0.005	0.081±0.053	126.130±1.624

Table 3: The average and standard deviations of intramolecular enzyme and enzyme-solvent hydrogen bonds during the last 30 ns.

System	Enzyme-Enzyme	Enzyme-Solvent
Free OXA-10	218.122 ±6.432	461.830±12.522
OXA-10/Curcumin	214.592±6.454	463.022±12.212

The bound state of all three enzymes had a relatively lower conformational fluctuation than the free form of enzymes. H-bond analysis confirmed the all-docking and MD simulation results. Therefore, this study would be helpful in designing new inhibitors of antimicrobial resistance for further in vivo and in vitro studies.

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