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Integrating molecular docking and molecular dynamics simulation approaches for investigation of the affinity and interactions of quercetin with Class D beta-lactamase, OXA-10

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ABSTRACT

Introduction: The misuse of β-lactam antibiotics has led to the development of β-lactamase-producing organisms, which inhibit β-lactam activity by hydrolyzing the peptide bond. This study aims to investigate the inhibitory effect of quercetin, a natural composite and isoquinoline alkaloid, on β-lactamase enzyme action, potentially increasing antibiotic effectiveness.

Materials and Methods: This study utilized computational techniques like molecular docking and MD simulation to predict the binding mode and possible conformation poses of quercetin with the OXA-10 β-lactamase enzyme. Autodock software was used for docking, while Gromacs 2019.6 package was used for MD simulations to study molecular complex behavior over time.

Results: The outcomes of the molecular docking analysis revealed a favorable interaction between quercetin and the OXA-10 β-lactamase enzyme, as evidenced by a binding energy of -5.95 kcal/mol and a suitable binding mode. MD simulations confirmed the docking results, showing stable hydrogen bonds between Quercetin and OXA-10, as well as comparable RMSD, RMSF, SASA values, and other parameters.

Discussion: This research shows the potential of quercetin, a natural compound with multiple medicinal effects, as a possible inhibitor of the class D type β-lactamase OXA-10. Therefore, this study maintains valuable intuition for designing new inhibitors of antimicrobial resistance to combat β-lactamase activity.

Kewwors: Quercetin, Class D type β-Lactamase, Molecular Docking Molecular Dynamics Simulation.

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1. Introduction

The global rise of drug-resistant bacteria is swiftly unfolding, posing a threat to the effectiveness of antibiotics that have revolutionized the field of medicine and rescued countless lives (1). In recent years, bacterial infections have resurfaced as a significant concern, despite the advancements made in treating them with antibiotics several decades ago (2). The overuse and misuse of antibiotics, along with a decline in new drug development by the pharmaceutical industry due to diminished economic incentives and stringent regulatory demands, have been identified as the primary causes of the antibiotic resistance crisis (3). The Centers for Disease Control and Prevention (CDC) have identified several bacteria that pose immediate, severe, and worrisome risks. These bacteria have already imposed significant clinical and financial challenges on the U.S. healthcare system, patients, and their families (4).

Incorrectly prescribed antibiotics contribute to the proliferation of antibiotic-resistant bacteria. Research studies have revealed that in 30% to 50% of cases, the treatment indication, choice of antibiotic, or duration of therapy is incorrect (5). A study conducted in the United States found that only 7.6% of 17,435 patients hospitalized with community-acquired pneumonia (CAP) had a defined pathogen (6). In contrast, researchers at the Karolinska Institute in Sweden were able to identify the probable pathogen in 89% of CAP patients using molecular diagnostic techniques such as polymerase chain reaction (PCR) and semiquantitative PCR. Furthermore, it has been observed that 30% to 60% of antibiotics prescribed in intensive care units (ICUs) are unnecessary, inappropriate, or suboptimal. Incorrectly prescribed antibiotics may not provide the intended therapeutic benefits and can potentially expose patients to complications associated with antibiotic therapy. In addition, the presence of sub-inhibitory and sub-therapeutic antibiotic concentrations can contribute to the development of antibiotic resistance by facilitating genetic alterations, including changes in gene expression, horizontal gene transfer (HGT), and mutagenesis. These genetic changes can lead to increased virulence through alterations in antibiotic-induced gene expression, while also promoting antibiotic resistance and its spread through increased mutagenesis and HGT. Furthermore, studies have demonstrated that low levels of antibiotics can contribute to the diversification of strains in organisms such as Pseudomonas aeruginosa. Additionally, subinhibitory concentrations of piperacillin and/or tazobactam have been found to induce broad proteomic alterations in Bacteroides fragilis (5).

Class D β-lactamases, commonly referred to as oxacillinase or OXA enzymes, were initially named based on their preference for hydrolyzing penicillin oxacillin over benzylpenicillin. However, it is important to note that this characterization is no longer universally applicable (7). The recent growth of the OXA β-lactamase class has been primarily driven by the significant increase in both new enzymes and new enzyme variants. This class has experienced the highest percentage of growth compared to other classes (8). Despite the diverse nature of enzymes within this class, they can still be categorized based on their amino acid composition. These enzymes are present in various bacterial species, including Acinetobacter, Shewanella, Pseudomonas, and Burkholderia, and can be found on both chromosomes and plasmids (9). The Class D

β-lactamase family exhibits a remarkable level of diversity, currently encompassing more than 400 recognized variants. This extensive diversity has led to confusion in the literature when describing class D β-lactamases, particularly in terms of amino acid identity. To address this issue and provide a more organized framework for this family, we have employed a criterion of ≥80% amino acid identity to categorize the Class D β-lactamase family into distinct groups. Within each group, we further identify subgroups consisting of enzymes that share at least 95% amino acid identity. While the selection of these cut-off values may be subject to debate, we firmly believe that they are adequate for establishing clearer relationships among the diverse enzymes that constitute this enzyme family (10). The issue of antibiotic resistance necessitates a comprehensive approach that involves dedicated research and development endeavors focused on creating groundbreaking medications and alternative therapeutic interventions. Nevertheless, there have been setbacks in the progress of producing new antibiotics in recent times, and the prospects for pioneering drugs are highly promising (11).

There are numerous benefits linked to the utilization of plants and their phytoconstituents in contrast to pharmaceutical products. The biological activities of plant extracts and their phytoconstituents, including anti-diabetic, anti-hyperlipidemic, free-radical scavenging, and anti-inflammatory activities, have been scientifically validated. Metabolic disorders, which significantly impact the quality of life, often involve the detrimental influence of free radicals (12). Quercetin, an essential bioflavonoid found in over twenty plant sources, is renowned for its various beneficial properties, including anti-inflammatory, anti-hypertensive, vasodilator effects, anti-obesity, anti-hypercholesterolemic, and anti-atherosclerotic activities. The presence of free radicals plays a pivotal role in the progression of diseases such as hypertension, vascular disorders, and metabolic syndrome (13). Given the significance of rare β-Lactamase inhibitors in addressing inflammation, along with the importance of natural compounds and the extensive therapeutic impact of quercetin, the objective of this research is to explore the inhibitory properties of quercetin on class β-Lactamase in the liver through computational methods.

2. Materials and Methods

2-1. Computational Methods

The utilization of both molecular docking and molecular dynamics simulations allows for a comprehensive comprehension of the binding mechanism between quercetin and class D β-lactamase. This approach provides intricate insights into the molecular interactions and dynamics of the complex compound, thereby aiding in the development of novel compounds with enhanced binding affinity and specificity. Additionally, these computational techniques can be employed to investigate the impact of mutations or structural alterations in class D β-lactamase on quercetin binding. This knowledge is vital in understanding the mechanisms underlying drug resistance and can serve as a foundation for devising strategies to combat resistance. The combination of molecular docking and molecular dynamics simulation methods serves as a potent tool for examining the interactions between small molecules like quercetin and target proteins such as class D β-lactamase. This investigation holds significant implications for the advancement of innovative antibiotics and the battle against antibiotic resistance.

2.2. Selection of enzyme and ligand structures

The class D β-lactamase (OXA-10) PDB file with the ID of *1K55* was acquired from the RCSB protein database (14) while the three-dimensional structure of quercetin was obtained from the PubChem database using the CID 5280343 in the SDF Format. To convert it to PDB format, the Open Babel software (15)

2.3. Molecular Docking

In order to explore the interactions and binding affinity between quercetin and the OXA-10 β-lactamase enzymes, the researchers employed docking techniques and utilized Autodock 4.2.2 software (16, 17). Initially, the water molecules and co-crystal ligands were present in pdb files, but with the removal of hydrogen atoms and the addition of Gasteiger charges, the system was prepared for docking (18, 19). To minimize the energy of the enzymes, the GROMACS 2019.6 package was employed, utilizing the AMBER99SB force field (23). The active sites of the enzyme were determined based on the co-crystal ligand specified in the PDB file, and a grid array with dimensions of 60 × 60 × 60 points and a grid point spacing of 0.375 Å was selected. Subsequently, 200 docking calculations were performed using the Lamarckian Genetic Algorithm (LGA) method, which involved 25 million energy assessments. Ultimately, the conformation with the lowest binding energy within the most highly populated cluster was chosen as the optimal docking position for further investigations.

2-4. Molecular dynamic simulation

The agitator underwent atomic dynamics simulations in a neutral state and in conjunction with quercetin in a solvated cubic box. These simulations were conducted using the GROMACS 2019.6 program on a Linux Kubuntu 2020 operating system. The force field parameters for quercetin were generated using the Python-based ACPYPE tool. To maintain charge neutrality, a sufficient number of ions were added to the system. The initial solvation systems were scaled down using the steepest descent method to eliminate excessively large forces. Subsequently, the simulated systems were equilibrated at a temperature of 310 K and a pressure of 1 bar through 1 ns simulation in the NVT (constant number of particles, volume, and temperature) and NPT (constant number of particles, pressure, and temperature) ensembles. Once the extended system reached equilibrium, a molecular dynamics (MD) run was performed with a time step of 2 fs for a total simulation time of 100 ns. The simulated trajectories were then analyzed to study the atomic structure of the enzyme, the ligand, and the resulting intermolecular interactions (20).

3. Results and discussion

The binding poses of the OXA-10 β-lactamase enzyme and the interactions of this complex with the key residues in the active site are displayed in Figure 1. This figure exhibits the amino acids Ser67, Val117, Leu155, Gly207, Phe208, Ser209, Glu244, Leu247, and Arg250 in the active site of the OXA-10 interact with the quercetin through van der Waals interactions. That is clear in this figure, in which the carbonyl groups of quercetin were found to interact with the carboxyl groups of Ser67 and Ser209 and form an H-bond with the amine group of Arg250, respectively. Table 1 indicates the binding energy and the inhibition constant of quercetin with OXA-10 and verifies the findings of the Figure 1 plots. Final findings indicate that the OXA-10/Quercetin complex has low binding energy, and Quercetin has a high affinity for enzymes, possibly playing an inhibitory role for OXA-10 β-lactamase.

Table 1. The obtained docking results, binding energy and inhibition constant predicted by AutoDock program

System	$\Delta G_{binding}$ (KCal/mol)	K_i (µM)
OXA-10-Quercetin	-5.95	43.34

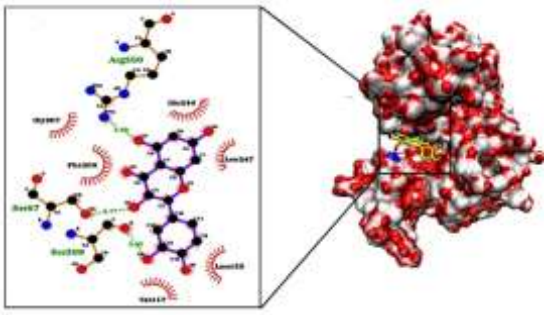

Fig 1. The best docking poses and molecular interactions of quercetin and the residues of the enzyme. The C, N, and O atoms are indicated in black, blue, and red, respectively. Hydrogen bonds are identified by green drops, and hydrophobic interactions are shown by red curves with spokes radiating towards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

Molecular dynamic simulation

Analysis of the root mean square deviation (RMSD)

RMSD evaluation helps us to find the stability and structural variation of the free enzyme and enzyme-ligand systems during the simulation time. Figure 2 displays the RMSD of the free protein and its complex form. According to this figure, the OXA-10 enzyme has reached equilibrium at about 80 ns for free and 70 ns for complex systems, and a shift from 0.174±0.042 nm to 0.211±0.056 nm was observed in the free form. Table 2 shows the averages of the MD parameters for the system in the last 20 ns. As can be seen in this table, the structural stability of OXA-10 has diminished as a result of a result of binding with quercetin.

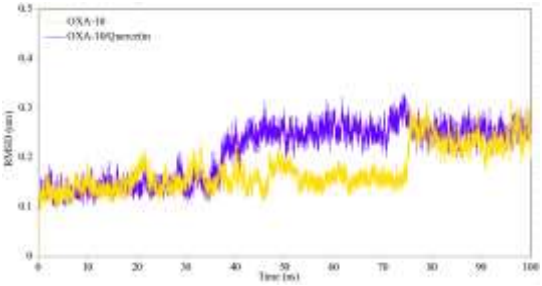

Fig. 2. RMSD plots of free and bound enzyme during the simulation as a function of time

Analysis of the root mean square fluctuation (RMSF)

RMSF puts information about the stability, fluctuations, and flexibility of each amino acid in different areas of the enzyme system in the free and bound states. Figure 3 displays the RMSF of free protein and its complex form. In this figure, the binding of quercetin to OXA-10 causes minor fluctuations in the system. The RMSF for the OXA-10 residues was observed at a minimum of 0.05 Å and a maximum of 0.55 Å. In Table 2, the mean RMSF value in the presence of quercetin for the enzyme has decreased from 0.112±0.073 nm in free form to 0.107±0.065 nm in complex form, indicating that the bound state of the OXA-10 enzyme had less structural fluctuation than the free form of the enzyme.

Analysis of the radius of gyration (Rg)

The Rg investigation presents the shape of the protein and its structural compression during the simulation time. Figure 4 exhibits the Rg of the free enzyme and the enzyme-quercetin complex. In this figure, the OXA-10 enzyme has reached equilibrium at about 80 ns for free and complex systems. The tertiary structure of the system has been compacted into the quercetin complex with OXA-10. Table 2 displays the average amount of R_g during the last 20 ns of simulation time and shows that the R_g of OXA-10 has declined in the presence of quercetin, which indicates further compression of the enzyme as it binds to quercetin.

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

System	Mean RMSD (nm)	Mean Rg (nm)	Mean RMSF (nm)	Mean SASA (nm^2)
Free OXA-10	0.234±0.022	1.821±0.009	0.112±0.073	122.163±2.051
OXA-10-Quercetin	0.252±0.017	1.806±0.009	0.107±0.065	128.093±1.708

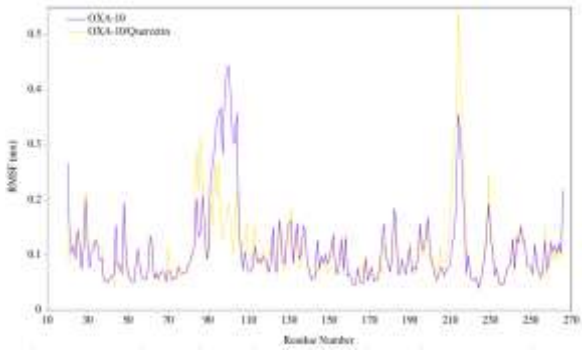

Fig. 3. RMSF plots of free and bound enzyme during the simulation as a function of time.

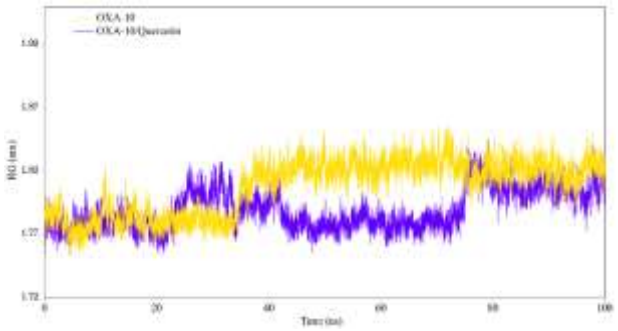

Fig. 4. RG plots of free and bound enzyme during as a function of time

Analysis of the solvent-accessible surface area (SASA)

The SASA calculation shows the surface space of enzymes that is available for solvent molecules in the system over the simulation time. Figure 5 represents the SASA plot. In this figure, the average amount of SASA for the enzyme has increased with binding to quercetin due to contact between quercetin and the residue of the enzyme in a cavity on the surface of OXA-10. The SASA for the OXA-10 residues was observed to be a minimum of 115 nm and a maximum of 130 nm. In table 2, the average amount of SASA by binding of quercetin to OXA-10 increased, which shows the surface of the enzymes for water molecules was extended in complex form.

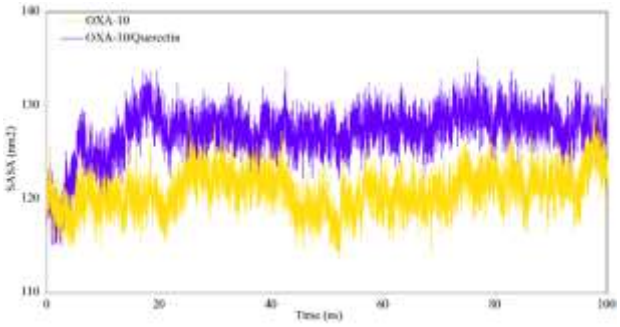

Fig. 5. SASA plots of free and bound enzyme during as a function of time.

Hydrogen bond analysis

Understanding the hydrogen bonds of enzymes and ligands presents the structural stability of the complexes as a function of time. Figure 6 represents the number of hydrogen bonds between quercetin and the enzyme over the 100 ns simulation time. During the simulation time, the maximum number of hydrogen bonds formed between quercetin and OXA-10 was 5, which shows the stability of complexes and validates previous studies of docking plots and MD simulation analysis. Quercetin has the highest binding affinity for this enzyme. Figures 7 and 8 target the enzyme-enzyme and enzyme-solvent hydrogen bond analyses for free and bound enzymes during the simulation time, respectively. The average number of hydrogen bonds between enzyme atoms in the presence of quercetin has slightly decreased, and the hydrogen bonds between the OXA-10 enzyme and the solvent molecules have increased in the presence of quercetin. Table 3 shows the average and standard deviations of intramolecular enzyme and enzyme-solvent hydrogen bonds during the last 30 ns.

Table 3: The average and standard deviations of intra molecular enzyme and enzyme-solvent hydrogen bonds during last 30 ns

System	Enzyme-Enzyme	Enzyme-Solvent
Free OXA-10	195.650 ±6.915	486.769±13.281
OXA-10/Quercetin	193.352±6.743	490.786±14.980

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

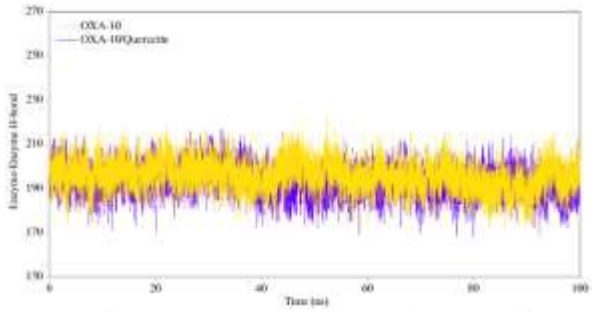

Fig. 7. Enzyme - Enzyme hydrogen-bond plots of free and bound enzyme during as a function of time

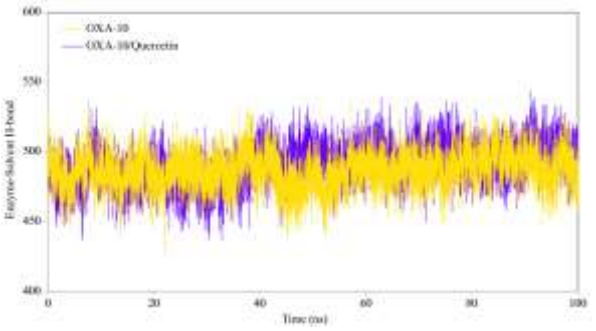

Fig. 8. Enzyme-Solvent hydrogen bond plots of free and bound enzyme during as a function of time.

Conclusions

The current investigation is based on a new molecular technique. The molecular docking and molecular dynamics simulation approaches were applied to identify the inhibitory effect of quercetin on OXA-10 β-lactamase class D enzymes. Molecular docking studies revealed Quercetin's favorable interactions with the β-lactamase enzyme through hydrogen bonds and van der Waals interactions, and MD simulation was performed to verify the overall stability of Quercetin as an inhibitor within the active site of OXA-10. Analysis of the RMSD depicts the stabilization of OXA-10 as Quercetin binding, and R_g plots present the compression of OXA-10 due to binding with Quercetin. The bound state of the enzyme had a relatively lower conformational fluctuation than the free form of enzymes, and H-bond analysis confirmed the all-binding and MD simulation results. Therefore, this identified potential inhibitor will be helpful in launching drug design and drug discovery for in vivo and in vitro experiments.

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