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Inferring the affinity and interactions of quercetin with Class C β-lactamase (AmpC, pdb code: 4HEF) by integrating molecular docking and molecular dynamics simulation approaches

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ABSTRACT

Introduction: Microbial drug resistance generated serious problems in treating infectious diseases and decreased the efficiency and efficacy of antibiotcs. Investigating for inhibitors of these pathogens is helpful to decrease microbial resistance against antibiotics and to decrease high mortality rates due to the resistance of microorganisms. In this paper, quercetin, which is a natural compound with significant medicinal effects, can be used to inhibit AmpC β-lactamase class C enzymes.

Materials and Methods: Molecular docking and molecular dynamic simulation were performed to determine the binding pose, structural integrity, stability, and binding energy of class C β-lactamase with quercetin 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 favorable hydrogen bonds and van der Waals interactions of quercetin with AmpC. These findings suggest that targeting β -lactamase using quercetin inhibitor analogs could provide a novel approach to treating antimicrobial resistance and could be used as a guide for future experimental studies.

Conclusion: By utilizing new molecular techniques, molecular docking, and molecular dynamics simulation, this paper suggests that quercetin, which has several medicinal effects, can be used to inhibit the AmpC- β-lactamase class C enzyme.

Keywords: Molecular docking, Molecular dynamics simulation, Quercetin, β-lactamase

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1. Introduction

To date, one of the serious predicaments of maintaining health is antibiotic resistance to pathogenic and innocuous bacteria (1, 2). Many people around the world have been treated and saved by antibiotics. Antibiotics are thus a key therapy option for several infectious disorders, including bacterial infections (3). The quest to synthesize penicillin G's various derivatives began after its discovery (2, 3). In the United States, antibiotic-resistant bacteria caused about 2 million infections and 23,000 deaths in 2014. The World Health Organization (WHO) named drug resistance against antibiotics as a major global threat in 2014. The WHO declared that drug resistance has increased globally and that the world has now entered the "post-antibiotic" age, based on data from 114 countries. A period when simple infections that were treatable for many years have become fatal (4).

There are actually many antibiotics called β-lactams that have a β-lactam ring in their molecular structure. Staphylococcus epidermidis, Haemophilus influenzae, Neisseria gonorrhea, Moracella catarrhalis, Escherichia coli, Proteus, Ceraschia, Pseudomonas, and Klebsiella species, as well as anaerobic bacteria like Bacteroides species, have all been linked to β-lactamase activity (5). The most frequent reasons for bacterial resistance to β-lactam antibiotics are hydrolase and β-lactamase enzymes. These enzymes deactivate almost all β-lactam antibiotics by hydrolyzing the amide bond in the β-lactam ring (4). Increasingly, the substrate range of β-lactamase is expanding. To date, β-lactamases are a serious threat to global health. B-lactamases are categorized into four groups: A, B, C, and D, based on their sequence of amino acids (6).

Bosch and Jacobi established a new classification in 1995, attempting to link the classification of these enzymes with their phenotype in clinical isolates utilizing the inhibitor profile and its substrate (7). Class A β-lactamases are coded by bacterial chromosomes and plasmids and include a wide range of enzymes, such as TEM-1, SHV-1, CTX-M, and gerbapenems (such as KPC-2) (8). Metallo-β-lactamase S, classified as class B, hydrolyzes penicillins, cephalosporins, and carbapenems. Class C enzymes are often encoded by the bacterial chromosome and are resistant to clavulanic acid (9). OXA-10 and OXA-23 are just two examples of the many cephalosporins and carbapenems that class D β-lactams hydrolyze. Non-fermenting bacteria such as Pseudomonas aeruginosa and Acinetobacter baumannii belong to this class (10). Classes A, B, and D have a protected serine nucleophile in their active sites. These three classes use the same mechanism to hydrolyze and break β-lactam antibiotics (10). There are certainly several inhibitors that have been designed and synthesized to overcome antibiotic resistance caused by β-lactamase S (11).

In these conditions, a β-lactamase inhibitor is prescribed along with the corresponding antibiotic. Clavulanic acid, sulbactam, and tazobactam are three irreversible inhibitors that cause enzyme suicide by forming an inactive acyl-enzyme complex (1). Since 1995, a new series of ESBL class A bacteria called CTX-M enzymes has appeared in the world, and now they have become the most abundant ESBL enzymes. The sequence homology of CTX-M enzymes and TEM and SHV-type enzymes is less than 40%, and they contain more than 40 different amino acids. These enzymes have wide-spectrum hydrolyzing effects and can hydrolyze penicillin and 1st, 2nd, and 3rd generation cephalosporins (12, 13).

Studies have shown that most CTX-M β-lactamase enzymes expressed by *Escherichia coli* cause very high resistance to oxyiminocephalosporins (cefotaxime and ceftriaxone) and varying levels of resistance to cefepime and cefirom (14, 12, and 15). Today, techniques such as practical screening, nuclear magnetic resonance, X-ray crystallography, mass spectrometry, bioinformatics, mathematics, molecular modeling, and

molecular quantum mechanics are used to design and discover new and powerful inhibitors (16, 10, and 1). The process of producing a new drug is estimated to cost 2.6 billion dollars (17).

2. Material and methods

2.1. Computational methods

The use of computational biology and molecular modeling can be both valuable and effective in drug discovery and design. Virtual assessment is a technique based on searching and screening the desired compounds among huge structural libraries in virtual and computer environments. Considering the importance of identifying β-lactamase inhibitors in the treatment of infections, the importance of natural compounds, and the broad and valuable medicinal properties of quercetin, this study aims to investigate the inhibitory behavior of this compound inside the active site of β-lactamase type C of AmpC type using computational methods. Therefore, computational biology and molecular modeling can be used as valuable and effective methods in drug discovery and design.

2.2. Enzyme and Ligand structures selection

The PDB file of class C β-lactamase (4HEF) was downloaded from the RCSB protein data bank (21) and the 3-dimensional structure of quercetin was obtained from the PubChem database with CID C15H10O5 in sdf format and converted to pdb format using open-babel software (22).

2.3. Molecular docking

To study the interactions and binding affinity between quercetin and the AmpC β-lactamase enzymes, docking techniques were applied to evaluate them using Autodock 4.2.2 software (23, 24). Initially, the water molecules and co-crystal ligands existed in pdb files, with hydrogen atoms removed and Gasteiger charges added to the system to be prepared for docking (25, 26). Energy minimization of enzymes was performed utilizing the GROMACS 2019.6 package using the AMBER99SB force field (27). The active sites of the enzyme were determined by the co-crystal ligand reported in the PDB file of the enzyme, and then the grid box with dimensions of 60×60×60 points and a grid point spacing of 0.375 Å was selected. Finally, 200 docking calculations consisted of the 25 million energy evaluations performed using the Lamarckian genetic algorithm (LGA) method. In the end, the lowest binding energy conformation in the highest-populated cluster was selected as the best docking pose and used for further investigations.

2.4. Molecular dynamic simulation

MD simulation was utilized for the enzyme in free and in complex with quercetin form in a cubic box solvated by water tip3p model by the GROMACS 2019.6 program using the AMBER99SB force field (26). Force field parameters of Quercetin were generated by the Python-based ACPYPE tool (Antechamber Python Parser Interface) (25). An adequate number of Na+ or Cl-ions were added to neutralize system charges and achieve a physiological ion concentration of 0.15 M. In the first step, the steepest descent method is utilized for the energy minimization process. Then energy-minimized systems were equilibrated with 1ns simulations in nvt and not ensembles in 310 K and 1 bar.

After the systems were well equilibrated, an MD run was performed with a time step of 2 fs for 100 ns of simulation time. Further simulated trajectories were used to study the molecular structure of enzymes, ligands, and intermolecular interactions during the simulation time. To analyze the system, plots for root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), and solvent accessible surface area (SASA), along with hydrogen bond analysis, were generated.

3. Results and Discussion

Figure 1 represents the binding mode of quercetin in the active site of the AmpC β-lactamase enzyme and the interactions of this compound with the key residues. This figure exhibits the amino acids Ser90, Leu145, Gln148, Arg175, Tyr177, Glu299, Gly313, Asn314, Ser315, Met318, Ala319, Lys342, Thr343, Gly344, Asn373, Ser345 and Gly344 in the active site of the enzyme with pdb code 4HEF interact with the quercetin through van der Waals interactions. As seen in this figure, the carbonyl group of quercetin was found to interact with the amine and carboxyl groups of Ser345, Arg175, Gly313, and the amine group of Asn 373, respectively.

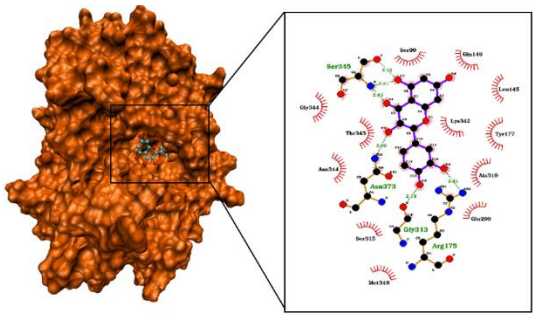


Fig. 1 shows the best docking pose and molecular interactions of the quercetin and the residues of the enzyme. The C, N, and O atoms are indicated in black, blue, and red, respectively. Hydrogen bonds are identified by green drops, and hydrophobic interactions are shown by red curves with spokes radiating 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

Table 1 represents the binding energies and inhibition constants of quercetin with the amp As shown in this table, the AmpC quercetin system has the lowest binding energy. The results indicate that quercetin has a high affinity for enzymes and can play an inhibitory role well, especially for AmpC β-lactamase.

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

System	ΔG binding (KCal/mol)	Ki (μM)
AmpC-Quercetin	-7.39	3.86

3.1. Molecular dynamic simulation Analysis of the root mean square deviation (RMSD)

RMSD analysis helps find the stability and structural variation of the free protein and protein-ligand systems. Figure 2 represents the RMSD of the free protein and its bond to the ligand. As shown in this figure, the AmpC enzyme has reached equilibrium at about 90 ns for free and 80 ns for bond systems. Table 2 shows the averages of the MD parameters for free and complex systems in the last 20 ns. According to Figure 2 and Table 2, quercetin binding causes changes in RMSD graphs and parameters of the AmpC enzyme, which signify stabilization by the interaction with quercetin.

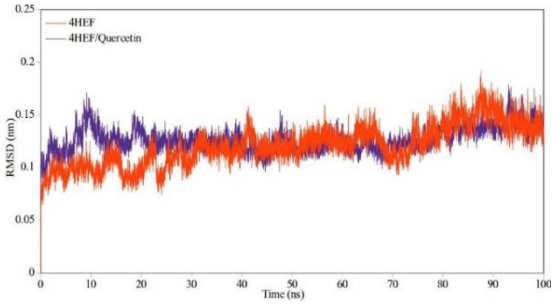


Fig. 2. RMSD plots of free and bound enzymes as a function of time

3.2. Analysis of the root mean square fluctuation (RMSF)

The analysis of RMSF was performed to understand the fluctuations and flexibility of each residue in different regions of the enzyme system in the free and bound states. Figure 3 demonstrates the RMSF of free protein and its bond to the ligand. According to this figure, for most of the amino acid residues except the limited regions, possibly related to random coils, the value of the RMSF is less than 0.32. Over the simulation time for most residues in the active sites, the RMSF value in the presence of quercetin has mildly diminished. According to Table 2, the mean RMSF value in the presence of quercetin for the enzyme has decreased, indicating that the bound state of the Ampc enzyme has a relatively lower conformational fluctuation than the free form of the enzyme.

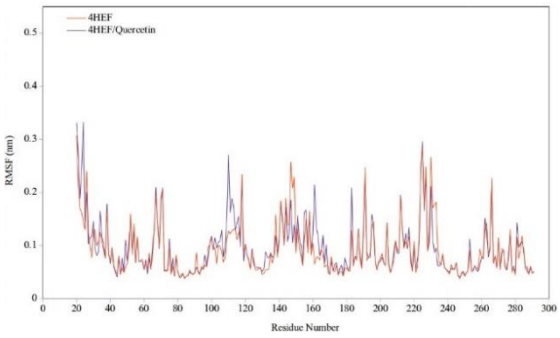


Fig. 3. RMSF plots of free and bound enzymes as a function of time.

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)
Free Ampc	0.139±0.026	1.996±0.006	0.093±0.049	155.525±2.292
Ampc-Quercetin	0.126±0.011	1.994±0.005	0.095±0.051	155.311±2.295

3.3. Analysis of the radius of gyration (Rg)

Rg calculation of a protein is helpful to assess the shape of the protein, its structural compactness during the simulation time, and its variation when complexed with ligand. Figure 4 exhibits the Rg of the free enzyme and the enzyme-quercetin complexes. According to this figure, the average amount of Rg during the first 80 ns of simulation time has increased due to binding with quercetin. The structure of the enzyme has been slightly compressed in the presence of quercetin. Moreover, the mean values of Rg are displayed in Table 2. According to Figure 4 and Table 2, the average amount of Rg during the last 20 ns of simulation time for AmpC has minimally increased due to binding with quercetin. This shows that in the presence of quercetin, the compression of the third structure of Ampc is reduced, and the structure of this enzyme is uncondensed.

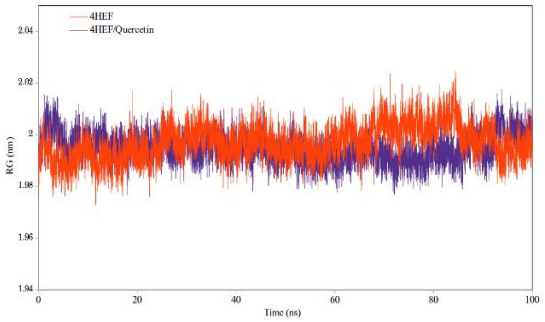


Fig. 4. RG plots of free and bound enzymes as a function of time

3.4. Analysis of the solvent accessible surface area (SASA)

Analysis of SASA helps evaluate the surface space of enzymes that is reachable to solvent molecules in each system over the simulation time. Figure 5 shows the SASA diagrams. Results indicate that the average amount of SASA for the enzyme has diminished due to the binding to quercetin due to contact of quercetin with the residue of the enzyme in a cavity on the surface of AmpC, which therefore reduces the available surface area of the enzyme for water molecules. According to Table 2, the average amount of SASA by binding Quercetin to AmpC possibly had not changed, which shows no alteration in the surface of the enzymes for water molecules as complexed with Quercetin.

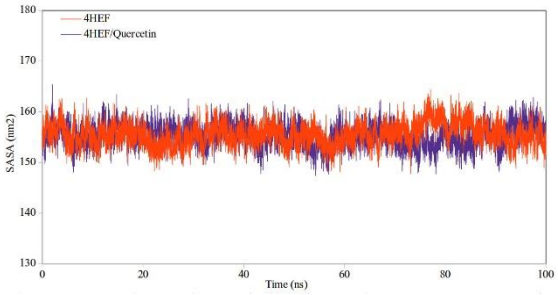


Fig. 5. SASA plots of free and bound enzymes as a function of time.

3.5. Analysis of the number of the hydrogen bonds

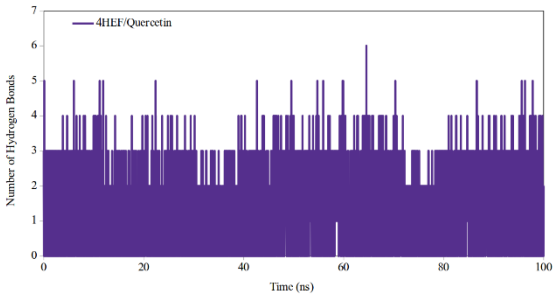
To calculate and understand the stability of the complexes the Exploring of the number of Hydrogen bonds between enzyme and ligand atoms are necessary and helpful. Figure 6

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 AmpC was six, which shows the stability of all complexes and shows that Quercetin 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. Table 3 provides the average number of enzyme-enzyme and enzyme-solvent hydrogen bonds for all systems during the last 20 ns. The results show that the average number of hydrogen bonds between enzyme atoms in the presence of quercetin has slightly increased, and the hydrogen bonds between the AmpC enzyme and the solvent molecules have decreased in the presence of quercetin.

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 Ampc	284.290 ±8.326	654.630±15.157
Ampc-Quercetin	288.467±8.588	650.101±14.737

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



Conclusions

Molecular docking and md simulation techniques were utilized to find the inhibitory effect of Quercetin on the AmpC β-lactamase class C enzyme, as docking results suggested

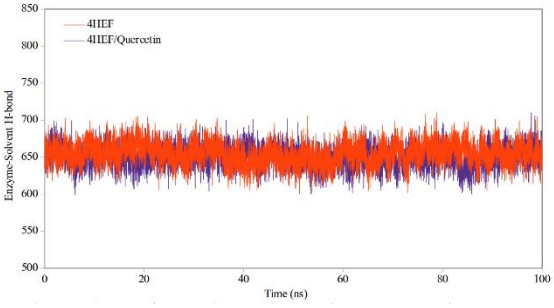


Fig. 7. Enzyme-enzyme hydrogen-bond plots of free and bound enzymes as a function of time.

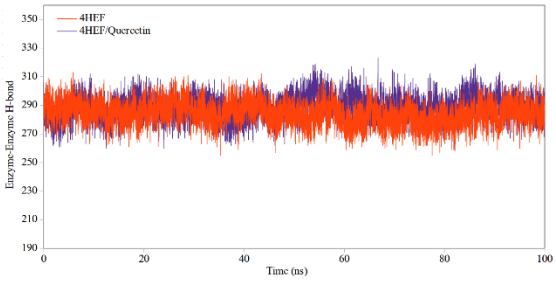


Fig. 8. Enzyme-solvent hydrogen bond plots of free and bound enzymes as a function of time.

that the interaction of Quercetin with AmpC showed an appreciable binding affinity and many intermolecular bonds. MD trajectory analyses (i.e., RMSD, RMSF, Rg, SASA, and hydrogen bonding) suggested that the Quercetin with AmpC docked complex was quite stable with minimal conformational changes. Analysis of the RMSD shows that in the presence of quercetin, the structures of AmpC become stabilized. Rg plots showed the compression of AmpC third structures when complexed with quercetin. Additionally, less conformational alteration is seen in the bound state of the enzyme than in the free form of the enzyme. The analysis of hydrogen bonds between the enzyme and quercetin confirmed the molecular docking results. Through MD simulation analysis, we have confirmed Quercetin's structural integrity, stability, and compactness, providing further evidence of their potential to target AmpC effectively. The findings of this paper may provide a new perspective to solve the problem of drug resistance caused by bacteria and help design new β-lactamase inhibitors in the future.

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