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Molecular docking and dynamics simulation of piperine as a potential inhibitor of class C beta-lactamase

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Abstract

Background: Antimicrobial resistance is a major concern of human being through the decades which are the cause of hundred thousand of death. β -lactamases secretion by bacteria is one of main resistant mechanism enzymes bacteria to fight antibiotics. Multiple investigation has performed to inhibit the β-lactamase enzyme activity which is one of the important ways to reduce microbial drug resistance and increase the effectiveness of antibiotics.

Methods: Molecular docking was performed to determine the binding pose and binding energy of class C beta lactamase with piperine using Autodock 4.2.2 software. Molecular dynamic simulation was carried out for enzyme utilizing GROMACS 2019.6 program applying AMBER99SB force field.

Results: Molecular docking results and interaction analysis of molecular dynamics simulations showed favorable hydrogen bonds and van der Waals interactions of Piperine with AmpC. The results of this paper may provide a new perspective to solve the problem of drug resistance caused by bacteria and help to design new beta-lactamase inhibitors in the future.

Conclusion: By using the valuable techniques of molecular docking and molecular dynamics simulation, this paper suggests that Piperine, which is the main component of black pepper and has significant medicinal effects, can be used to inhibit AmpC β -lactamase class C enzyme.

Key words: Molecular docking, Molecular dynamics, Simulation, Piperine, β-Lactamase Class C.

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Introduction

penicillin discovery in 1929 and its introduction to clinic in 1940, created a revolution in treating bacterial infection in which these infections cause lethal diseases and have large impact on public health(1). Antimicrobial drugs such as beta-lactams used in clinical medicine to treat bacterial infection despite it undergo continuous development since their introduction to improve their properties (2). Beta-lactam antibiotics react with target penicillin-binding protein that prevent formation of the peptidoglycan transpeptide Crosslink and finally lead to inhibition of bacterial cell wall synthesis (2). Due to extensive use of beta-lactam antibiotics, resistance against these antimicrobial drugs (Penicillin, Monobactams, Carbapenems, Cephalosporins and so on) emerged in which it is important to note that clinical resistance is often multifactorial and derived by a combination of multiple mechanism that includes β-Lactamase enzyme secretion, permeability modification or efflux pump upregulation(3). Antimicrobial resistance is a rapid growing issue with potentially devastating consequences. Primary mechanism of bacterial resistance is producing enzyme such as beta lactamase that hydrolyzed amid bonds of beta-lactam ring (4). B-Lactamase is divided into four classes; the active-site serine β-Lactamases (class C, C and D) and the Metallo beta lactamase or zinc dependent (MBL; class B). Class C are the abundant β-lactamases that include TEM, SHV, CTX-M, PC1 and KPC, B are associated with metallo-β-lactamase, C contains of CMY and GC1 enzyme and D includes OXA β -lactamases(5).

Along with emergence of antimicrobial resistance, using β-Lactamase inhibitor combined with beta-lactam antibiotics were a major strategy and interesting way for treating β-Lactamase mediated resistance, therefore multiple research and investigation have been conducted to use β-Lactamase Inhibitors with antibiotics such as amoxicillin-clavunate, ampicillin-sulbactam, piperacillin-tazobactam and have been found to be wide application as treatment of associated infection by Beta lactamase producing organism(6-9).

Natural products are the basis of obtaining the source of medicines that are used to treat different types of diseases (10). Recently, natural products have received a lot of attention from scholars and continue to increase during the last 20 years, many bases for maintaining and researching these products have been created and are increasing and more than 120 bases and collections of different natural products have been published since 2000 and have been widely used, 98 of which are still are available and 50 of them are in free access (10). The essence of medicines is natural products, which has been given special attention since the past, the majority of medicines are derived from natural products, the rest are derived from a combination of natural products, comparing the new information that was obtained in the years 1981 to 2007. It shows that half of the drugs approved since 1994 are based on natural products (11).

Medicinal plants have been playing an important and significant role in foods and spices for many years as *Piper nigrum*, which belongs to the *piperaceae* family, is one of the drugs that has significant use around the world in which it has pungent smell and taste, despite the other existing materials, piperine is still used more and more(12). Piperine has different pharmacological effects such as anti-cancer, anti-obesity, anti-microbial, anti-aging, heart protection, effects especially on the immune system, anti-virus, anti-diabetes, and other effects that have been the focus of scholars' attention. It also has the effects of liver protective, anti-allergenic, anti-inflammatory and neuroprotective properties (12-14).

Computational biology and molecular modeling methods are powerful techniques which known for their acceleration in research, cost and time effective in drug design and to identify new and more efficient biomolecules with potential inhibitory action against β-lactamase. This study was designed to assess piperine as a potential inhibitor of AmpC class C β-lactamase enzyme utilizing computational method including molecular docking and molecular dynamic (MD) simulation.

Materials and Methods

Enzyme and Piperine structure selection

The structures of class C β-lactamase with PDB code 4HEF, downloaded from RCSB protein data bank(15) and the 3D structure of Piperine with CID 638024 code was obtained from PubChem database in sdf format and converted to pdb format using open babel software(16).

Molecular docking

Docking technique was applied to evaluate the interactions and binding affinity between Piperine and the 4HEF β-lactamase enzyme utilizing Autodock 4.2.2 software (17). Enzyme structure initially prepared for docking by removing water molecules and co-crystal ligands existed in pdb files and hydrogen atoms with Gasteiger charges added to the system (18). Before docking procedures, energy minimization of enzyme performed utilizing GROMACS 2019.6 package using AMBER99SB force field (19). The active sites of enzyme were determined by co-crystal ligand reported in PDB file of enzyme and then the grid box with the 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 by using Lamarckian genetic algorithm (LGA) method were performed. Ultimately, the lowest binding energy conformation in the highest populated cluster was selected as the best docking pose and used for molecular dynamic simulation input files.

Molecular dynamic simulation

Investigation of complexes between Piperine and 4HEF carried out applying MD simulation technique with more details. MD simulation performed for the enzyme in the free form and in complex with Piperine

in a cubic box solvated by water tip3p model, by GROMACS 2019.6 program using the AMBER99SB force field executed on Kubuntu 2020.4 LINUX operating system (20). Force field parameters of Piperine were generated by Python based ACPYPE tool (AnteChamber Python Parser Interface) (19). Enough number of Na+ or Cl- ions were added to neutralize system charges and achieve to the physiological ion concentration of 0.15 M. In the first step steepest descend method utilized for 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, MD run was performed with a time step of 2 fs for 200 ns simulation time. Eventually simulated trajectories were used to study the molecular structure of enzyme, ligand and intermolecular interactions during the simulation time. For analysis purposes, 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 and analysed.

Results

The binding energies and the inhibition constants of Piperine with the selected enzyme is represented in Table 1. As can be seen in this table, that 4HEF-Piperine system shows the lowest binding energy. The results show that Piperine has a high affinity for enzyme and can play an inhibitory role well especially for AmpC β-lactamase.

The binding mode of Piperine in the active site of 4HEF β-lactamase enzyme and the interactions of this compound with the key residues represented in Figure 1. This figure exhibits the amino acids Ser90, Leu145, Gln146, Arg175, Met292, Glu299, Gly313, Asn314, Met318, Ala319, Lys342, Thr343, Gly344, Ser345 and Gly344 in the active site of the enzyme with pdb code 4HEF interact with the Piperine through van der Waals interactions. As seen in this figure hydroxyl group of Piperine formed two hydrogen bonds with amine group of Ser345 and amine group of Arg175.

Molecular dynamic simulation

Analysis of the root mean square deviation (RMSD)

Analysis of the RMSD is helpful in understanding the stability of the free protein and protein–ligand systems. RMSD calculations of the simulated enzyme and enzyme–ligand complexes were done to find the structural variation and presented in Figure 2. According to this figure , the 4HEF enzyme has been reached to equilibrium in about 70 ns for both free and bond systems.

Analysis of the root mean square fluctuation (RMSF)

The RMSF analysis was conducted on system in the free and bound states to understand the fluctuations as well as the flexibility of each residue in different regions of the enzyme. The RMSF results of understudy system exhibited in Figure 3, 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 0.3. For enzyme over the simulation time for most residues in the active sites the RMSF value in the presence of the Piperine has decreased slightly.

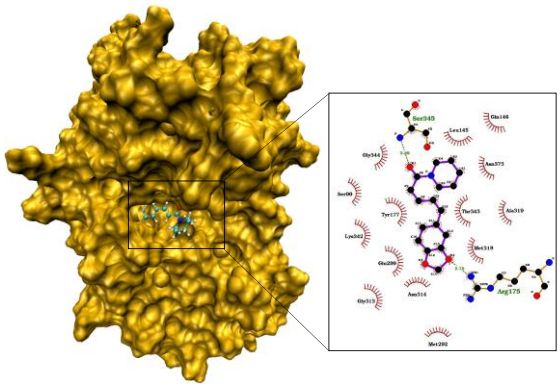

Fig. 1 The best docking modes and molecular interactions between the Piperine and the residues of the enzyme. The C, N and O atoms are indicated in black, blue and red respectively. Hydrogen bonds are identified by green drops and hydrophobic interactions are shown by red curves with spokes radiating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

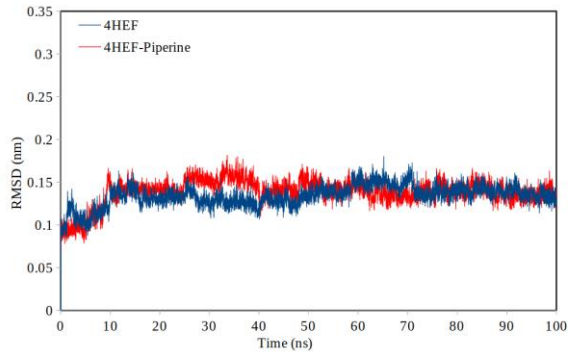

Fig. 2. RMSD plots of free and bound enzyme during as a function of time

Analysis of the radius of gyration (Rg)

Calculating and examining the Rg of a protein is useful to measure the shape of

the protein and its variation when complexed with ligand. The Rg of the free enzyme and the enzyme–Piperine complexes were calculated to assess their structural compactness during the simulation time and shown in Figure 4. According to this figure, the average amount of Rg during the first 80 ns simulation time has increased due to binding with Piperine. This shows that in the presence of Piperine, the structures enzyme has slightly compressed in the presence of Piperine.

Analysis of the solvent accessible surface area (SASA)

SASA plot generated to evaluate the surface space of enzyme that is reachable to solvent molecules in each system over the simulation time. Figure 5 shows the SASA diagrams. All results show that the average amount of SASA for enzyme has decreased due to the binding to Piperine. This is due to the fact that Piperine in a cavity on the surface AmpC enzyme is in contact with some amino acids of the enzyme and therefore reduces the available surface of the enzyme for water molecules.

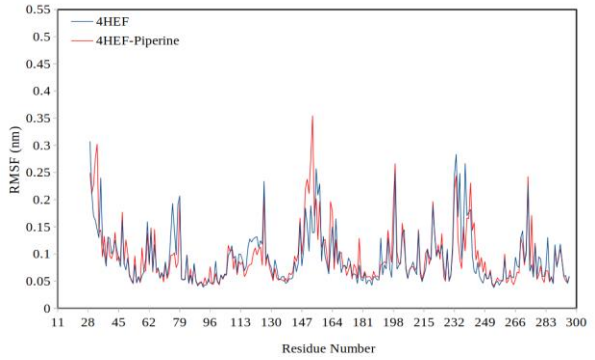

Fig. 3. RMSF plots of free and bound enzyme during as a function of time.

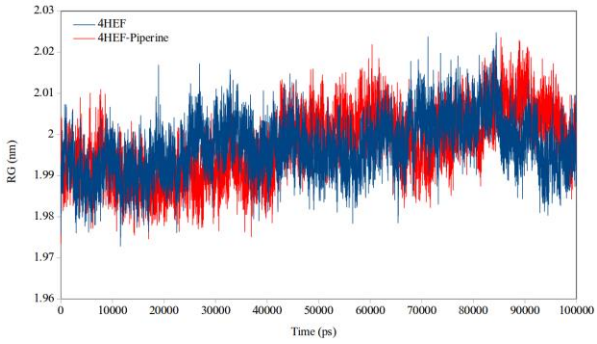

Fig. 4. RG plots of free and bound enzyme during as a function of time

Analysis of the number of the hydrogen bonds

Exploring of the number of hydrogen bonds between enzyme and ligand atoms is helpful to evaluate the stability of the complexes. Figure 6 represents the number of hydrogen bonds between Piperine and the enzyme over the 100 ns simulation time. During the simulation time, maximum number of hydrogen bonds formed between Piperine and AmpC were two which shows the stability of all complexes, which showed that Piperine has the highest binding tendency to this enzyme.

Figure 7 and 8 represent the Enzyme-Enzyme and Enzyme-Solvent hydrogen bonds for free and bound enzyme during the simulation time, respectively.

The results show that the average number of hydrogen bonds between enzyme atoms in the presence of Piperine has slightly increased, the hydrogen bonds between 4HEF enzyme and the solvent molecules decreased in the presence of Piperine.

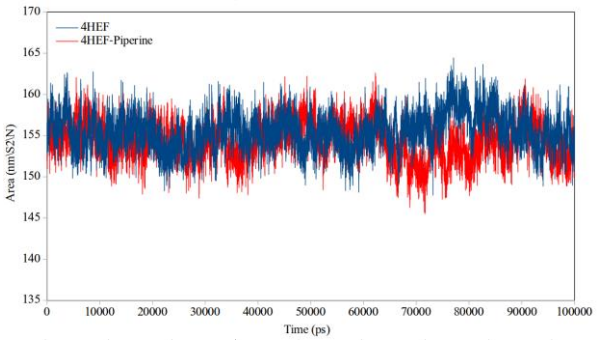

Fig. 5. SASA plots of free and bound enzyme during as a function of time.

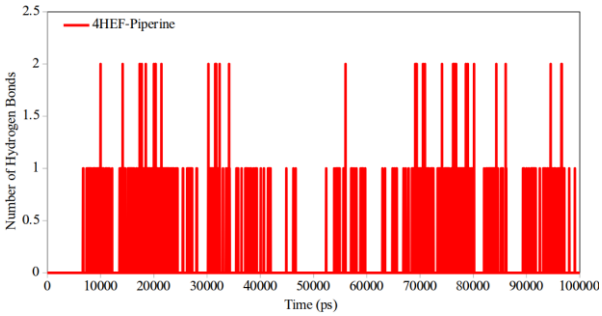

Fig. 6. Time dependence of the number of hydrogen bonds between Piperine and enzyme during the simulation time

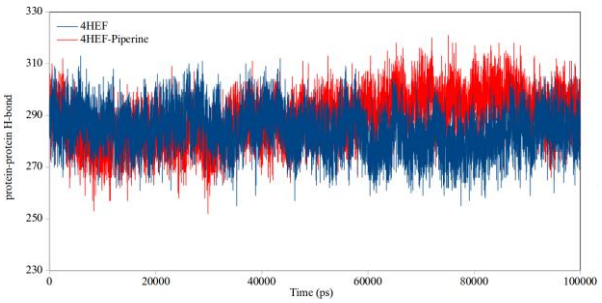

Fig. 7. protein-protein hydrogen-bond plots of free and bound enzyme during as a function of time.

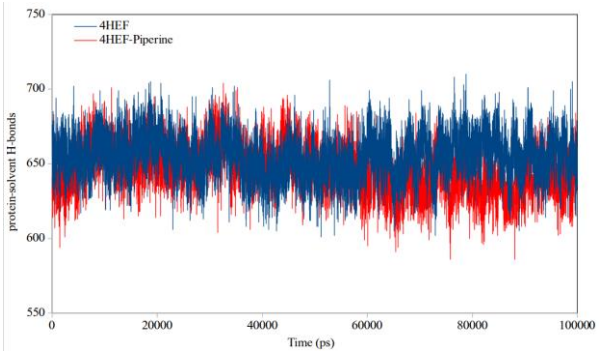

Fig. 8. protein-Solvent hydrogen bond plots of free and bound enzyme during as a function of time.

Conclusions

The possible binding of Piperine with AmpC was explored to study the inhibitory effect of the Piperine on AmpC β-lactamase class C enzyme by utilizing molecular docking, MD simulation. Docking results indicated that the binding of Piperine with AmpC showed an appreciable binding affinity and many intermolecular interactions. MD trajectory analyses (i.e., RMSD, RMSF, Rg, SASA, and hydrogen bonding) suggested that the Piperine with AmpC docked complex was quite stable with minimal conformational alterations. Analysis of the RMSD shows that in the presence of the Piperine the structures of AmpC become stabilized. Rg plots indicated that due to binding with Piperine the third structures of AmpC become a slightly compressed. Moreover, bound state of enzyme had a relatively less conformational fluctuation than the free form of enzyme. Analysis of hydrogen bonds between enzyme and Piperine confirmed the molecular docking results. The results contribute in many ways to our knowledge and provide a base for setting up an experimental platform of Piperine interactions with AmpC which could be used as a guide for future experimental studies.

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

System	ΔG binding (KCal/mol)	Ki (μM)
4HEF-Piperine	-7.57	2.81

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