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Novel mutant of *Escherichia coli* asparaginase II to reduction of the glutaminase activity in treatment of acute lymphocytic leukemia by molecular dynamics simulations and QM-MM studies

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ABSTRACT

L-Asparaginases (ASNase) belong to a family of amidohydrolases, have both asparaginase and glutaminase activity. Acute lymphocytic leukemia (ALL) is an outrageous disease worldwide. Bacterial ASNase has been used for the treatment of ALL. Glutaminase activity of enzyme causes some side effect and it is not essential for anticancer activity. The aim of this study was engineering of *Escherichia coli* asparaginase II to find a mutant with reduced glutaminase activity by molecular docking, molecular dynamics (MD) and QM-MM (Quantum mechanics molecular dynamics) simulations. Residues with low free energy of binding to Asn and high free binding energy to Gln were chosen for mutagenesis. Then, a mutant with higher glutaminase free binding energy was selected for further studies. Additionally, the MD simulation and QM-MM computation of wild type (WT) were employed and the selected mutated ASNase were analyzed and discussed. Our data showed that V27T is a good candidate to reduction the glutaminase activity, while has no remarkable effect on asparaginase activity of the enzyme. The simulation analysis revealed that V27T mutant is more stable than WT and mutant simulation was successful completely. QM-MM results confirmed the successfulness of our mutagenesis.

Introduction

L-Asparaginase (with Enzyme Commission number 3.5.1.1) catalyze the conversion of L-asparagine (L-Asn) to L-aspartate and ammonia [1]. In the four last decades, ASNase used as an enzymatic drug and chemotherapy complementary to the treatment of disease such as acute lymphoblastic leukemia (ALL), Hodgkin's disease, reticulosarcoma, chronic lymphocytic leukemia, acute myelomonocytic leukemia, acute myelogenous leukemia, lymphosarcoma and melanosarcoma [2]. Its medical importance causes the ASNase market value as it donates nearby 40% of total worldwide enzyme sale [3]. ASNase is an intracellular enzyme that obtained from microorganisms, animal and plants. ASNase extracted from *Escherichia coli* and *Erwinia carotovora* used in medicine for the treatment of ALL [4]. The *E.coli* ASNase has a higher affinity for Asn compared to others [5]. ALL is the most common childhood cancer and since the treatments are in progress, survival rates for children diagnosed with ALL now account for over 90% [6] But treatment in infants (children < 12 months) and adults need improvement [7]. ALL comprises less than 1% of adult's cancers [8]. The discovery of asparaginase as an anticancer drug began in 1953 by Kidd, then Broome (1963) found that lymphomas in rat and mice returned to their previous condition after treatment with guinea pig serum [9,10]. This drug depletes L-Asn in the serum and takes away some tumor cells of the required amino acid. Results of this process are quick inhibition of protein synthesis and postponed inhibition of DNA and RNA synthesis. Therefore, certain tumor cells function, especially lymphocytes that lack L-asparagine synthetase, are impaired resulting in apoptosis and cytoprotective autophagy [11,12].

Among the agents used in treatment for ALL, ASNase is one of the most effective with comparatively mild side effects [13]. Nevertheless, Some of ASNase side effects includes hypersensitivity, coagulation abnormalities, impaired liver function, pancreatitis and neurological dysfunctions prevent their clinical application [14]. Apart from hypersensitivity, all side effects are mostly associated with L-glutaminase (GLNase) activity of the enzyme [15–17]. Unfortunately, many ASNase also hydrolyzes L-glutamine (L-Gln) to L-glutamic acid (up to 9%), but to a much lower extent than Asn. Asparaginases showed other limitations including insufficient serum half-life, low trypsin tolerance and

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causing the producing of anti-asparaginase antibodies. Because of these limitations, most of the treatment has been interrupted [18]. There is two way to improve the function and also to reduce the side effects of the enzyme. The first approach is to find microorganisms that producing ASNase with less glutaminase activity and the second is the modification of current enzyme by protein engineering. Chan et al., (2014) reported that leukemic cells that lack asparagine synthetase were more sensitive to L-asparaginase treatment without glutaminase activity (Q59L mutant) [19]. There are some limitations to the evaluation of protein properties just by experimental protein engineering. These limitations including high cost, low efficiency, time-consuming and start work without assurance of getting the result. Computational methods can be used to overcome some of these limitations. The most widely used computational method is MD, which is used to study of equilibration systems and dynamic interactions of proteins. MD is the most widely used computational technique to study the equilibration structures and dynamic interactions of biological systems [20]. Since 2007, QM-MM methods are widely applied to enzymes. Over 100 paper have been published every year [21]. Here, we report an *Escherichia coli* ASNase mutant by MD simulations of that enzyme with less glutaminase activity and no change in asparaginase activity (based on molecular dynamics simulation). Residues that interact with Gln over Asn, which are not essential for catalytic activity, were chosen for mutagenesis. Finally, mutant and WT ASNases were elevated with MD simulations and QM-MM studies.

Methods

Preparation of structures and molecular docking studies

The crystal structure of *E. coli* II ASNase (PDB ID 1NNS) was employed for our molecular modeling simulations [22]. Water molecules and non-polar hydrogen atoms were removed, and atom charges were assigned with Gasteiger-Marsili method [23]. Before starting the docking process, the 3D structure of ASNase was introducing a 500 cycle of steepest descent method-based minimization to relax any closed and unreal contact [24]. For Molecular docking of substrates (asparagine, glutamine) into the active site of ASNase, AutoDock Vina was utilized. This software is a molecular docking software and is free for the academic user. AutoDock Vina operates based on empirical scoring functions and is able automatically to calculate the grid maps in the pre-defined docking box [25]. The crystal structure of ASNase had an aspartic acid as a ligand and hence, its surrounding residues were allocated as the binding site [22]. The gid spacing parameter of AutoDock Vina was changed from 0.375 Å to 1 Å. All remaining parameters were kept at their default values. For each substrate, the pose with the minimum binding energy was picked up for MD studies.

MD simulations of ASNase WT

All MD runs were performed using GROMACS 5.1 package with AMBER 99SB force field [24]. PROPKA 2.0 server was employed to estimate the correct ionization states of ASNase ionizable groups [26]. The required files of substrates including partial charge and topology files were produced by ACPYPE software, which is a tool on the basis of AnteChamber package [27]. MD simulation for each substrate was done, separately. Each ASNase:substrate complex was simulated under the periodic boundary condition (PBC) in a cubic box and TIP3P water model [28]. The surface of solvated ASNase was covered with a water shell of 10 Å. As it is necessary the neutralization of each biologic system for MD simulation, the ASNase:substrate complex in both WT and V27T mutant was neutralized with three sodium ions. As mentioned in the docking method, the steepest descent algorithm was occupied to minimize each system to achieve a maximum force of less than 1000 kJ mol^{-1} nm^{-1} on each atom. Then, each system was equilibrated under 100 ps of NVT and 100 ps of NPT ensemble. In the NVT, the constant number of particles, volume, and the temperature is assigned whereas in NPT, the MD is conducted with constant number of particles, pressure and temperature. In the following, the 20 ns MD simulations with a time step of 2 fs at constant 300 k and 1 bar for ASNase:substrate complexes were done. In our MD simulation, to constrain the bond length and calculation of long-range electrostatics interaction, the LINCS algorithm and Particle Mesh Ewald (PME) method and were employed, respectively [29,30]. The produced trajectories from MD simulations were examined and analyzed utilizing visual molecular dynamics software (VMD), along with the standard tools implemented in the GROMACS software [31].

Binding free energy computation of WT ASNase:substrates

It has been shown that Molecular Mechanics–Poisson Boltzmann Surface Area (MM-PBSA) is a robust and widely used method to calculate the binding free energy between protein and substrate in biomolecular complexes [32]. In the MM-PBSA algorithm, the binding energy is computed based on the following formula:

$$\Delta G_{bind} = G_{complex} - G_{receptor} - G_{ligand} = \Delta E_{MM} + \Delta G_{sol} - T\Delta S$$

$$\Delta E_{MM} = \Delta E_{bonded} + \Delta E_{nonbonded} = \Delta E_{bonded} + (\Delta E_{vdw} + \Delta E_{ele})$$

$$\Delta G_{sol} = \Delta G_{polar} + \Delta G_{nonpolar}$$

The nonbonded interaction ($E_{nonbonded}$) is comprised of Van der Waals (E_{vdw}) and electrostatic interaction (E_{ele}), which could be estimated from molecular mechanics method (MM). The bonded interaction is comprised of four essential interactions: bond, angle, dihedral and improper interactions [32,33]. The summation of polar and nonpolar solvation free energies constitutes the free energy of solvation (G_{sol}). This type of energy can be computed with the Poisson–Boltzmann (PB) equation and the solvent-accessible surface area (SASA). The term T△S demonstrates the changing of conformational entropy due to substrate binding. Since, the calculation of T△S is accompanied by high computational cost and low prediction accuracy; it is usually neglected in the computation of the binding free energy. So, the final formula for calculation of the binding free energy is:

$$\Delta G_{bind} = \Delta G_{vdw} + \Delta E_{ele} + \Delta G_{polar} + \Delta G_{nonpolar}$$

We used MM-PBSA for estimation of binding free energy between ASNase and its relative substrate (Gln and Asn) for each frame of MD simulation. Finally, the residues with the high free energy of binding to Asn and low free binding energy to Gln were chosen to build mutant.

QM-MM study on WT

The density functional theory (DFT) calculations were carried out using jaguar implemented in Schrodinger's Maestro Molecular modeling software. Snapshot created by GROMACS was used as input file in QM-MM calculations. WT and V27T ASNase were minimized and optimized with DFT using Becke's three parameters exchange potential and Lee-Yang-Parr correlation functional (B3LYP) gradient corrected exchange correlation functional together with 6–31 G^* basis set [34,35]. OPLS 2005 force field was used in the MM part of QM-MM studies. Highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO) and energy gaps were computed.

Substrates docking, MD, binding free energy computation, QM-MM on mutant

Mutations of interest were built into the crystal structure. Mutants were prepared like WT and substrates docked into the active site. MD simulation, binding free energy and QM-MM computation were done in the same manner of WT.