Interaction of β-Lactoglobulin with Resveratrol: Molecular Docking and Molecular Dynamics Simulation Studies

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In this work, the interaction of *trans*-resveratrol, as a natural polyphenolic compound, and Bovine β-lactoglobulin (BLG), was studied using molecular docking and molecular dynamics simulation methods. The molecular dynamics study makes an important contribution to understanding the effect of the binding of resveratrol on conformational changes of BLG and the stability of a protein-drug complex system in aqueous solution. Molecular docking studies revealed that the resveratrol was bound to the surface of the protein by two hydrogen bond interactions. The binding constant and free energy change, ΔG°, for the binding of resveratrol to BLG were about 6.6×10^5 mol L^{-1} and –33.4 kJ mol^{-1}, respectively. Furthermore, the results of molecular dynamics simulation represented that the rmsd of unliganded BLG and BLG-resveratrol complex reached equilibration and oscillated around the average value after 600 ps simulation time. The study of the radius of gyration (R_g) revealed that BLG and BLG-resveratrol complexes were stabilized around 1500 ps and also exhibited no conformational change. Finally, analyzing the rms fluctuations suggested that the structure of the ligand binding site remains approximately rigid during the simulation.

Key words:

Bovine beta lactoglobulin, resveratrol, molecular docking, binding, molecular dynamics simulation

Introduction

Resveratrol (3,5,4′-trihydroxystilbene) (Fig. 1) was first isolated in 1940 as an ingredient of the roots of white hellebore (Veratrum grandiflorum O. Loes) and has since been found in about 70 plant species, including grapes, mulberries and peanuts.[1] It can also be found in food products and beverages such as peanut butter, red wine and grape juice.[2,3] It provides cardioprotection in red wine through multiple routes like antioxidant action,[4] activating NO production,[5] inhibiting low-density lipoprotein,[6] hindering platelet aggregation,[7] and promoting anti-inflammatory effects.[4] Resveratrol protects the heart at a relatively low concentration (about 2.5 to 10 mg kg^{-1} doses).[8] In contrast, resveratrol destroys cancer cells at relatively higher doses.[8–11] For example, it causes death of cancer cells by apoptosis at 100–1000 mg kg^{-1} doses. Resveratrol exists as *trans*- and *cis*-isomers. Most of its biological activities are attributed to the *trans*-isomer. Although a few biological activities of *cis*-resveratrol have been reported, which include the inhibition of colla-

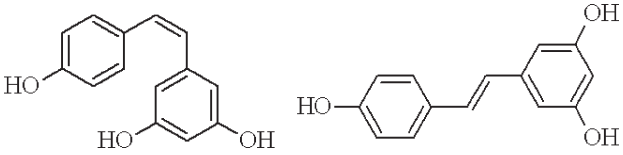

Fig. 1 – *Chemical structures of cis-resveratrol, (left) and trans-resveratrol (right)*

gen-induced platelet aggregation and kinase activity related to cancer,[7,12] it is unclear whether *cis*-resveratrol might extensively exhibit biological activities comparable to those of the *trans*-isomer. In solution, *trans*-resveratrol converts to its *cis*-isomers under the exposure to light.[13,14] The stability of *trans*-resveratrol, which ranges from hours to several days, depends on the pH of the solution.[13] It may form complexes with natural and modified cyclodextrins, increasing both its stability and water solubility.[14] Due to the low water solubility of resveratrol, it must be bound to protein or conjugated to remain at a high concentration in serum.[15]

Bovine β-lactoglobulin (BLG) has been one of the most extensively studied proteins in the history of protein science.[16–19] The major reason for this is simply its abundance in cow's milk; the concentra-

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tion of BLG in milk is about 0.2 g/100 mL following casein (2.9 g/100 mL),[20] which makes BLG easily accessible for researchers. BLG is a globular protein with a monomer molecular weight of about 18300 Da. This small globular protein has a three-dimensional structure consisting of eight strands of antiparallel β-sheet twisted into a cone-shaped barrel that constitutes a hydrophobic pocket.[19] BLG is known for binding a great variety of hydrophobic ligands, such as fatty acids, retinoids and sodium dodecyl sulfate (SDS).[21] As reviewed by Sawyer et al.,[22] it has been determined from crystallographic studies (X-ray diffraction) that the majority of these hydrophobic ligands bind within the central cavity of BLG in the pH range of 6.0 and 8.1. Moreover, the nuclear magnetic resonance (NMR) spectra of BLG solutions obtained[23] have revealed that at neutral pH palmitate (PA) is bound within the central cavity of the protein, while, at pH lower than 6.0, PA starts to be released. Like retinol binding protein (RBP), BLG is able to bind a wide variety of hydrophobic molecules such as retinoids, alkenes and fatty acids.[18,24] Binding to BLG provides protection for retinol and β-carotene from degradation due to heat, oxidation and irradiation.[25] It has been proposed therefore that BLG could be used as a versatile carrier of hydrophobic molecules in the controlled delivery applications.[26] The binding constants for different compounds with BLG vary widely from as little as 1.5×10^2 mol L^{-1} for 2-heptanone to 6.8×10^5 mol L^{-1} for palmitate and 5×10^7 mol L^{-1} for retinol.[27] It has been shown that there are no considerable changes in retinol binding properties of BLG in the presence of various amounts of sodium n-dodecyl sulfate (SDS) and Triton X-100 [28]. The interaction of dodecyltrimethylammonium bromide (C_{12}TAB) with BLG has also been investigated at various pH levels that represent increase of the binding strength of BLG/C_{12}TAB complex with increasing the pH, although C_{12}TAB binding has no significant effect on the retinol binding affinity of BLG.[29] It has been shown recently that BLG binds polyphenolic compounds like resveratrol from grapes[30] and resveratrol interacts with BLG in order to form 1 : 1 complexes. The binding constant for the resveratrol-BLG interaction is between 10^4 and 10^6 mol L^{-1} as determined by protein or polyphenol fluorescence. The BLG-resveratrol interaction may compete with the self-association of both polyphenol and protein. It has no apparent influence on the BLG secondary structure but it partially disrupts the tertiary structure. Complexing with BLG provides a slight increase in the photostability of resveratrol and a significant increase in its hydrosolubility.

Considering the fact that resveratrol exhibits many physiological effects associated with health benefits, it is important to understand the interactions of this compound with a major carrier protein such as BLG. In the present work, the interaction of BLG with resveratrol was studied using molecular dynamics (MD) simulation and molecular docking studies. Molecular dynamics studies have made an important contribution to understanding the effect of the binding of resveratrol on the conformational changes of BLG and the stability of a protein-ligand complex system in aqueous solution.

Methods

Molecular modeling and docking

The molecular docking program ArgusLab 4.0.1[31] was employed for generating an ensemble of docked conformations. Earlier ArgusLab 4.0.1 validation studies have reported very little difference in the docking accuracies between ArgusLab and Genetic optimization for ligand docking (GOLD).[31–33] Flexible ligand docking of ArgusLab is available by describing the ligand as a torsion tree. Groups of bonded atoms that have no rotatable bonds are nodes, while torsions are connections between the nodes. Topology of a torsion tree is a determinative factor influencing efficient docking. In the docking calculations, the scoring method Ascore from the ArgusLab 4.0.1 suite is employed. Ascore is based on the decomposition of the total protein–ligand binding free energy, taking into account the following contributions: the van der Waals interaction between the ligand and the protein, the hydrophobic effect, the hydrogen bonding between the ligand and the protein, the hydrogen bonding involving charged donor and/or acceptor groups, the deformation effect, and the effects of the translational and rotational entropy loss in the binding process, respectively.[31,32]

Preparation of the protein and the ligand

Experimental results showed that the resveratrol binds to unusual binding site on the surface of BLG.[30] Therefore, we have to use an apo-protein (unliganded protein) for docking studies. The known crystal structure of BLG (PDB ID: 3NPO) was obtained from the RCSB Protein Data Bank and the water molecules were removed. In order to obtain the most stable conformations of *trans*-resveratrol, the structure-optimizing calculations were carried out at 6–31G** level by employing the Becke three-parameter Lee–Yang–Parr (B3LYP) hybrid density functional theory using the GAMESS quantum chemistry software.[39] The ArgusDock docking engine, implemented in ArgusLab 4.0, approximated an exhaustive search method with similarities to DOCK and Glide. ArgusDock exhaustive

search docking engine with grid resolution of 0.40 Å was used. Docking precision was set to regular precision, and flexible ligand docking mode was employed for docking run. To recognize the binding sites in BLG, blind docking was carried out and the grid size was set to 146 Å, 156 Å, and 132 Å along the *X*, *Y*, and *Z* axes, respectively. During docking, a maximum number of 10 conformers were considered. The lowest energy conformation was used for further analysis (Table 1). Equation 1 was used to convert the ΔG° to *K*.

$$\Delta G^\circ = -RT \ln K \quad (1)$$

Table 1 – *Docking summary of BLG with resveratrol by the ArgusLab program.*

Conformation	$\Delta G°$/kJ mol^{-1}	K_a/mol L^{-1}
1	**–33,4**	**6.6×10^5**
2	–32.6	4.8×10^5
3	–30.1	2.3×10^5
4	–29.5	1.3×10^5
5	–28.9	1.1×10^5
6	–28.0	7.6×10^4
7	–26.8	4.6×10^4
8	–25.0	2.2×10^4
9	–24.3	1.6×10^4
10	–22.3	7.6×10^3

MD simulations

A 6000 ps MD simulation of the BLG-resveratrol complex was carried out with the GROMACS 4.0 [34,35] package using the GROMOS96 43a1 force field.[36,37] The initial conformation was taken from the docking result. The topology parameters of BLG were created using the Gromacs program. The topology parameters of resveratrol were built by the Dundee PRODRG2.5 server (beta).[38] The partial atomic charges of resveratrol were subsequently determined by using the GAMESS[39] at the level of HF/6–31G**. Then, the complex was immersed in a cubic box (6.65049 × 6.65049 × 6.65049 nm^3) of extended simple point charge (SPC) water molecules.[40] The solvated system was neutralized by adding sodium ions to the simulation and the entire system was composed of 1594 atoms of BLG, one resveratrol, 8 Na^+ counterions and 26709 solvent atoms. To release conflicting contacts, energy minimization was performed using the steepest descent method of 2000 steps, followed by the conjugate gradient method for 2000 steps. The MD simulation study consisted of equilibration and production phases. In the first stage of equilibration, the solute (protein, counterion and resveratrol) was fixed and the position-restrained dynamics simulation of the system, in which the atom positions of BLG restrained at 300 K for 40 ps, proceeded. Finally, the full system was subjected to 6000 ps MD at 300 K temperature and 1 bar pressure. The periodic boundary condition was used and the motion equations were integrated by applying the leaf-frog algorithm with a time step of 2 fs. The atomic coordinates were recorded every 0.5 ps during the simulation for the latter analysis. The MD simulation and the results analysis were performed on the openSUSE Linux cluster with 8 nodes, the Isfahan University of Technology, Iran.

Results and discussion

Molecular docking studies

BLG consists of a single polypeptide chain of 162 amino acid residues and has a three-dimensional structure consisting of one α-helix and nine anti-parallel β-strands with eight β-sheets folded into a cone-shaped barrel forming a hydrophobic pocket.[19] Three potential binding sites have been reported for ligand binding to BLG: the internal cavity of the β-barrel, the surface hydrophobic pocket in a groove between the α-helix and the β-barrel and the outer surface near Trp19-Arg124.[41] Polar aromatic compounds, such as p-nitrophenyl phosphate, 5-fluorocytosine, ellipticine and protoporphyrin, bind to this outer surface site.[22,42]

In this study, the molecular docking program ArgusLab 4.0.1 program was chosen for examining the binding mode of resveratrol at the active site of BLG. During docking, a maximum number of 10 conformers were considered. The lowest energy conformation was used for further analysis. The docking results showed that resveratrol binds to the surface of protein by two hydrogen bond interactions. This observation is consistent with the previously reported experimental results.[30] Trp(19), Lys(100), Lys(101), Arg(124) and Glu(127) are near amino acids for resveratrol (Fig. 2). There is one hydrogen bond between resveratrol and Glu(127) BLG with the distance of 1.8 Å. OH (4') group of an aromatic ring of the ligand hydrogen bonded with a carbonyl group of Glu(127). Where the OH group is a hydrogen bond donor and Glu(127) is hydrogen bond acceptor. Also, there is another hydrogen bond between resveratrol and Lys(100) BLG with the hydrogen bond distance of 2.1 Å. OH (3) group of an aromatic ring of the ligand hydrogen bonded with a amino group of Lys(100). Where the OH group is a hydrogen bond donor and Lys(100) is hydrogen bond acceptor.

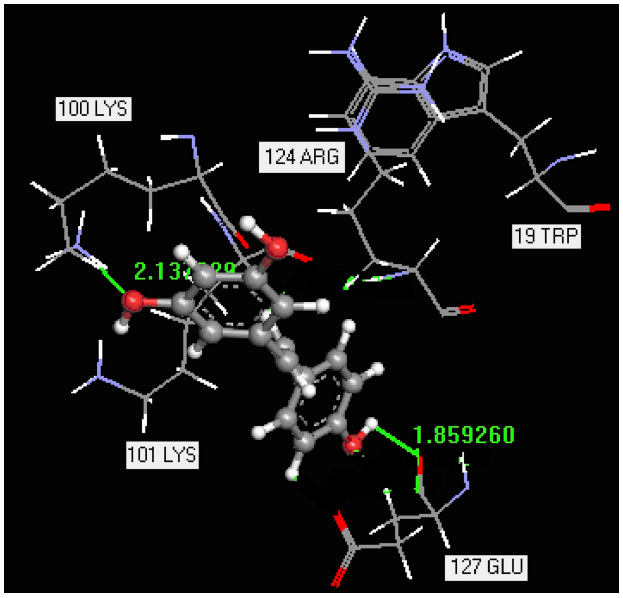

Fig. 2 – *The docking poses of the BLG-resveratrol complex. Resveratrol is rendered as sticksTwo H-bonds (as highlighted by the line in green colors) formed between resveratrol and BLG*

The binding constant and free energy change, $\Delta G°$, for the binding of resveratrol to BLG were about 6.6×10^5 mol L^{-1} and –33.4 kJ mol^{-1}, respectively. These data are very similar to the experimental results.[30] They suggested that resveratrol possibly binds to the surface of BLG with the binding constant value of 10^4 to 10^6 mol L^{-1}. Also, in their results, the hydrophobic interactions had no dominant role in the binding of resveratrol to BLG. The results of molecular docking indicate that the interaction between resveratrol and BLG are dominated by hydrogen bonds.

Analysis of the dynamics trajectories

To investigate the stability of the system, (protein, ligand, water, ions, etc.) properties were examined by means of rms deviations (rmsd's) of protein and resveratrol with respect to the initial structure, rms fluctuations (rmsf's) and the radius of gyration (R_g) of protein. In addition, the stability of the system proved the credibility of the docking result (Fig. 2), where resveratrol bound to the surface of BLG near Trp19-Arg124 was used for MD simulations.

The rmsd values of atoms in unliganded BLG and BLG-resveratrol complexes were plotted from 0 to 6000 ps as shown in Fig. 3. Analysis of Fig. 3 indicates that the rmsd of both systems reaches equilibration and oscillates around in the average value after 600 ps simulation time. The rmsd values of atoms in BLG and BLG-resveratrol complexes were calculated from a 600–6000 ps trajectory, where the data points were fluctuated for BLG,

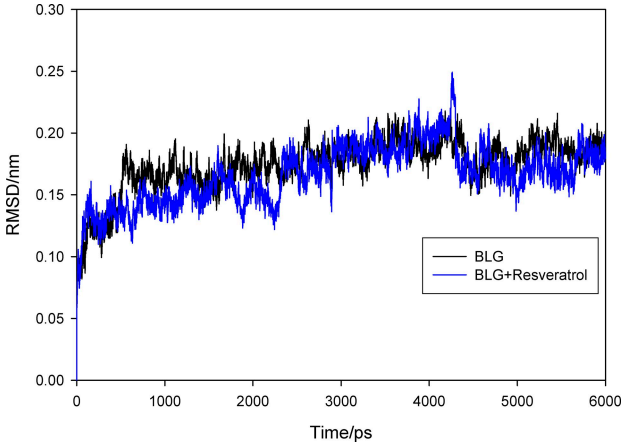

Fig. 3 – *Time dependence of rmsd's. Rmsd values for unliganded BLG and BLG-resveratrol complex during 6000 ps MD simulation using GROMACS 4.0 package and the GROMOS96 43a1 force field*

0.18 ± 0.014 nm and BLG-resveratrol, 0.17 ± 0.024 nm, respectively.

In the present MD study, the radius of gyration (R_g) values of unliganded BLG and BLG-resveratrol complex was determined as shown in Fig. 4. In both systems, R_g values were stabilized at about 1500 ps, indicating that the MD simulation achieved equilibrium after 1500 ps. Initially, the R_g values of both unliganded BLG and BLG-resveratrol complex were 1.44 nm. The unliganded BLG and BLG-resveratrol were stabilized at 1.40 ± 0.008 and 1.41 ± 0.008 nm, respectively (Fig. 4). The earlier report showing that the R_g value of BLG was experimentally 1.39 ± 0.04 nm, indicated that the present MD simulations matched the experimental values reported earlier.[43] The above results suggest that the radius of gyration value does not change tangibly upon the resveratrol complexation with respect to free BLG. This clearly indicates that the resveratrol

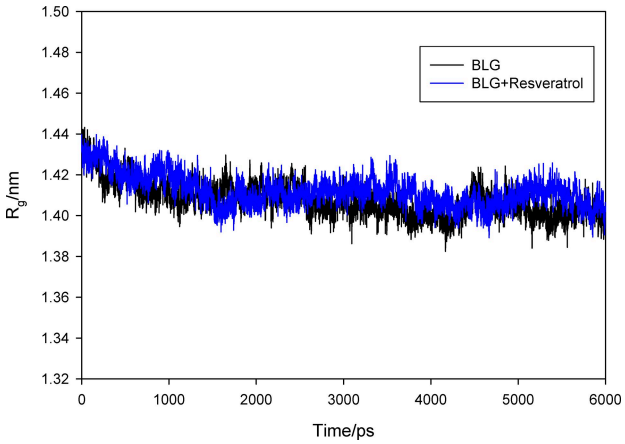

Fig. 4 – *Time evolution of the radius of gyration (R_g) during 6000 ps of MD simulation of BLG and BLG-resveratrol complex using GROMACS 4.0 package and the GROMOS96 43a1 force field*

does not change the microenvironment of BLG and does not lead to the conformational changes in the BLG during the MD simulation. These results matched the experimental values reported earlier.[30] It is clearly shown in the MD simulation that, around 1500 ps, the BLG + resveratrol complexes were stabilized.

Local protein mobility was analyzed by calculating the time averaged rmsf values of free BLG and BLG-resveratrol complex. The results were plotted against residue numbers based on the 6000 ps trajectory data shown in Fig. 5. The profiles of atomic fluctuations were found to be very similar to those of BLG and BLG-resveratrol complexes. These results suggest that the structure of ligand binding site remains approximately rigid during the simulation.

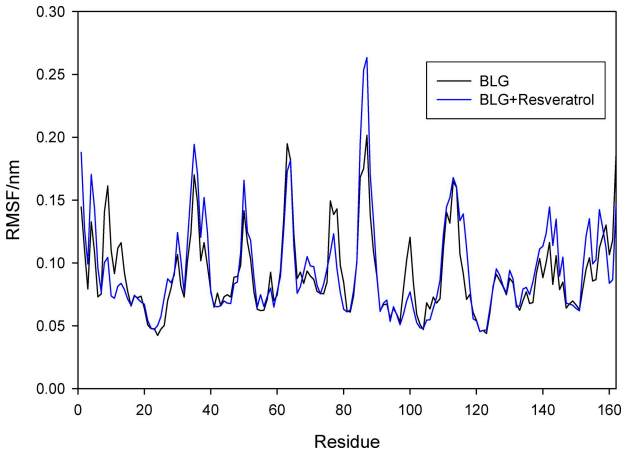

Fig. 5 – *The rmsf values of unliganded BLG and BLG-resveratrol complex were plotted against residue numbers. The results are based on the 6000 ps trajectory*

Conclusions

Resveratrol exhibits many physiological effects associated with health benefits. Here, this compound was examined with BLG since it plays a major role in transporting the ligands to the target places. The molecular docking studies indicated that the resveratrol binds to the surface of BLG near Trp(19) and Arg(124) with two hydrogen bonding interactions. Therefore, it can be concluded that the interaction of resveratrol with BLG is not mainly hydrophobic. The binding constant and free energy change, $\Delta G°$, for the binding of resveratrol to BLG were about 7.3×10^5 mol L^{-1} and –33.4 kJ mol^{-1}, respectively. These results are strongly supported by the experimental data. Specifically, the MD study made an important contribution to understanding the effect of the binding of resveratrol on the conformational changes of BLG and the stability of the BLG-resveratrol complex system in the aqueous solution. MD simulation studies revealed that the rmsd of both systems reached equilibration and oscillated around the average value after 600 ps simulation time. Analyzing the R_g represented that BLG and BLG-resveratrol complexes were stabilized around 1500 ps and exhibited no a conformational change. Initially, the R_g values of both unliganded BLG and BLG-resveratrol complex were 1.44 nm. The unliganded BLG was stabilized at 1.40 ± 0.008 nm, which matched the experimental values reported earlier. The similarity of the profiles of atomic fluctuations of BLG and BLG-resveratrol complexes suggested that the structure of ligand binding site remains approximately rigid during the simulation. The molecular docking, and MD simulation study described herein, is a promising approach for probing the interactions of ligands with relevant target proteins. Accurate measurements of BLG binding properties and knowledge of its binding site locations are important for preventing adverse drug reactions leading to life-threatening diseases, such as heart disease, cancer, diabetes, and so forth.

ACKNOWLEDGMENTS

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Abbreviations

BLG – β-lactoglobulin
$\Delta G°$ – Gibbs free energy change at 25 °C
K_a – binding constant
MD – Molecular dynamics
Rmsd – root mean square deviation
R_g – radius of gyration
Rmsf – root mean square fluctuation

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