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Computational Studies on the Interaction of Arctiin and Liquiritin With β -lactoglobulin

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The study of the interaction of drugs purified from natural sources and a transport protein, such as β -lactoglobulin (BLG), at the atomic level could be a valuable factor to control their transport to biological sites. In the present study, molecular docking and molecular dynamics simulation methods were used to study the interaction of arctiin and liquiritin as natural drugs and BLG as the transport protein. The molecular docking results indicated that these drugs bind in the internal cavity of BLG and the BLG affinity for binding the liquiritin is greater than arctiin. The docking results also indicated that the hydrogen bond interactions have a dominant role in the BLG-drug complex stability. The analysis of MD simulation trajectories showed that the root mean square deviation (RMSD) of BLG-liquiritin, unliganded BLG, and BLG-arctiin reached equilibrium and fluctuated around the mean value at about 1000, 3500, and 4000 ps, respectively. The time evolution of the radius of gyration and total solvent accessible surface of the protein showed that BLG-arctiin and BLG-liquiritin complexes became stable around 2500 and 5000 ps, respectively. Also, the profiles of atomic fluctuations during the simulation showed the rigidity of the ligand binding sites.

Keywords arctiin, binding affinity, β -lactoglobulin, liquiritin, molecular docking, molecular dynamics simulation

Introduction

Drugs purified from natural sources have significant biological properties.^[1-3] Arctiin (Fig. 1), found in *Arctium lappa* Linne (*Arctium lappa* L), is used for treatment of tumefaction, urination, hemorrhoids, and many infectious diseases. Arctiin displays antiinflammatory,^[4] and antitumor activity.^[5] Also, recent studies have shown that arctiin might be beneficial for diabetic nephropathy.^[6]

Liquiritin (Fig. 2) is the important bioactive constituent purified from licorice and has been demonstrated to display multiple pharmacological activities, including a neuroprotective effect and an antitumor effect.^[7]

The interaction of these drugs and a transport protein, such as β -lactoglobulin (BLG), could be a valuable factor to control and promote their transport to biological sites. BLG is a

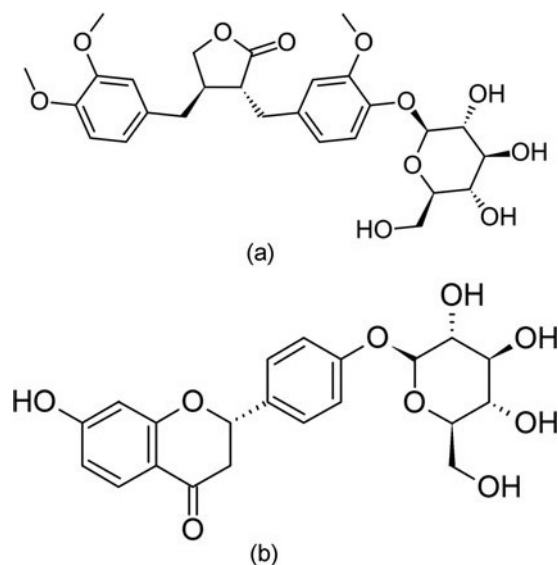


Figure 1. Chemical structure of arctiin (a), and liquiritin (b).

member of the lipocalin super-family whose ability to bind and transfer small hydrophobic ligands has been confirmed.^[8,9] The core structure of BLG consist of eight antiparallel β -sheets that forms an internal cavity as a central binding site for hydrophobic ligands (see Fig. 2). BLG is the major whey protein, consisting of 162 amino acids with an about 18.4 kDa molecular weight.^[10–12]

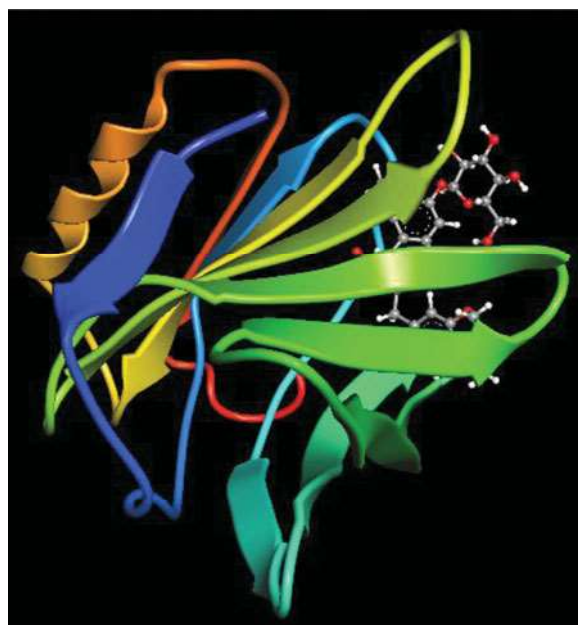
In our previous paper, we studied the interaction of three polyphenol flavonoids (quercetin, quercitrin, and rutin) with BLG.^[13] In the present work, we study the interaction and protein conformation of BLG with arctiin and liquiritin using molecular docking and molecular dynamics simulation studies. Finding new biologically active ligands that bind to BLG is very important and not only could contribute to better understanding of the molecular properties of this protein, but also might pave the way for future studies about the transport of drugs to biological sites.

Experimental

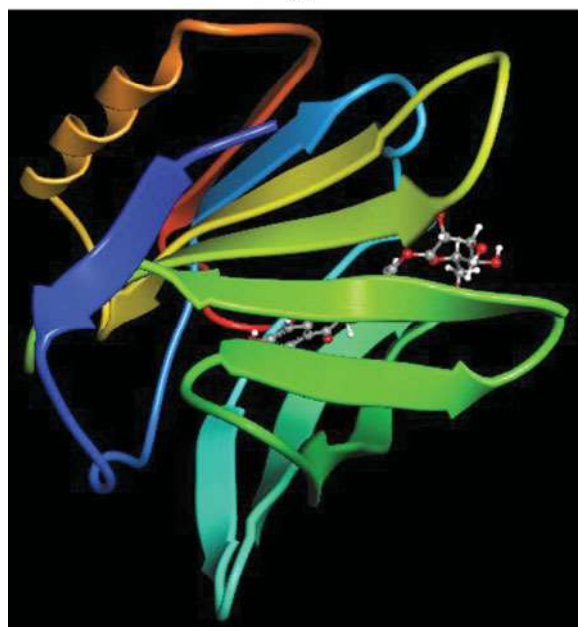
Molecular Docking

All of the docked conformations of drug-BLG complexes were generated using ArgusLab 4.0.1 (<http://www.ArgusLab.com>, Planaria Software LLC, USA) molecular docking program.^[14] Comparison between the results of ArgusLab and GOLD (Genetic Optimization for Ligand Docking) has shown little difference in their docking accuracies.^[14–16]

The known crystal structure of BLG (PDB ID: 3NPO) was taken from the RSCB protein data bank (www.rcsb.org). The ArgusLab suite was used to remove its water molecules. The Becke three-parameter Lee–Yang–Parr (B3LYP) hybrid density functional theory was employed to optimize the drugs structures, using the GAMESS quantum chemistry software (USA).^[17] The blind docking approach was used and the whole protein was taken as a potential binding site. The Ascore scoring method in ArgusLab, using high precision, 0.4 Å grid resolution, and the maximum 200 candidate poses, was employed for docking



(a)



(b)

Figure 2. Arctiin (a), and liquiritin (b) docked in BLG using ArgusLab 4. 0. 1. Ligands, depicted in a ball and cylinder model, and BLG, represented in cartoon ribbon.

calculations. The Ascore scoring function ranked the docked conformations on the basis of their free binding energy. The lowest free binding energy conformation of each drug-BLG complex was considered for further studies.

Molecular Dynamics Simulation

The lowest free binding energy conformation of each complex was considered as the initial conformation for the MD studies. All MD studies were carried out using the GROMACS 4.5.6 (University of Groningen, Netherlands) package [18,19] and the GROMOS96 43a1 force field.[20,21] The Dundee PRODRG2.5 server [22] was used to generate the topology parameters of the drugs. The partial atomic charges of the drugs were calculated using the GAMESS quantum chemistry software (USA) [17] at the level of HF/6–31G**. The complex was located in a cubic box with periodic boundary conditions. The box volume was 294.14 nm³ and filled with extended simple point charge (SPC) water molecules [23] and the solvated systems were neutralized by adding 8 sodium ions. Energy minimization was done using the steepest descent method for 8 ps. Then, the system was equilibrated for 40 ps at the temperature of 300 K. Finally, a 10 ns MD simulation was carried out at 1 bar and 300 K. The Berendsen thermostat [24] at 300 K, the particle mesh Ewald (PME) method [25,26] for long range electrostatics, and a 7 Å cut off for van der Waals interactions and Coulomb interactions were used. The equation of motions was integrated by the leap-frog algorithm with 2 fs time steps. The atomic coordinates were recorded to the trajectory file every 0.5 ps for later analysis.

Results and Discussion

Molecular Docking Studies

BLG has three potential binding sites with the internal cavity being the main binding site for hydrophobic ligands.[27] Our previous studies on the interaction of polyphenol flavonoids and BLG showed that quercetin and quercitrin bind in this cavity.[13] In this study, the binding mode of arctiin and liquiritin at the active site of BLG was examined. The docking results showed that arctiin and liquiritin bind in the internal cavity of BLG (Fig. 2). Figure 3a shows that there are 3, 1, and 1 hydrogen bond interactions between arctiin and Ser (116), Glu (114), and Asn (90), respectively. Also, there are 2 and 1 hydrogen bond interactions between liquiritin and Asn (90) and Asn (88), respectively (Fig. 3b). The near residues for each drug are listed in Table 1.

It can be concluded that the interaction of BLG and these drugs are not primarily hydrophobic, and the hydrogen bond interactions have a significant role in the binding of these drugs to BLG. The binding constant (K_a), free binding energy (ΔG°), and the structural volume (V) of the drugs are listed in Table 2. The results indicate a decrease in the binding constant with an increase in the structural volume of the drugs. It is to be added that as the structural volume of the drug increases, the orientation of the drug in its binding site becomes inappropriate, and this can affect the fitting of the drug in the binding site. The inappropriate orientation of the drug leads to the reduction of both the hydrophobic interactions and the stability of the BLG-drug complex. Similar results have been observed in the interaction of rutin and BLG.[13]

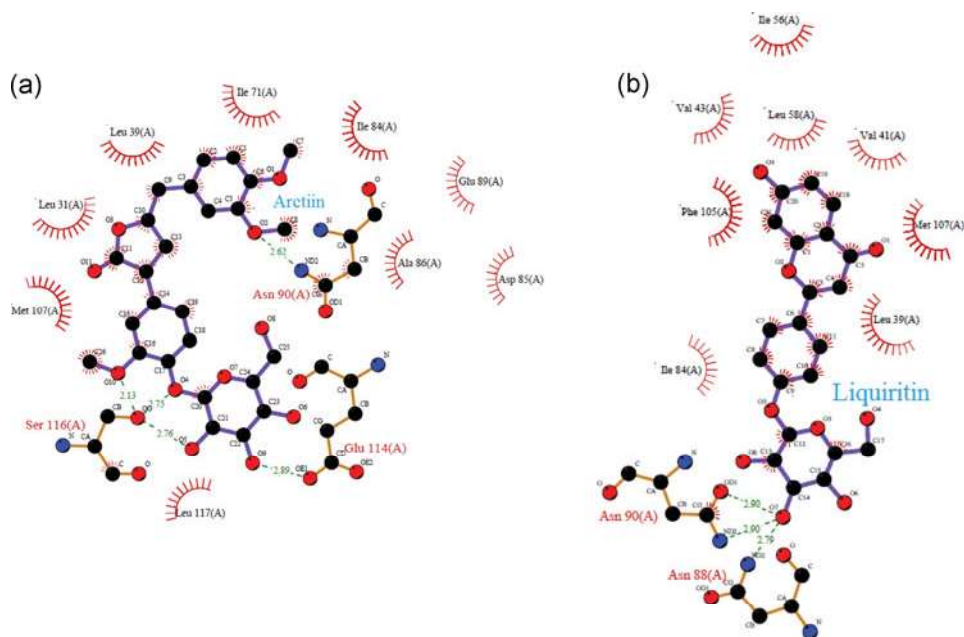


Figure 3. The docking poses of the BLG-arctiin (a), and BLG-liquiritin (b) complexes. H-bonds (as showed by dotted lines) formed between ligands and BLG.

MD Simulation Studies

The root mean square deviation (RMSD) of the backbone of BLG, potential energy of the system, system temperature, and the density of the system were obtained to examine the stability of trajectories for unliganded BLG and the BLG-drugs complexes (Fig. 4). The analysis of Fig. 4a indicates that the RMSD of the various systems reached equilibrium and fluctuated around the mean value after various times. These times were about 1000, 3500, and 4000 ps for BLG-liquiritin, unliganded BLG, and BLG-arctiin complex, respectively.

The radii of gyration (R_g) for the BLG and BLG-drug complexes were also determined and plotted versus simulation time to examine the protein compactness (Fig. 5). The results showed that the R_g of unliganded BLG and BLG-arctiin systems stabilized after about 2500 ps and the R_g of BLG-liquiritin complex stabilized after about 5000 ps, which

Table 1
Residues within 3 Å distance from each Drug

Drug	Residues
Arctiin	Ala (26), Ala (86), Ala (118), Asn (88), Asn (90), Asn (109), Asp (85), Glu (89), Glu (108), Glu (114), Ile (71), Ile (84), Leu (31), Leu (39), Leu (117), Met (107), Ser (27), Ser (116), Val (92)
Liquiritin	Asn (88), Asn (90), Asn (109), Ile (56), Ile (71), Ile (84), Leu (46), Leu (31), Leu (39), Leu (46), Leu (54), Leu (58), Leu (122), Met (107), Phe (82), Phe (105), Pro (38), Ser (116), Val (41), Val (43), Val (92)

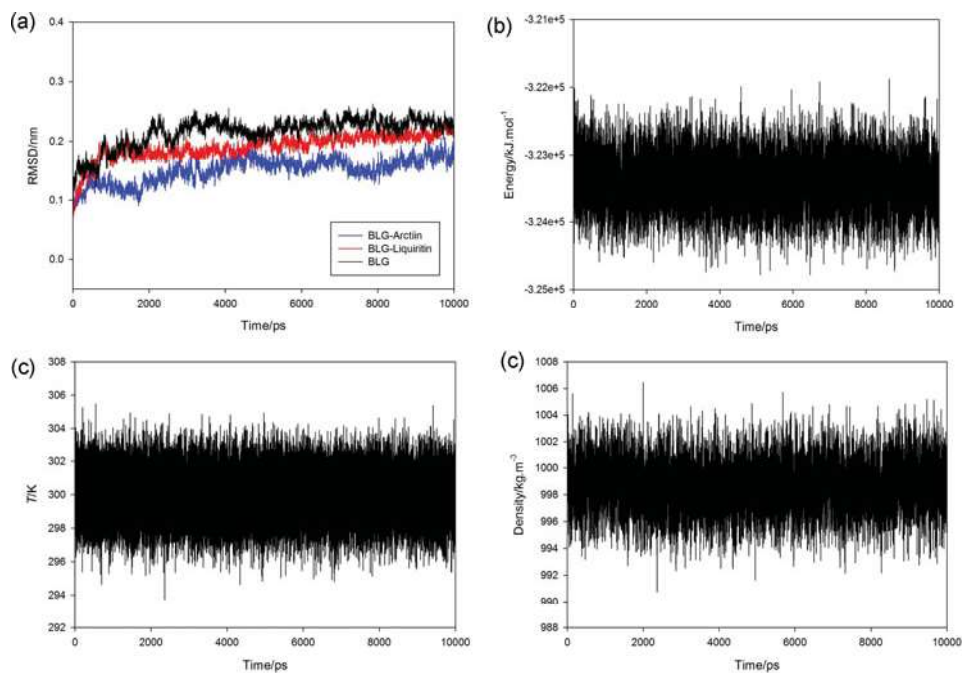


Figure 4. (a) RMSD values for nonliganded BLG and BLG-drug complexes during 10,000 ps MD simulation using GROMACS 4.5.6 package and the GROMOS96 43a1 force field at 300 K and 1 bar. (b) The variation of the total energy for BLG-arctiin complex during 10,000 ps MD simulation (The data for unliganded BLG and BLG-liquiritin are not shown). (c) Time dependence of the temperature during 10,000 ps MD simulation for BLG-arctiin complex (The data for unliganded BLG and BLG-liquiritin are not shown). (d) Time dependence of the density during 10,000 ps MD simulation for BLG-arctiin complex (The data for unliganded BLG and BLG-liquiritin are not shown).

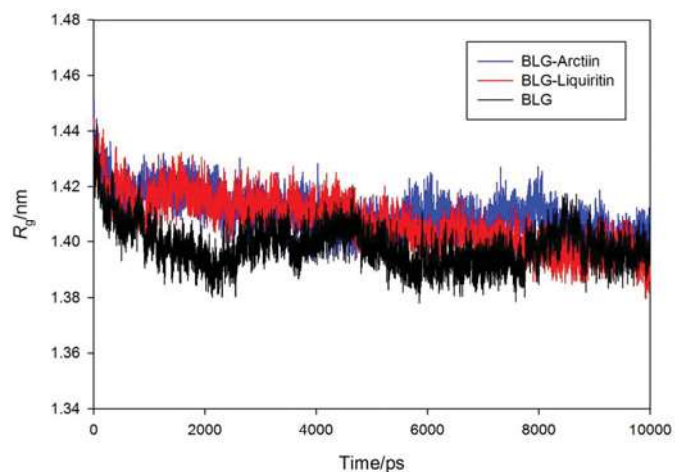


Figure 5. Time evolution of the radius of gyration (R_g) during 10,000 ps of MD simulation of BLG and BLG-drug complexes using the GROMACS 4.5.6 package and the GROMOS96 43a1 force field at 300 K and 1 bar.

Table 2
Docking summary of BLG with drugs

Drug	K_a/M^{-1}	$\Delta G^\circ/kcal.mol^{-1}$	$V/\text{\AA}^3$
Arctiin	1.6×10^7	−9.88	1362.12
Liquiritin	2.2×10^7	−10.09	1093.63

indicates that the MD simulation equilibrated after 2500, 2500, and 5000 ps for unliganded BLG, BLG-arctiin, and BLG-liquiritin, respectively. The initial value of R_g for all of the systems was about 1.44 nm, matching the earlier experimental results.^[28] Also, Figure 5 shows that the R_g value of BLG does not depend upon the complexation with the drugs. This indicates that the environment of BLG did not change during its interaction with the drugs.

Figure 6 shows the changes in the total solvent accessible surface (SAS) of the protein during the 10 ns simulation time. The variation of SAS indicates that the trends for unliganded BLG and BLG-drug complexes were similar to the variation of their R_g . This similarity validates the accuracy of the MD simulation results.

The time averaged root mean square fluctuations (RMSF) of the protein residues was obtained to analyze the local mobility of the protein. The RMSF values versus residue numbers are shown in Figure 7. These values are based on the 9000, 6500, and 6000 ps of trajectory for BLG-liquiritin, unliganded BLG, and BLG-arctiin complex, respectively. All of the atomic fluctuation profiles were similar and the fluctuations of the residues in the internal cavity were lower than the others. This clearly indicates that the binding sites remain approximately rigid during the MD simulation.

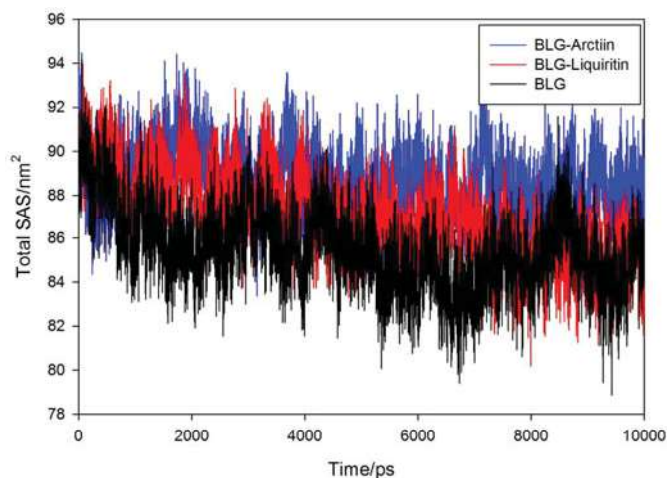


Figure 6. Time evolution of the total solvent accessible surface (SAS) during 10,000 ps of MD simulation of BLG and BLG-drugs complexes using the GROMACS 4.5.6 package and the GROMOS96 43a1 force field at 300 K and 1 bar.

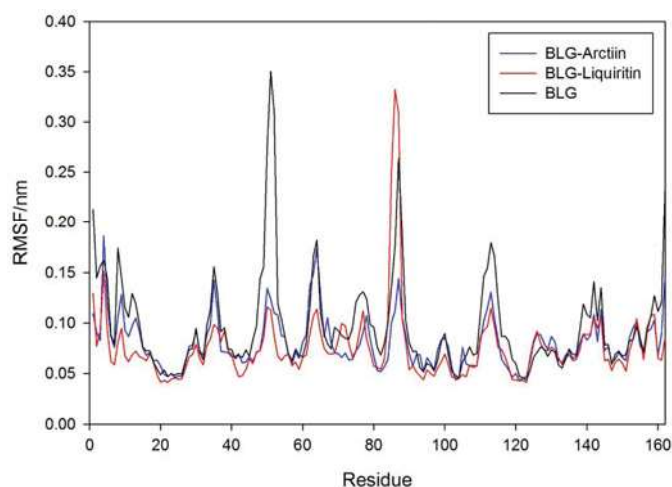


Figure 7. The RMSF values of unliganded BLG and BLG-drugs complexes plotted against residue numbers. The results are based on the 10,000 ps trajectory.

Conclusions

Based on our molecular docking results, liquiritin and arctiin bind in the internal cavity of BLG. Liquiritin has a lower structural volume than arctiin and its free binding energy was more negative than the arctiin. Also, the docking results indicate that the hydrogen bond interactions have the dominant role in the BLG-drug complex stability. MD simulation studies showed that BLG did not exhibit conformational changes during the liquiritin and arctiin binding. Also, the profiles of atomic fluctuations show the rigidity of the ligand binding site during the simulation.

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