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The Interaction of Polyphenol Flavonoids with β -lactoglobulin: Molecular Docking and Molecular Dynamics Simulation Studies

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The interaction of quercetin, quercitrin, and rutin, as natural polyphenolic compounds, with β -lactoglobulin (BLG) using molecular docking and molecular dynamics simulation methods was examined. Molecular docking studies showed that quercetin and quercitrin were bounded to the internal cavity of protein, while rutin was bounded to the entrance of the cavity because of its large structural volume. It was found that there were one-, three-, and four-hydrogen bond interactions between BLG and quercetin, quercitrin, and rutin respectively. This showed that with an increase in the number of OH groups in the flavonoid structure, there was an increase in the number of hydrogen bond interactions. The binding constants for the binding of quercetin, quercitrin, and rutin to BLG were 1.2×10^6 , 1.9×10^6 , and $7.4 \times 10^4 \text{ M}^{-1}$ respectively. The results of molecular dynamics simulation showed that the root mean square deviation (RMSD) of non-liganded BLG and BLG–ligand complexes reached equilibration after 3500 ps. The study of the radius of gyration revealed that BLG and BLG–ligand complexes were stabilized around 2500 ps, and unlike the two other complexes, there was no conformational change for BLG–quercetin. Finally, analyzing the RMS fluctuations suggested that the structure of the ligand binding site remained approximately rigid during simulation.

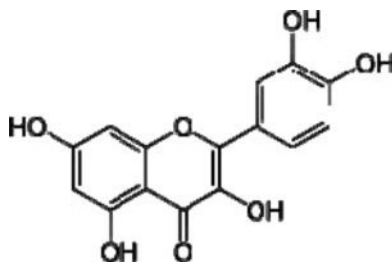
Keywords β -lactoglobulin, molecular docking, molecular dynamics simulation, polyphenolic flavonoids

1. Introduction

In recent years, antioxidants have been the subject of many epidemiological studies, which have related the consumption of fruits and vegetables to reduction in the incidence of cardiovascular diseases and several types of cancers; of these are those antioxidants in which phenolic compounds in particular play a very important role.^[1–4] The beneficial effects of phenolic compounds (a large amount of which is found in all kinds of plant foods) are thought to result from their ability to scavenge reactive oxygen and nitrogen species, which are formed in large amounts, during intracellular oxidative stress induced by the extraneous attack of pro-oxidants.^[5,6] However, little attention has been directed to the interactions

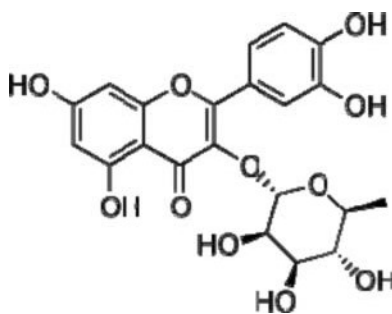
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of phenolic compounds with other components, which are also present in food or the organism (e.g., proteins). The phenolic compounds have multiple chemical and biological actions, besides being an antioxidant;^[7,8] for instance, chelating,^[9] anticarcinogenic,^[10,11] bacteriostatic,^[9] and secretory activities.^[12] For one polyphenol flavonoid, quercetin (see Scheme 1(a)), over a dozen biological effects can be listed. Quercetin is particularly interesting because of its anticarcinogenic activities^[13] and its significant quantitative presence in human foods.^[14] The biological activity of quercetin includes the inhibition of cell proliferation in animals and cell cultures.^[15]



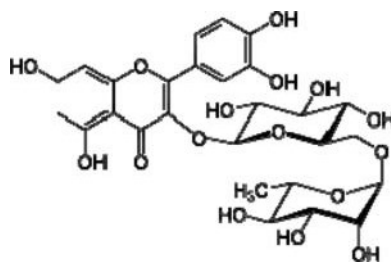
Scheme 1(a). Chemical structure of quercetin.

Another polyphenol flavonoid, Quercitrin (quercetin 3-O- α -L-rhamnoside, see Scheme 1(b)) is a glycoside extracted from the oak bark as a bitter and yellow dye citron. Quercitrin shows stronger peroxy radical-scavenging capacity than trolox, a water-soluble vitamin E analogue.^[16] In addition, quercitrin is effectively prevented from allergic reactions.^[17] Cherries are rich in two important polyphenol flavonoids: isoquercitrin and quercitrin, as well as anthocyanins. Consuming foods such as cherries has been shown to help neutralize cancer-causing agents and decrease oxidative stress.^[18]



Scheme 1(b). Chemical structure of quercitrin.

Rutin ((3-(6-Deoxy- α -L-mannopyranosyl)- β -glucopyranosyl)oxy)-2-(3',4',5,7-tetrahydroxyflavone) (see Scheme 1(c)) is another commonly found flavonol glycoside and is widely present in many plants, especially the buckwheat plant. Rutin has been reported to have clinically relevant functions, including antioxidant, antihypertensive, anti-inflammatory, and antihemorrhagic activities, strengthening the capillaries of blood vessels and regulating the capillary permeability and stabilization of platelets.^[19,20] These properties are potentially beneficial in preventing diseases and protecting the stability of genome. Many of these activities have been related to its antioxidant actions. Nevertheless, due to its poor solubility (solubility at $T = 298.15$ K is $0.66 \pm 0.03 \times 10^{-5}$ mole fraction value)^[21], rutin shows a slow dissolution rate in solid oral forms and the drug absorption from the



Scheme 1(c). Chemical structure of rutin.

gastrointestinal tract is generally slow and irregular, thus restricting its use in therapy.^[22] Therefore, it is important to improve its solubility and bioavailability.

The lipocalin family consists of small secreted proteins that typically bind and transfer small hydrophobic ligands and interact with cell-surface receptors.^[23,24] The natural lipocalin member, bovine β -lactoglobulin (BLG), is a major bovine whey protein. Several genetic variants of BLG have been determined, among which the bovine phenotypes A and B are the most predominant ones.^[25,26] Variant A differs in amino acid sequence from variant B at positions 64 (Asp_A→Gly_B) and 118 (Val_A→Ala_B).^[25] Around neutral pH, BLG is mainly dimeric, whereas between pH 2 and 3, it is dissociated into monomers due to electrostatic repulsion between subunits.^[27] The crystal structure shows that the 162-amino acid-long single polypeptide chain of bovine BLG forms a calyx composed of an eight-stranded antiparallel β -sheet.^[27] An important physicochemical property of this lipocalin protein is its ability to bind *in-vitro* several physiologically relevant ligands such as steroids, fatty acids, retinoids, vitamin D, and cholesterol.^[28–30] So BLG can serve as a potential transport and depot protein, especially for hydrophobic non-water soluble molecules. Finding novel bioactive ligand molecules of BLG is of biotechnological and pharmacological importance and could contribute to the deeper understanding of molecular recognition properties of this lipocalin.

Considering the fact that the mentioned polyphenol flavonoids exhibit many biological effects, it was deemed important to evaluate the putative interactions of these compounds with a major carrier protein such as BLG. In the present work, the interaction of BLG with quercetin, quercitrin, and rutin was studied using molecular dynamics (MD) simulation and molecular docking studies. The molecular docking and the MD simulation described here are a promising approach for probing the interactions of ligands with relevant target proteins.

2. Experiment

2.1. Molecular Docking

The ArgusLab 4.0.1 molecular docking program (Planaria Software, LLC, USA)^[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 do not have rotatable bonds are nodes, while torsions are connections between the nodes. The topology of a torsion tree is a determinative factor which influences efficient docking. In the docking calculations, the Ascore scoring method from the ArgusLab

4.0.1 suite was employed. Ascore is based on the decomposition of total protein–ligand binding free energy and takes the following contributions into account: van der Waals interaction between the ligand and the protein, hydrophobic effects, hydrogen bonding between the ligand and the protein, hydrogen bonding involving the charged donor and/or acceptor groups, deformation effects, and the effects of translational and rotational entropy loss in the binding process.^[31,32]

2.1.1. Preparing protein and ligand. The known crystal structure of BLG (PDB ID: 3NPO) was obtained from the RCSB Protein Data Bank (www.rcsb.org), and water molecules were removed. In order to get the most stable conformations of quercetin, quercitrin, and rutin, 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 (USA).^[34] The ArgusDock exhaustive search docking engine was used, having a grid resolution of 0.40 Å. Docking precision was set to regular precision and the 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, 156, and 132 along the *x*-, *y*-, and *z*-axes respectively. During the docking, a maximum number of 10 conformers were considered. The lowest energy conformations were used for further analyses (Table 1).

2.2. Molecular Dynamics Simulation

A 10,000-ps MD simulation of BLG–flavonoids complexes was carried out with the GRO-MACS 4.0 (University of Groningen, Netherlands)^[35,36] package using the GROMOS96 43a1 force field.^[37,38] The lowest binding energy conformation for each complex (Table 1) was considered as the initial conformation. The topology parameters of BLG were created using the Gromacs program. The topology parameters of quercetin, quercitrin, and rutin were built by the Dundee PRODRG2.5 server (beta) (UK).^[39] The partial atomic charges of ligands were subsequently determined using the GAMESS^[34] at the level of HF/6-31G**. Then the complex was immersed in a cubic box ($6.65049 \times 6.65049 \times 6.65049$ nm³) of extended simple point charge (SPC) water molecules.^[40] The solvated systems were neutralized by adding sodium ions to the simulation and the entire systems were composed of 1594 atoms of BLG, one ligand, 8 Na⁺ counterions, and 26754, 26718, 26700, and 26634 solvent atoms for BLG, BLG–quercetin, BLG–quercitrin, and BLG–rutin respectively. Energy minimization was performed using the steepest descent method of 2000 steps, followed by the conjugate gradient method for 2000 steps. In the first stage of MD simulation (equilibration), the solute (protein, counterion, and ligand) was fixed and the position-restrained dynamics simulation of the system proceeded at 300 K for 40 ps. Finally, the full system was subjected to 10,000-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 leap-frog algorithm with the time-step of 2 fs. The atom coordinates were recorded every 0.5 ps during the simulation for later analysis.

3. Results and Discussion

3.1. Molecular Docking Studies

Three potential binding sites have been reported for ligand binding to BLG: (i) The internal cavity of β -barrel, (ii) the surface hydrophobic pocket in a groove between α -helix and

Table 2
Residues within 3-Å distance from each polyphenol flavonoid

Polyphenol flavonoid	Residues
Quercetin	Pro(38), Val(41), Val(43), Leu(46), Leu(54), Ile(56), Leu(58), Gln(59), Lys(60), Lys(69), Ile(71), Ala(73), Ile(84), Val(92), Phe(105), Met(107)
Quercitrin	Pro(38), Leu(39), Val(41), Val(43), Leu(58), Lys(60), Lys(69), Ile(71), Ile(84), Asn(90), Val(92), Phe(105), Met(107)
Rutin	Pro(38), Leu(39), Val(41), Leu(58), Lys(60), Lys(69), Lys(70), Ile(71), Ile(84), Asp(85), Ala(86), Asn(88), Asn(90), Glu(108), Asn(109), Ser(116)

β -barrel, and (iii) the outer surface near Trp19–Arg124.^[41] There is a large hydrophobic cavity present in β -barrel and many ligands can bind in this location. In this study, the molecular docking program, ArgusLab 4.0.1, was chosen for examining the binding mode of quercetin, quercitrin, and rutin at the active site of BLG. The docking results showed that quercetin and quercitrin bind to the internal cavity of protein by one and three hydrogen bond interactions respectively (Figs. 1(a) and (b)). All near residues for each ligand are listed in Table 2.

There was one hydrogen bond between quercetin and Lys(69) of BLG with a 2.9-Å distance (Fig. 2(a)). Also, there were three hydrogen bonds between quercitrin and Pro(38), Lys(69), and Ile(71) of BLG, with 2.8-, 2.8-, and 2.3-Å distance respectively (Fig. 2(b)). While rutin did not bind to the internal cavity, there were four hydrogen bond interactions between rutin and Lys(70), Ile(71), Asn(88), and Ser(116) at the entrance of internal cavity with a distance of 2.5-, 2.7-, 1.1, and 2.5-Å respectively (Figs. 1(c) and 2(c)).

Thus, it can be concluded that the interaction of these polyphenol flavonoids with BLG is not primarily hydrophobic and the phenolic OH groups in their structure have a significant role in the binding process. The above results indicate that the number of hydrogen bond interactions increases with the increase of OH groups in the polyphenol flavonoid structure. Similar results were obtained for the interaction of bisdemethoxycurcumin and diacetyl-bisdemethoxycurcumin with human serum albumin (HSA) by a fluorescence quenching study.^[42]

The binding constants (K_a) for the binding of quercetin, quercitrin, and rutin to BLG were 1.2×10^6 , 1.9×10^6 , and $7.4 \times 10^4 \text{ M}^{-1}$ respectively. Also, the free energy change values, ΔG° , for the binding of quercetin, quercitrin, and rutin to BLG were -8.29 , -8.55 , and $-6.64 \text{ kcal mol}^{-1}$ respectively (Table 1).

The structural volume of these polyphenol flavonoids was calculated using the HyperChem-7 package program (Hypercube, Inc., USA), and 780.45, 1039.70, and 1491.79 \AA^3 were obtained for quercetin, quercitrin, and rutin respectively. Therefore, rutin cannot enter the internal cavity of BLG because of its large structural volume. In comparison with quercetin and quercitrin, the obtained lower value of K_a for binding rutin to BLG is because of its binding to the entrance of internal cavity, where the hydrophobic interactions do not have a dominant role in ligand binding.



(a)



(b)



(c)

Figure 1. (a) Quercetin, (b) Quercitrin, and (c) Rutin docked in BLG using ArgusLab 4.0.1. Ligands are depicted in a ball-and-stick model (light gray), and BLG are represented in cartoon ribbon.

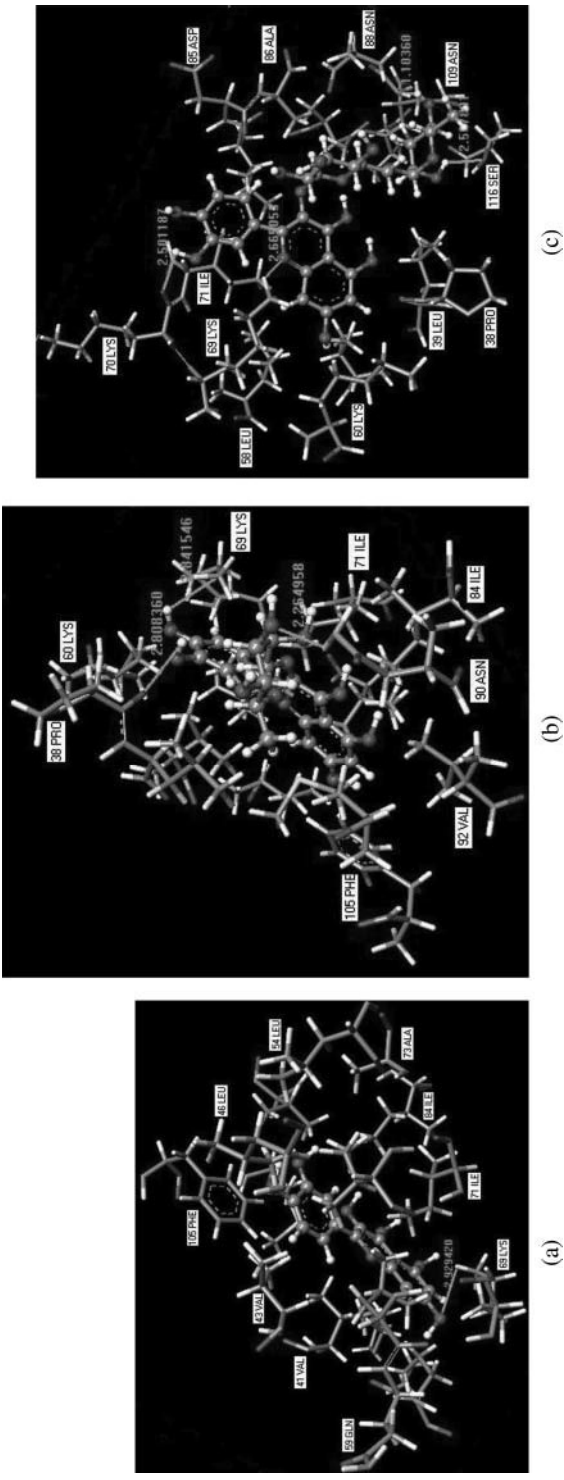


Figure 2. The docking poses of (a) BLG–quercetin, (b) BLG–quercitrin, and (c) BLG–rutin complexes. Ligands are rendered as ball-and-stick models. H-bonds (as highlighted by lines in green color) are formed between ligands and BLG.

3.2. Analysis of Dynamics Simulation Trajectories

The trajectories were stable during the whole production part of the 10,000-ps MD simulation run. The trajectory stability was checked and corroborated by the analysis of the root mean square deviation (RMSD) (Fig. 3) as functions of time for BLG and its complexes with polyphenol flavonoids. The analysis of Fig. 3 indicates that the RMSD of all systems reached equilibrium and oscillated around the average value after about 3500-ps simulation time. This evidence clearly indicates that the whole system was stable and equilibrated. The RMSD values of atoms in BLG and BLG–ligand complexes were calculated from a 3500–10,000-ps trajectory, where the data points for BLG, BLG–quercetin, BLG–quercitrin, and BLG–rutin fluctuated by about 0.23 ± 0.010 , 0.20 ± 0.017 , 0.16 ± 0.009 , and 0.19 ± 0.010 nm respectively.

In the present MD study, the radius of gyration (R_g) values of non-liganded BLG and BLG–ligand complexes were determined and plotted as a function of time, as shown in Fig. 4. In these systems, the R_g values were stabilized at about 2500 ps, indicating that the MD simulation achieved equilibrium after 2500 ps. Initially, the R_g values of the non-liganded BLG and BLG–ligand complexes were 1.44 nm. The non-liganded BLG, BLG–quercetin, BLG–quercitrin, and BLG–rutin stabilized at 1.40 ± 0.006 , 1.40 ± 0.007 , 1.41 ± 0.005 , and 1.42 ± 0.006 nm respectively. An earlier report showing that the experimental R_g value of BLG was 1.39 ± 0.04 nm indicated that the present MD simulations matched the reported experimental values of R_g for non-liganded BLG.^[43]

The above results suggest that the radius of gyration value increased slightly upon quercitrin and rutin complexation and did not change tangibly upon quercetin complexation with respect to free BLG. This clearly indicates that, unlike quercetin, quercitrin and rutin changed the microenvironment of BLG leading to conformational changes in BLG during MD simulation.

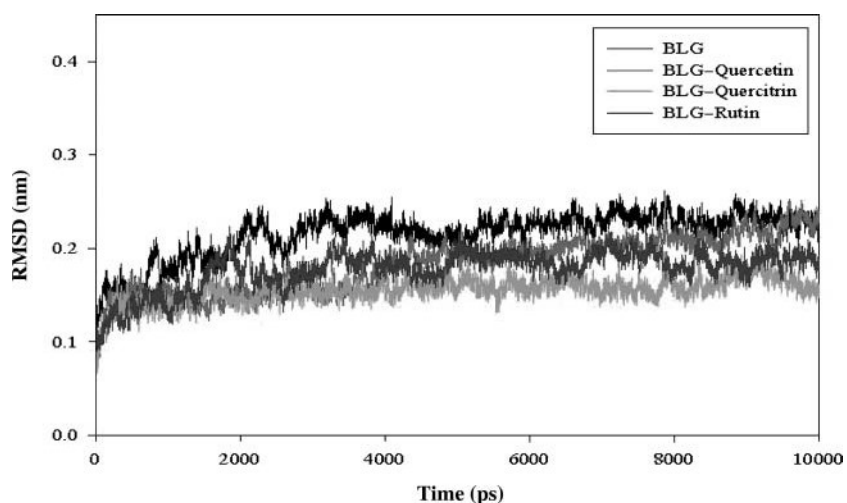


Figure 3. Time dependence of RMSD. RMSD values for non-liganded BLG and BLG–ligand complexes during 10,000-ps MD simulation using GROMACS 4.0 package and the GROMOS96 43a1 force field at 300 K and 1 bar.

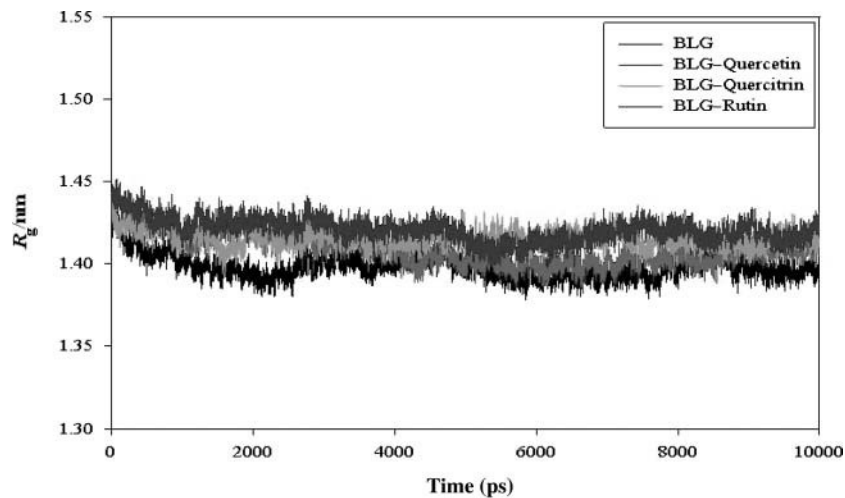


Figure 4. Time evolution of the radius of gyration (R_g) during 10,000-ps of MD simulation of BLG and BLG–ligand complexes using GROMACS 4.0 package and the GROMOS96 43a1 force field at 300 K and 1 bar.

Local protein mobility was analyzed by calculating the time-averaged root mean square fluctuation (RMSF) values of free BLG and BLG–ligand complexes. The results were plotted against residue numbers based on 10,000-ps trajectory (Fig. 5). The profiles of atomic fluctuations were found to be very similar for BLG and BLG–ligand complexes. The obtained results clearly indicate that the residues at the binding site had low fluctuations for each ligand. Also, the structure of ligand binding site remained approximately rigid during simulation.

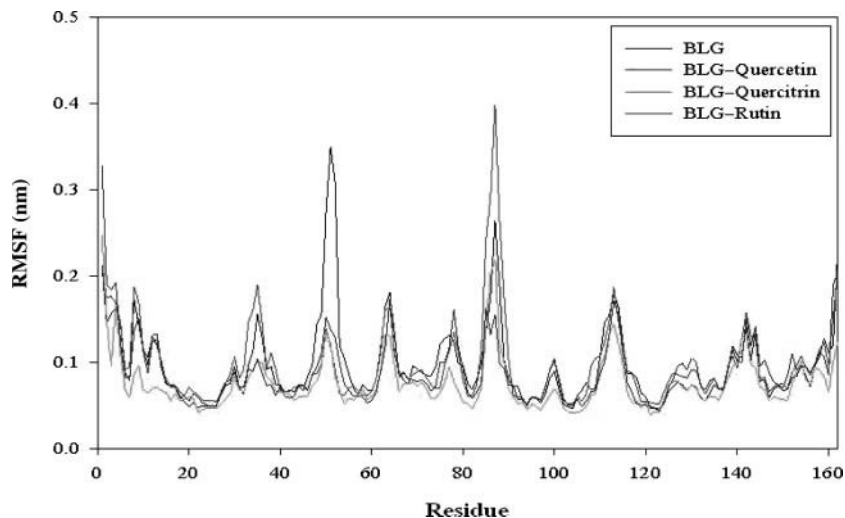


Figure 5. The RMSF values of non-liganded BLG and BLG–ligand complexes were plotted against residue numbers. The results are based on the 10,000-ps trajectory.

4. Conclusions

Interaction of polyphenol flavonoids, quercetin, quercitrin, and rutin with BLG was examined since BLG plays a major role in transporting ligands to target places. The molecular docking studies indicated that quercetin and quercitrin bind to the internal cavity of BLG with one and three hydrogen bonding interactions respectively. Rutin, however, cannot enter the cavity because of its large structural volume. This ligand binds to the entrance of internal cavity of BLG with four hydrogen bond interactions. Therefore, it can be concluded that the interaction of these polyphenol flavonoids with BLG is not mainly hydrophobic and the number of hydrogen bond interactions increases with the increase of OH groups in polyphenol flavonoid structure. The K_a values for the binding of quercetin, quercitrin, and rutin were 1.2×10^6 , 1.9×10^6 , and $7.4 \times 10^4 \text{ M}^{-1}$ respectively. These K_a values gave the ΔG° values of -8.29 , -8.55 , and $-6.64 \text{ kcal mol}^{-1}$ for the binding of quercetin, quercitrin, and rutin to BLG respectively. The greater K_a values for quercetin and quercitrin are because of their binding in the internal cavity of BLG, which is more hydrophobic than its entrance. Thus, it can be judged that quercetin and quercitrin bind to BLG by hydrophobic and hydrogen bond interactions, and the only interactions that stabilize the BLG–rutin complex are the four hydrogen bonds. The MD study, in particular, made an important contribution toward understanding the effect of the binding of these ligands on the conformational changes of BLG and the stability of BLG–ligand complex systems in aqueous solution. The MD simulation studies revealed that the RMSD of the systems reached equilibrium and oscillated around the average value after 3500 ps simulation time. Analysis of R_g indicated that BLG and BLG–ligand complexes were stabilized around 2500 ps, and, unlike quercetin, quercitrin and rutin changed the conformation of BLG during MD simulation. Initially, the R_g value for both non-liganded BLG and BLG–ligand complexes was 1.44 nm. The non-liganded BLG was stabilized at $1.40 \pm 0.006 \text{ nm}$, which matched the experimental value reported earlier. The similarity of profiles of atomic fluctuations of BLG and BLG–ligand complexes suggested that the structure of the ligand-binding site remained approximately rigid during the simulation.

Acknowledgments

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References

1. Rimm, E.B.; Ascherio, A.; Giovannucci, E.; Spiegelman, D.; Stampfer, M.; Willett, W. Vegetable, fruits, and cereal fiber intake and risk of coronary heart disease among men. *JAMA* **1996**, *275*, 447.
2. Sesso, H.D.; Gaziano, J.M.; Liu, S.; Buring, J.E. Flavonoid intake and the risk of cardiovascular disease in women. *Am. J. Clin. Nutr.* **2003**, *77*, 1400.
3. Block, G.; Patterson, B.; Subar, A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer* **1992**, *18*, 1.
4. Hertog, M.G.; Feskens, E.J.; Hollman, P.C.; Katan, M.B.; Kromhout, D. Dietary flavonoids and cancer risk in the Zutphen Elderly Study. *Nutr. Cancer* **1994**, *22*, 175.
5. Kono, Y.; Kobayashi, K.; Tagawa, S.; Adachi, K.; Ueda, A.; Sawa, Y.; Shibata, H. Antioxidant activity of polyphenolics in diets: rate constants of reactions of chlorogenic acid and caffeic acid with reactive species of oxygen and nitrogen. *Biochim. Biophys. Acta* **1997**, *1335*, 335.

6. Duthie, S.J.; Johnson, W.; Dobson, V.L. The effect of dietary flavonoids on DNA damage (strand breaks and oxidised pyrimidines) and growth in human cells. *Mutat. Res.* **1997**, *390*, 141.
7. Bors, W.; Heller, W.; Michel, C.; Saran, M. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. *Methods Enzymol.* **1990**, *186*, 343.
8. Lee, Y.; Howard, L.R.; Villalón, B. Flavonoids and antioxidant activity of fresh pepper (*Capsicum annuum*) cultivars. *J. Food Sci.* **1995**, *60*, 473.
9. Gross, M.; Pfeiffer, M.; Martini, M.; Campbell, D.; Slavin, J.; Potter, J. The quantitation of metabolites of quercetin flavonols in human urine. *Cancer Epidemiol. Biomarkers Prev.* **1996**, *5*, 711.
10. Okuda, T. Natural polyphenols as antioxidants and their potential use in cancer prevention, In *Polyphenolic phenomena*; Scalbert, A., Ed.; INRA: Paris, **1993**, pp. 221–235.
11. Middleton, E.; Kandaswami, C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In *The Flavonoids: advances in research since 1986*; Harbourne, J.B., Ed.; Chapman and Hall: London, **1994**, pp. 619–652.
12. Nguyen, T.; Canada, A. Citrus flavonoids stimulate secretion by human colonic T84 cells. *J. Nutr.* **1993**, *123*, 259.
13. Deschner, E.E.; Ruperto, J.; Wong, G.; Newmark, H.L. Newmark, Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. *Carcinogenesis* **1991**, *12*, 1193.
14. Hertog, M.G.L.; Hollman, P.C.H.; van de Putte, B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J. Agric. Food Chem.* **1993**, *41*, 1242.
15. Weber, G.; Shen, F.; Prajda, N.; Yang, H.; Li, W.; Yeh, A.; Csokay, B.; Olah, E.; Look, K.Y. Regulation of the signal transduction program by drugs. *Adv. Enzyme Regul.* **1997**, *37*, 35.
16. Tung, N.H.; Ding, Y.; Kim, S.K.; Bae, K.; Kim, Y.H. Total peroxy radical-scavenging capacity of the chemical components from the stems of *Acer tegmentosum maxim.* *J. Agric. Food Chem.* **2008**, *56*, 10510.
17. Cruz, E.A.; Da-Silva, S.A.; Muzitano, M.F.; Silva, P.M.; Costa, S.S.; Rossi-Bergmann, B. Immunomodulatory pretreatment with *Kalanchoe pinnata* extract and its quercitrin flavonoid effectively protects mice against fatal anaphylactic shock. *Int. Immunopharmacol.* **2008**, *8*, 1616.
18. Traustadottir, T.; Davies, S.S.; Stock, A.A.; Su, Y.; Heward, C.B.; Roberts, L.J.; Harman, S.M. Tart cherry juice decreases oxidative stress in healthy older men and women. *J. Nutr.* **2009**, *139*, 1896.
19. Hertog, M.G.L.; Hollman, P.C.H.; Kantan, M.B.; Kromhout, D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. *Nutr Cancer* **1993**, *20*, 21.
20. Formica, J.V.; Regelson, W. Review of the biology of quercetin and related bioflavonoids. *Food Chem. Toxicol.* **1995**, *33*, 1061.
21. Chung, M.I.; Gan, K.H.; Lin, C.N.; Ko, F.N.; Teng, C.M. Antiplatelet effects and vasorelaxing action of some constituents of formosan plants. *J. Nat. Prod.* **1993**, *56*, 929.
22. Lauro, M.R.; Torre, M.L.; Maggi, L.; De Simone, F.; Conte, U.; Aquino, R.P. Fast- and slow-release tablets for oral administration of flavonoids: rutin and quercetin. *Drug Dev. Ind. Pharm.* **2002**, *28*, 371.
23. Flower, D.R.; North, A.C.; Sansom, C.E. The lipocalin protein family: structural and sequence overview. *Biochim. Biophys. Acta* **2000**, *1482*, 9.
24. Schlehuber, S.; Skerra, A. Lipocalins in drug discovery: from natural ligand-binding proteins to anticalins. *Drug Discov. Today* **2005**, *10*, 23.
25. Sawyer, L.; Kontopidis, G. The core lipocalin, bovine β -lactoglobulin. *Biochim. Biophys. Acta.* **2000**, *1482*, 136.
26. Dong, A.; Matsuura, J.; Allison, S.D.; Chrisman, E.; Manning, M.C.; Carpenter, J.F. Infrared and circular dichroism spectroscopic characterization of structural differences between β -Lactoglobulin A and B. *Biochemistry* **1996**, *35*, 1450.
27. Brownlow, S.; Cabral, J.H.M.; Cooper, R.; Flwoer, D.R.; Yewdall, S.J.; Polikarpov, I.; North, A.C.T.; Sawyer, L. Bovine [beta]-lactoglobulin at 1.8 Å resolution—still an enigmatic lipocalin. *Structure* **1997**, *5*, 481.

28. Kontopidis, G.; Holt, C.; Sawyer, L. The ligand-binding site of bovine [beta]-lactoglobulin: evidence for a function? *J. Mol. Biol.* **2002**, *318*, 1043.
29. Kontopidis, G.; Holt, C.; Sawyer, L. [Beta]-lactoglobulin: binding properties, structure, and function. *J. Dairy Sci.* **2004**, *87*, 785.
30. Wu, S.Y.; Pérez, M.D.; Puyol, P.; Sawyer, L. β -lactoglobulin binds palmitate within its central cavity. *J. Biol. Chem.* **1999**, *274*, 170.
31. Thompson, M.A. *ArgusLab 4.0*, Planaria Software, LLC: Seattle, WA, <http://www.ArgusLab.com>.
32. Thompson, M.A. Molecular docking using arguslab: an efficient shape-based search algorithm and the AScore scoring function, Poster presentation, presented at the Fall ACS Meeting, Philadelphia, PA, **2004**.
33. Katarina, N.; Slavica, F.; Danica, A. QSAR study of imidazoline antihypertensive drugs. *Bioorganic Med. Chem.* **2008**, *16*, 7134.
34. Schmidt, M.W.; Baldrige, K.K.; Boatz, J.A.; Elbert, S.T.; Gordon, M.S.; Jensen, J.H.; Koseki, S.; Matsunaga, N.; Nguyen, K.A.; Su, S.J.; Windus, T.L.; Dupuis, M.; Montgomery, J.A. General atomic and molecular electronic structure system. *J. Comput. Chem.* **1993**, *14*, 1347.
35. Berendsen, H.J.C.; Van derSpoel, D.; Van Drunen, R. GROMACS: A message-passing parallel molecular dynamics implementation. *Comput. Phys. Commun.* **1995**, *91*, 43.
36. Lindah, E.; Hess, B.; Van derSpoel, D. GROMACS 3.0: a package for molecular simulation and trajectory analysis. *J. Mol. Model.* **2001**, *7*, 306.
37. Van Gunsteren, W.F.; Billeter, S.R.; Eising, A.A.; Hünenberger, P.H.; Krüger, P.K.H.C.; Mark, A.E.; Scott, W.R.P.; Tironi, I.G. *Biomolecular simulation: the GROMOS96 manual and user guide*. Vdf Hochschulverlag AG: Zürich, Switzerland, **1996**.
38. Van Gunsteren, W.F.; Daura, X.; Mark, A.E. The GROMOS force field. In *Encyclopedia of computational chemistry*, Vol. 2; Von Rague Schleyer, P., Ed.; Wiley: Chichester, UK, **1998**, pp. 1211–1216.
39. Schüttelkopf, A.W.; Van Aalten, D.M.F. PRODRG: a tool for high-throughput crystallography of protein-ligand complexes. *Acta Crystallogr.* **2004**, *60*, 1355.
40. Berendsen, H.J.C.; Postma, J.P.M.; Van Gunstretren, W.F.; Hermans, J. Interaction models for water in relation to protein hydration. In *Intermolecular forces*; Pullman, B., Ed.; Reidel: Dordrecht, Netherlands, **1981**, pp. 331–342.
41. Roufik, S.; Gauthier, S.F.; Leng, X.J.; Turgeon, S.L. Thermodynamics of binding interactions between bovine β -lactoglobulin A and the antihypertensive peptide β -Lg f142-148. *Biomacromolecules* **2006**, *7*, 419.
42. Mohammadi, F.; Bordbar, A.K.; Mohammadi, K.; Divsalar, A.; Saboury, A.A. Circular dichroism and fluorescence spectroscopic study on the interaction of bisdemethoxycurcumin and diacetyl-bisdemethoxycurcumin with human serum albumin. *Can. J. Chem.* **2010**, *88*, 155.
43. Renard, D. Etude de l'agrégation et de la gélfication des protéines globulaires: Application à la beta-Lactoglobuline. Dissertation thesis. Université de Nantes, Faculté des Science et des Techniques, France, **1994**.