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“Interaction of Saffron Ligands and β -Lactoglobulin”

In-Silico Study on the Interaction of Saffron Ligands and beta-Lactoglobulin by Molecular Dynamics and Docking Approach

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Abstract

Safranal, crocetin and dimethylcrocetin are secondary metabolites found in saffron and have a wide range of biological activities. An investigation of their interaction with a transport protein, such as β -lactoglobulin (β -lg), at the atomic level could be a valuable factor in controlling their transport to biological sites. The interaction of these ligands and β -lg, as a transport protein, was investigated using molecular docking and molecular dynamics simulation methods. The molecular docking results showed that safranal and crocetin bind on the surface of β -lg. However, dimethylcrocetin binds to the internal cavity of β -lg. The β -lg affinity for binding the saffron ligands decreases in the following order: crocetin > dimethylcrocetin > safranal. The analysis of MD simulation trajectories showed that the β -lg and β -lg–ligand complexes became stable at approximately 3000 ps and that there was little conformational change in the β -lg–safranal and β -lg–dimethylcrocetin complexes over a 10 ns timescale. In addition, the profiles of atomic fluctuations showed the rigidity of the ligand binding site during the simulation time.

Keywords

Saffron ligands, β -lactoglobulin, Binding affinity, Molecular docking, Molecular dynamics simulation

1. Introduction

Natural ligands have low toxicity and potent efficacy and are of particular interest as chemo-preventive compounds [1]. Saffron (*C. sativus* L.) is a plant in the Iridaceae family, planted in Iran, China, Turkey, India, Spain and Algeria; it has a wide range of biological activities including oxytocic, anti-carcinogenic, exhilarant, anti-depressant and anti-asthma effects [2]. It has been widely used as a herbal medicine and spice from the distant past. Saffron enhances the bioavailability and absorption of other drugs [2]. Previous studies have shown that there are more than 150 components in saffron stigmas [3]. The characterization of saffron showed the presence of vitamins, minerals, sugars, fats, and secondary metabolites. Among the secondary metabolites, carotenoids are the most important species because they control the color and taste of saffron and also have medicinal effects [4]. Safranal, crocetin and dimethylcrocetin are the three main chemical constituents of the carotenoids (Figure 1).

Safranal (2, 6, 6-trimethyl-1, 3-cyclohexadiene-1-carboxaldehyde, $C_{10}H_{14}O$) is an aromatic aldehyde which has anti-oxidant effects and reduces oxidative damages in various organs such as skeletal muscle, kidney and hippocampus [2, 5]. Some studies have shown that the genotoxic potency of methyl methanesulfonate (MMS)-induced DNA damage in the liver and lung of mice was inhibited by safranal [6]. Furthermore, in-vitro studies showed the minor groove binding of safranal to DNA [7].

Crocetin is a natural carotenoid dicarboxylic acid whose chemical structure is the central core of crocins [8]. Circular dichroism (CD) studies have shown that crocetin binds to the I-motif and G-quadruplex, unique secondary structures of telomeric DNA [9]. Recently, crocetin has been

shown to inhibit pancreatic cancer cell proliferation and tumor progression in a xenograft mouse model [10]. Furthermore, crocetin has a protective effect on the cyclophosphamide induced bladder toxicity [11]. Crocetin scavenging free radicals can protect cells from oxidative stress [12]. Crocetin reduces the growth of tumors by inhibiting nucleic acid synthesis, improving anti-oxidative systems, inducing apoptosis and hindering growth factor signaling pathways [13].

Dimethylcrocetin is synthesized from extracts of saffron [8]. The hydroxyl unit in the carboxylic groups in crocetin makes it suitable for reaction with free radicals. Hence, crocetin is more effective than dimethylcrocetin. Furthermore, the antioxidant activity of crocetin increases as the concentration increases; but for dimethylcrocetin, there is a maximum point in its activity as a function of concentration [14–16]. However, safranal has even lower antioxidant activity than crocetin and dimethylcrocetin.

The delivery of safranal, crocetin and dimethylcrocetin are poorly understood. The interaction of these natural ligands and a transport protein, such as bovine β -lactoglobulin (β -lg), could be a valuable factor to control their transport to biological sites and could increase their bioavailability.

β -lg is one of the natural lipocalin members that bind and transfer small hydrophobic ligands such as steroids, fatty acids, retinoids, vitamin D, and cholesterol [17-21]. β -lg is the major whey protein and has several genetic variants [22, 23]. The crystal structure shows that the 162 amino acids along the single polypeptide chain of bovine β -lg forms a calyx composed of an eight stranded antiparallel β -sheet [24].

Unfortunately, the clinical application of saffron ligands is limited due to their poor water solubility and low bioavailability caused by their hydrophobic structure. However, an important physicochemical property of β -lg is its ability to bind physiologically relevant ligands. Hence, β -lg can serve as a potential transport protein, especially for poor water-soluble molecules such as the saffron ligands. Furthermore, finding novel bioactive ligand molecules that interact with β -lg is of biotechnological and pharmacological importance. Such knowledge would contribute to a deeper understanding of molecular applications of this lipocalin. β -lg shows significant resistance against both gastric and simulated duodenal digestions. Therefore, it seems an appropriate candidate for the safe delivery and protection of pH sensitive drugs to the stomach. Moreover, the high stability of β -lg under acidic condition guarantees the safe delivery of hydrophobic ligands through the acidic condition of the stomach.

We have previously studied the interaction of flavonoids and biguanides with β -lg [25-28]. The molecular docking results revealed that citrus flavonoids bind in the internal cavity of β -lg and the β -lg–flavonoid complexes are stabilized through hydrophobic interactions, hydrogen bond interactions and π - π stacking interactions. In the present study we examined the interaction and protein conformation of β -lg with safranal, crocetin and dimethyl crocetin using molecular docking and molecular dynamics simulation studies. Finding new biologically active ligands to bind β -lg is very important, and not only can contribute to a 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.

2. Experimental

2.1. Molecular docking

The geometry of the ligands was optimized using Gaussian 03 at the level of B3LYP/6-31G** [29]. The crystal structure of β -lg (PDB ID: 3NPO) was taken from the Brookhaven Protein Data Bank (<http://www.rcsb.org/pdb>). The R-value of this file was 0.216, with the protein crystal prepared at pH=7.5. The water molecules of the protein .pdb file were removed, and missing hydrogen atoms and Gasteiger charges were added. Flexible-ligand docking was done using molecular docking software (AutoDock Version 4.2), the implemented empirical free energy function and the Lamarckian Genetic Algorithm (LGA) [30]. A blind docking with 126 lattice points along each of the X, Y, and Z axes was performed to find the active site of the ligands related to the β -lg. After determination of the active site, the dimensions of the grid map were selected as 60 points with a grid point spacing of 0.375 Å, to allow the ligand to rotate freely. 200 docking runs with 25,000,000 energy evaluations for each run were performed for each molecule. Cluster analysis (RMS tolerance equal to 0.5 Å) was then carried out on the docked results.

2.2. Molecular dynamics simulation

The lowest binding free 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 [31, 32] and the GROMOS96 43a1 force field [33, 34]. The Dundee PRODRG2.5 server was used to generate the topology parameters of the ligands [35]. The partial atomic charges of saffron ligands were calculated using Gaussian 03 [29] at the level of B3LYP/6-31G**. The complex was located in the cubic box with the periodic

boundary conditions. The box volume was 294.14 nm^3 ($6.6504 \times 6.6504 \times 6.6504 \text{ nm}^3$) and the minimum distance between the protein surface and the box was 1.0 nm. The box, filled with extended simple point charges (SPC), water molecules [36], and the solvated systems, were neutralized by adding 8 sodium ions (Na^+). Energy minimization was done through 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. A Berendsen thermostat [37] at 300 K, the particle mesh Ewald (PME) method [38,39] 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 the 2 fs time steps. The atomic coordinates were recorded to the trajectory file every 0.5 ps for later analysis. Finally, an all-bond constraint was used to keep the ligand from drifting in the MD.

3. Results and discussion

3.1. Molecular docking studies

β -lg consists of 162 amino acids, with three potential binding sites: 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 [40]. The initial crystal structure analyses have shown that most ligands are bound to the internal cavity of the β -barrel [41–43]. Besides the primary drug binding site in the internal cavity of the β -barrel, some compounds, such as p-nitrophenyl phosphate, 5-fluorocytosine, ellipticine and protoporphyrin, bind to the outer surface site [44, 45]. The best energy ranked results of the interaction between the ligands and β -lg in the docking procedures are shown in Fig. 2. As shown, safranal and crocetin were placed on the surface of β -

lg and dimethylcrocetin was placed within the internal cavity of β -lg with the binding energies ($\Delta G^\circ_{\text{bind}}$) of -4.9, -7.67 and -7.26 kcal.mol⁻¹ for safranal, crocetin, and dimethylcrocetin, respectively. All of these compounds were near some of the hydrophobic residues of the β -lg. Furthermore, the drugs were able to form hydrogen bond interactions with residues of the β -lg. Figure 3a shows that Gln(59) interacts with safranal by one hydrogen bond interaction. Also, as can be seen in Fig. 3b, Leu(1), Gly(9) and Lys(91) interact with crocetin by three hydrogen bond interactions. Finally, Fig. 3c shows that Asn(88), Asn(90) and Lys(69) also interact with dimethylcrocetin by three hydrogen bond interactions. Thus, it may be concluded that, in addition to the hydrophobic interactions which stabilize the protein-ligand complexes, the hydrogen bond interactions also play an important role in the stability of β -lg–ligand complexes. The comparison of the $\Delta G^\circ_{\text{bind}}$ values with the results of the our previous study on the interaction of β -lg with citrus flavonoids (-10.91, -10.44, -9.15 and -7.93 kcal.mol⁻¹ for naringenin, hesperetin, tangeretin and nobiletin, respectively [28]) indicates that β -lg has less affinity for saffron ligands than for the flavonoids.

3.2. Analysis of the MD simulation trajectories

The beginning structures for the MD analyses were selected from the conformations with lowest docking energies. The trajectories were analyzed in terms of root mean square deviation (RMSD), radius of gyration (R_g), total solvent accessible surface (SAS) and root mean square fluctuation (RMSF) using the GROMACS routines.

Root mean square deviation

The stability of trajectories for unliganded β -lg and β -lg–ligands was examined using the RMSD of the backbone of β -lg (Figure 4). A glance at the analysis in Fig. 4 shows that the RMSD of all systems reached equilibrium and fluctuated around the mean value at about 3000 ps. In other words, the RMSD of the back bone atoms increased up to about 0.20 nm and then stabilized around 7 ns and remained stable till the end of the simulation, demonstrating that the molecular system was well behaved afterward.

Radius of gyration

The R_g s for all systems were also quantified and plotted versus simulation time to examine the protein compactness (Figure 5). The results showed that all of the systems stabilized after about 2500 ps, indicating that the MD simulation equilibrated after 2500 ps. It can be clearly seen that the R_g was larger upon binding of the safranal and dimethylcrocetin than for β -lg and the bound crocetin, suggesting a less compact structure after the binding of these two ligands to β -lg. Also, Fig. 5 shows that the R_g value of β -lg did not depend upon the complexation with crocetin. This indicates that the environment of β -lg did not change during its interaction with crocetin.

Solvent accessible surface (RMSF)

The changes in the total solvent accessible surface (SAS) of β -lg during the 10 ns simulation time are shown in Fig. 6. The variations of SAS were similar to the variations of R_g . This similarity confirms the correctness of the MD simulation results.

Root mean square fluctuation

The mobility of the β -lg residues was evaluated by analysis of the RMSF of the C_α atoms of β -lg in the absence and presence of the saffron ligands (Figure 7). The results indicate that the fluctuations larger than 0.2 nm corresponded to the residues that are far from the binding sites of each ligand. Moreover, the residues that were in contact with the saffron ligands are the most stable and have lower RMSF values.

In addition, the RMSF of the atomic positions of the saffron ligands was calculated to examine their conformational variations (Figure 8). The results indicate that the saffron ligands atoms showed limited fluctuations (<0.30 nm). Hence, it can be concluded that the interactions of β -lg and the saffron ligands were stable during the simulation time.

4. Conclusions

In this work the interaction between saffron ligands and β -lg has been investigated using molecular docking and molecular dynamics simulation techniques. On the basis of the molecular docking results, safranal and crocetin bind on the surface of β -lg. However, dimethylcrocetin binds in the internal cavity of β -lg. The β -lg affinity for binding the saffron ligands decreases in the following order: crocetin > dimethylcrocetin > safranal. In addition, the docking results revealed that the hydrophobic and hydrogen bond interactions play a major role in the β -lg–ligands complex stability. MD simulation studies showed the stabilization of β -lg and β -lg–ligand complexes at around 3 ns. It can be clearly seen that the R_g and SAS increase upon binding of the safranal and dimethylcrocetin suggesting a less compact structure after the binding of these two ligands to β -lg. Also, the environment of β -lg did not change during its interaction

with crocetin. Furthermore, the profiles of the atomic fluctuations showed the interactions of β -lg and saffron ligands were stable during the simulation time.

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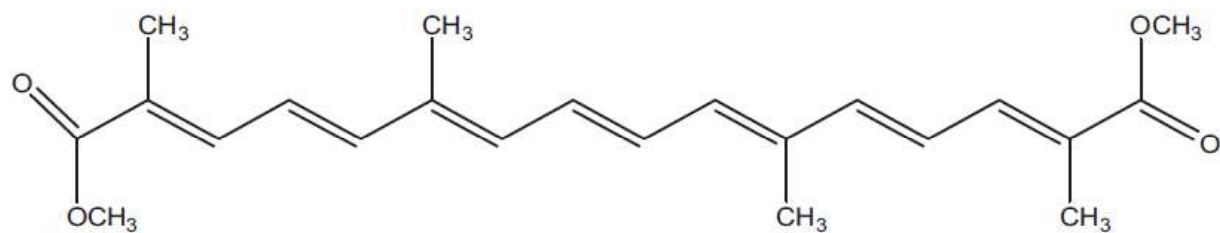
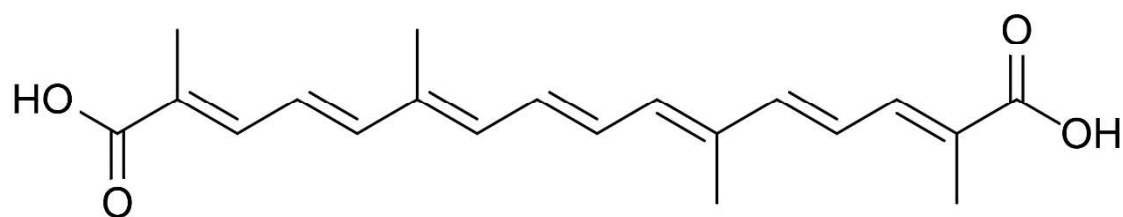
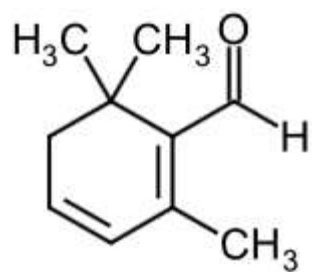


Figure 1. The chemical structures of a) safranal; b) crocetin and c) dimethylcrocetin.



Figure 2. a) safranal; b) crocetin and c) and dimethylcrocetin docked in β -lg using AutoDock. Ligands, depicted as a cylinder model, and β -lg, represented in cartoon ribbon.

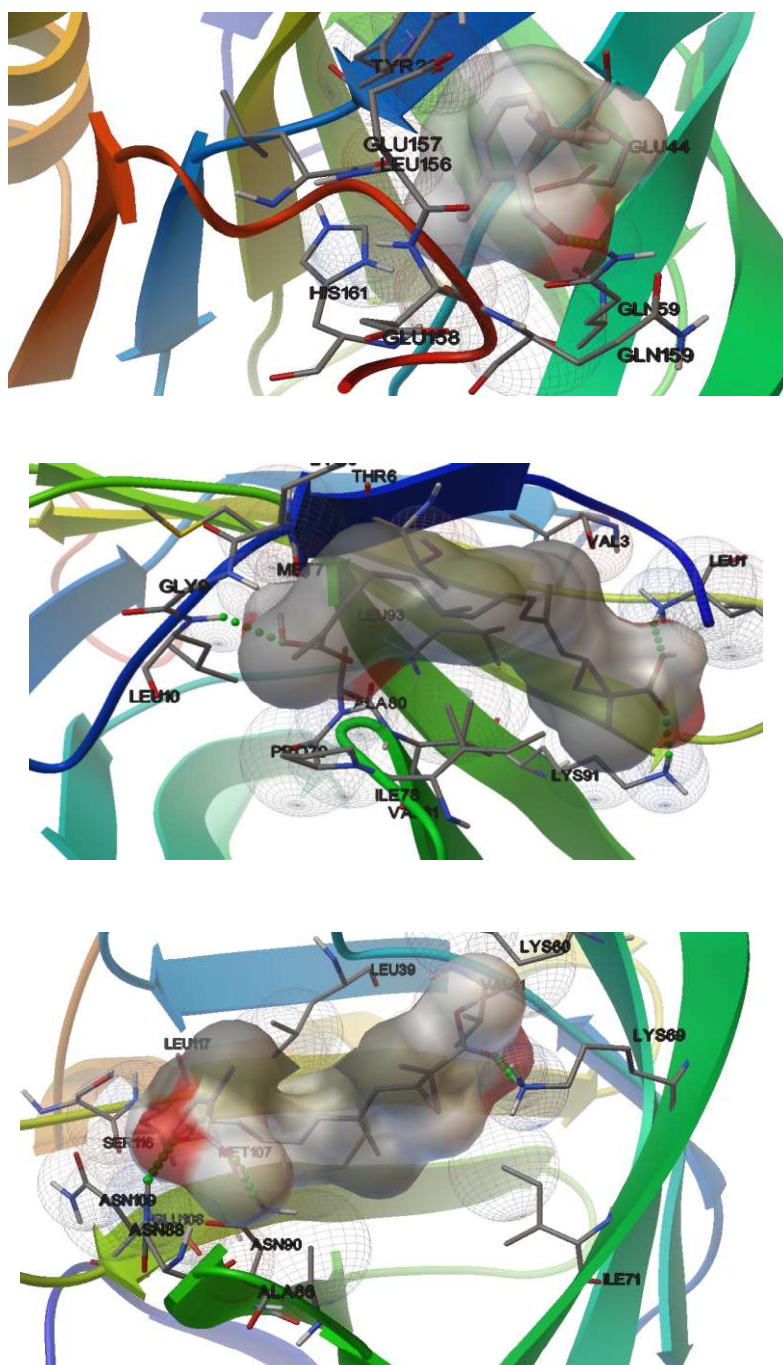


Figure 3. The docking poses of the β -lg–ligand complexes. a) safranal; b) crocetin and c) dimethylcrocetin. Hydrogen bond interactions are shown as spheres.

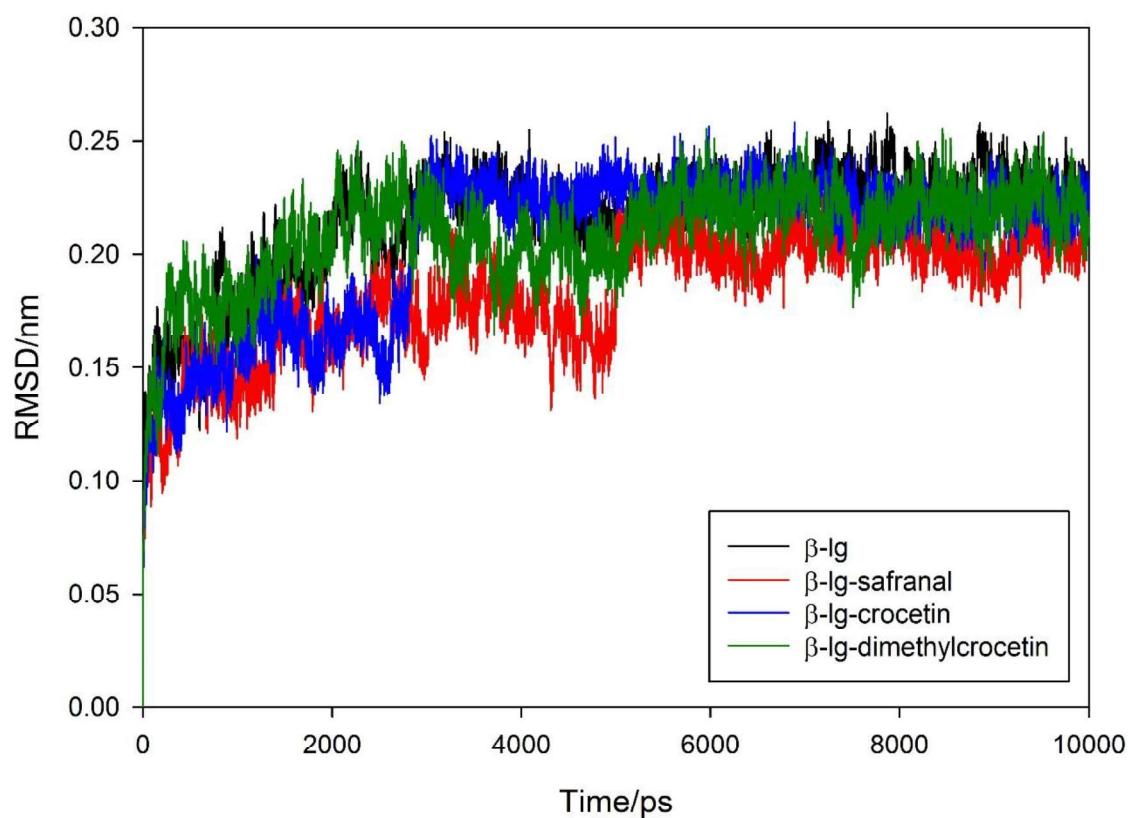


Figure 4. Time dependence of RMSD. RMSD values for unliganded β -lg and β -lg–ligand complexes. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

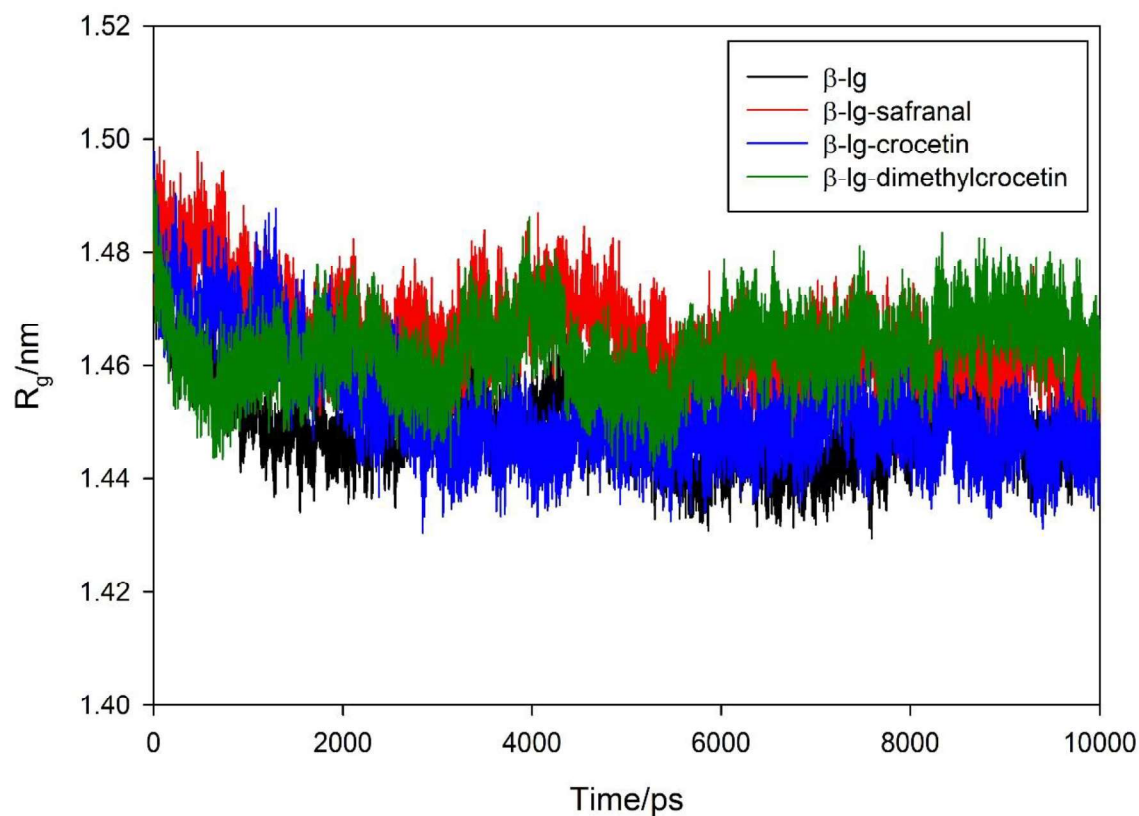


Figure 5. Time evolution of the radius of gyration (R_g) during 10000 ps of MD simulation of unliganded β -lg and β -lg–ligand complexes. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

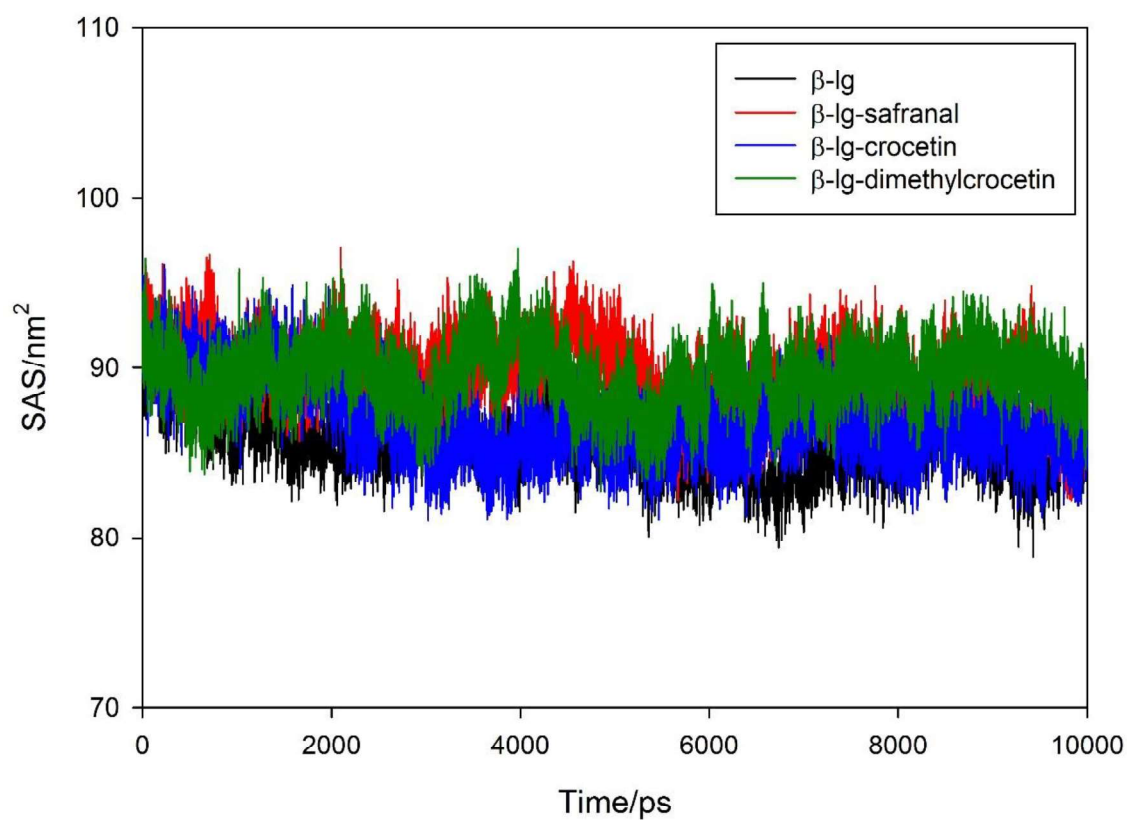


Figure 6. Time evolution of the total solvent accessible surface (SAS) during 10000 ps of MD simulation of unliganded β -lg and β -lg–ligand complexes. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

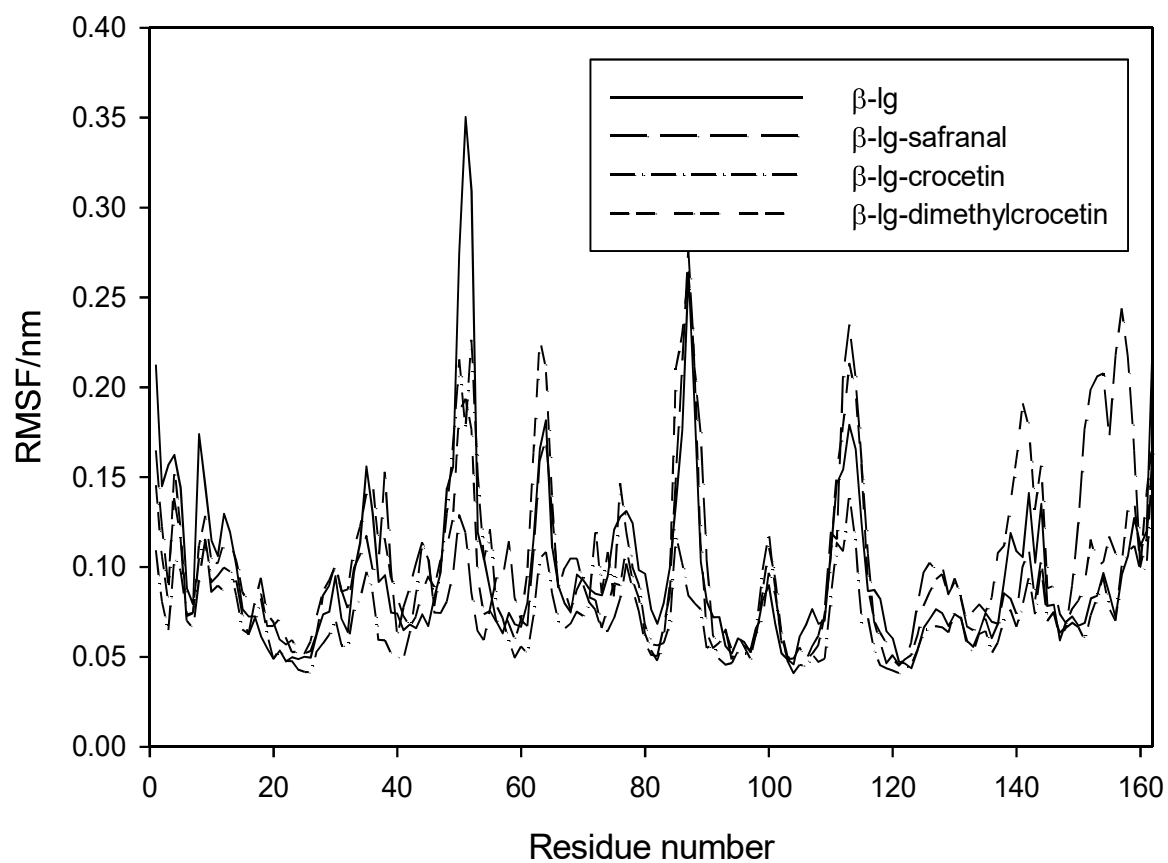


Figure 7. The RMSF values of unliganded β -lg and β -lg–ligand complexes were plotted against residue numbers.

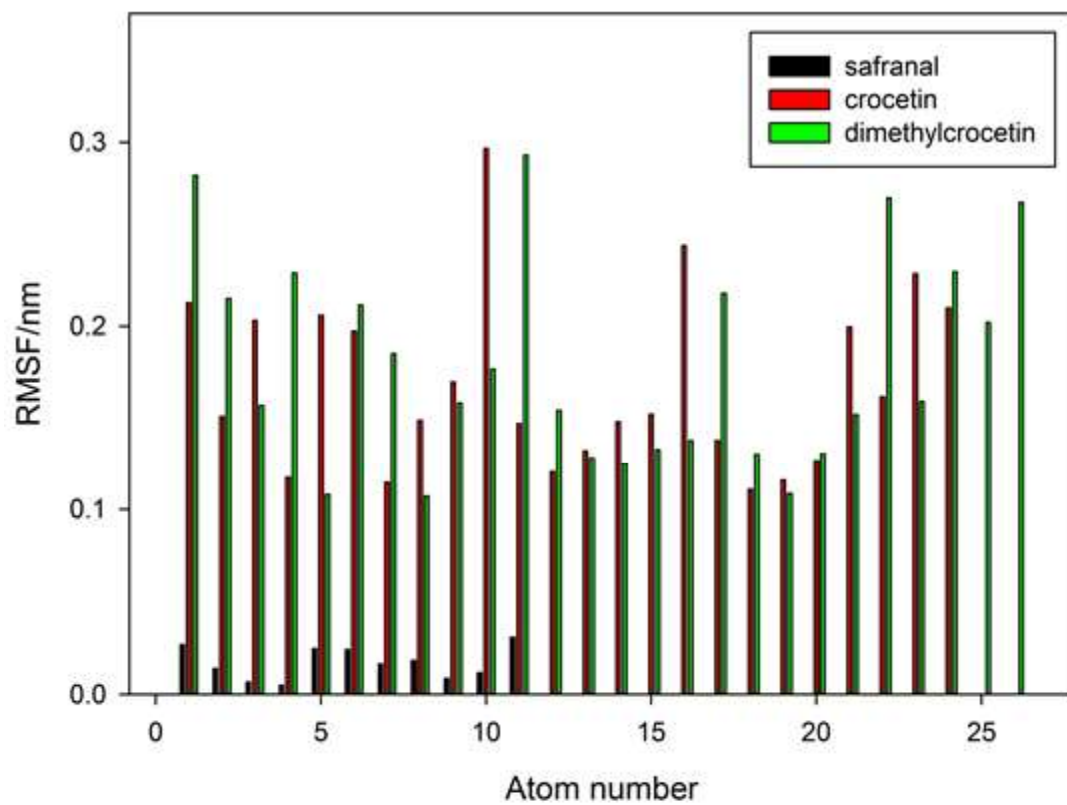


Figure 8. RMSF values of saffron ligands were plotted against atom numbers.