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Protein-ligand Interaction Modeling between Bovine Serum Albumin (BSA) with M3A and M3GA using Molecular Docking Simulations

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ABSTRACT

Introduction: Anthocyanins are natural pigments with potential applications as food colorants; however, their instability under environmental conditions limits their utilization. Protein–anthocyanin interactions, particularly with Bovine Serum Albumin (BSA), may improve anthocyanin stability through non-covalent complex formation. This study aimed to investigate the interaction between BSA and two anthocyanin derivatives, namely Malvidin-3-O-arabinoside (M3A) and Malvidin-3-O-galactoside (M3GA), using molecular docking simulations.

Materials and Methods: Molecular docking simulations were performed using the crystal structure of BSA (PDB ID: 4F5S). Ligand structures of M3A and M3GA were obtained from the PubChem database and optimized using the MMFF94 force field. Protein preparation and visualization analyses were conducted using BIOVIA Discovery Studio Visualizer and ChimeraX, while docking simulations were carried out using the CB-Dock2 platform. Binding affinity and ligand–receptor interaction profiles were analyzed based on binding free energy (ΔG) values and amino acid interactions.

Results: Both M3A and M3GA exhibited favorable binding affinity toward BSA with identical binding free energy values of −8.2 kcal/mol, indicating spontaneous and thermodynamically stable interactions. Interaction analysis revealed that the complexes were stabilized through hydrogen bonds, hydrophobic interactions, electrostatic interactions, Pi–Pi stacked interactions, and van der Waals forces. M3GA formed more hydrogen bond interactions, whereas M3A showed dominant hydrophobic and electrostatic interactions.

Conclusion: The results suggest that both anthocyanin derivatives can form stable complexes with BSA, indicating the potential role of BSA as a stabilizing carrier protein for anthocyanins in food and pharmaceutical applications. Further experimental studies are needed to validate the stability of these complexes under physiological and processing conditions.

Keywords: Anthocyanin, Bovine Serum Albumin, Molecular Docking, Malvidin-3-O-arabinoside, Malvidin-3-O-galactoside

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INTRODUCTION

Anthocyanins are natural water-soluble pigments widely distributed in fruits, flowers, and other plant tissues. These compounds are responsible for red, purple, and blue coloration and have attracted considerable attention as potential natural food colorants due to increasing concerns regarding the safety of synthetic dyes [1]. In addition to their coloring properties, anthocyanins also exhibit various biological activities, including antioxidant and anti-inflammatory effects, making them valuable compounds for food and pharmaceutical applications [2].

Chemically, anthocyanins belong to the flavonoid class of polyphenolic compounds and are characterized by a flavylium cation structure consisting of two aromatic rings (A and B) linked by a heterocyclic ring (C). The stability of anthocyanins is strongly influenced by their chemical structure and environmental conditions such as pH, temperature, light exposure, oxygen, and metal ions [3]. Despite their beneficial properties, anthocyanins are highly unstable under neutral and alkaline conditions, leading to rapid degradation during processing and storage. This instability remains one of the major limitations for their practical application in food systems [1,8].

One promising strategy to improve anthocyanin stability is through complex formation with proteins. Protein–anthocyanin interactions can protect anthocyanin molecules from environmental degradation by forming non-covalent interactions such as hydrogen bonding, hydrophobic interactions, and van der Waals forces [4]. Among various food-grade proteins, Bovine Serum Albumin (BSA) has been extensively studied because of its high ligand-binding capacity, structural stability, and ability to interact with polyphenolic compounds [5]. BSA is known to possess several hydrophobic binding sites that facilitate interaction with anthocyanin molecules, potentially enhancing their stability and bioavailability [4].

Molecular docking simulation is a computational approach widely used to predict interaction mechanisms and binding affinity between ligands and target proteins. This method provides valuable information regarding binding orientation, interaction energy, and amino acid residues involved in complex formation [6,12]. Previous studies have demonstrated that BSA exhibits favorable binding interactions with several anthocyanin compounds compared with other proteins, suggesting its potential role as a stabilizing agent for anthocyanins [7].

Therefore, the present study aimed to investigate the protein–ligand interactions between Bovine Serum Albumin (BSA) and two anthocyanin compounds, namely Malvidin-3-O-Arabinose (M3A) and Malvidin-3-O-galactoside (M3GA), using molecular docking simulations. The findings of this study are expected to provide insights into the binding characteristics and interaction mechanisms of BSA with anthocyanin derivatives, which may contribute to the development of more stable natural colorant systems for food applications.

MATERIALS AND METHODS

Research Design

This study employed an in silico molecular docking approach to investigate protein–ligand interactions between Bovine Serum Albumin (BSA) and two anthocyanin compounds, namely Malvidin-3-O-Arabinose (M3A) and Malvidin-3-O-galactoside (M3GA). The study consisted of ligand preparation, protein preparation, ligand structure optimization, docking validation, and molecular docking simulations.

Materials and Software

The target protein used in this study was Bovine Serum Albumin (BSA), obtained from the Protein Data Bank (PDB) with PDB ID 4F5S in PDB format. The ligands used were anthocyanin compounds identified from “Linmai 1”, namely Malvidin-3-O-Arabinose (M3A) and Malvidin-3-O-galactoside (M3GA), selected based on previously reported binding affinity values [7].

The computational analysis was performed using an Advan TBook N100 laptop equipped with an Intel® Celeron® Alder Lake-N N100 processor and 4 GB DDR4 RAM. The software and online platforms used in this study included PubChem, UCSF ChimeraX, Avogadro, BIOVIA Discovery Studio Visualizer, CB-Dock2, and SwissADME.

Ligand Preparation

The chemical structures of Malvidin-3-O-Arabinose (M3A) and Malvidin-3-O-galactoside (M3GA) were obtained from the PubChem database in Structure Data File (SDF) format. The downloaded two-dimensional ligand structures were imported into UCSF ChimeraX and converted into Protein Data Bank (PDB) format for further computational analysis [8].

Protein Preparation

The crystallographic structure of Bovine Serum Albumin (BSA) with PDB ID 4F5S was downloaded from the RCSB Protein Data Bank in PDB format. Protein preparation was performed using BIOVIA Discovery Studio

Visualizer by removing co-crystallized ligands and water molecules from the protein structure to obtain the purified macromolecule for docking simulations. Protein preparation is essential to improve docking accuracy and minimize interference from non-essential molecules [12].

Ligand Structure Optimization

Ligand structure optimization was conducted using Avogadro version 1.2.0 for Windows [9]. The ligand structures were energy-minimized using the Merck Molecular Force Field 94 (MMFF94) method to obtain stable conformations with lower potential energy. The optimized structures were subsequently saved in MOL2 format for molecular docking analysis. The MMFF94 force field is widely used for geometry optimization of small organic molecules due to its reliability in predicting molecular conformations [10].

Molecular Docking Simulation

Molecular docking simulations were performed using the CB-Dock2 web server. The prepared protein and optimized ligand structures were uploaded into the CB-Dock2 platform. CB-Dock2 automatically identified potential binding cavities and performed blind docking simulations based on cavity-guided docking algorithms [11]. The docking results were evaluated based on binding affinity values and interaction profiles between ligands and amino acid residues within the active site of the protein.

Ethical Consideration

This study did not involve human participants or experimental animals; therefore, ethical approval was not required.

Generative Artificial Intelligence (GenAI) Disclosure

Generative artificial intelligence (GenAI) tools were used solely for language refinement, grammar correction, and improvement of scientific writing clarity. No AI tools were used for data generation, experimental design, data analysis, or interpretation of results.

RESULTS AND DISCUSSION

Molecular docking simulations were performed to evaluate the binding affinity and interaction patterns between anthocyanin compounds and Bovine Serum Albumin (BSA; PDB ID: 4F5S). Two-dimensional interaction visualization demonstrated that both ligands, namely Malvidin-3-O-galactoside (M3Ga) and Malvidin-3-O-arabinoside (M3A), occupied the binding pocket of the protein, although they exhibited different interaction profiles. Differences in interaction types and amino acid residues involved are known to influence the stability of protein–ligand complexes [13].

Binding Affinity Analysis

According to the molecular docking simulations (Table 1), the binding free energy (ΔG) values of both anthocyanin derivatives against Bovine Serum Albumin (BSA) were successfully obtained. Binding free energy is widely used to evaluate the strength and stability of protein–ligand interactions, where more negative ΔG values indicate stronger binding affinity and a more stable complex formation (Trott & Olson, 2010). The docking results demonstrated that both Malvidin-3-O-arabinoside (M3A) and Malvidin-3-O-galactoside (M3GA) exhibited similar binding affinities toward BSA, with ΔG values of −8.2 kcal/mol.

Table 1. Comparison of Binding Free Energy (ΔG) Values between Anthocyanin Compounds and Control Ligand toward BSA

Protein Target	Ligand	Binding free energy (ΔG) of Compound test (kcal/mol)
Bovine Serum Albumin (BSA)	Malvidin-3-O-arabinoside (M3A)	−8.2
Bovine Serum Albumin (BSA)	Malvidin-3-O-galactoside (M3Ga)	−8.2
Bovine Serum Albumin (BSA)	Control	−8.2

Interestingly, the binding energy values of both anthocyanin compounds were comparable to that of the control ligand, suggesting that M3A and M3GA possess favorable interaction capabilities with the target protein. In molecular docking studies, binding energies within this range generally reflect spontaneous interactions stabilized

through non-covalent forces such as hydrogen bonding, hydrophobic interactions, electrostatic interactions, and van der Waals forces [13].

These findings indicate that both anthocyanin derivatives have binding affinities comparable to the control ligand, highlighting their potential to interact stably with BSA. The comparable ΔG values also suggest that slight structural differences between arabinoside and galactoside substituents did not substantially affect the thermodynamic stability of the resulting protein–ligand complexes.

Ligand–Receptor Interaction Analysis

a. Interaction between Bovine Serum Albumin (BSA) and Malvidin-3-O-galactoside (M3Ga)

Visualization of the molecular docking results demonstrated that Malvidin-3-O-galactoside (M3Ga) was able to bind stably within the binding cavity of Bovine Serum Albumin (BSA; PDB ID: 4F5S) with a binding free energy (ΔG) value of −8.2 kcal/mol (Figure 1). The negative ΔG value indicates that the formation of the protein–ligand complex occurred spontaneously and was thermodynamically favorable. In molecular docking studies, lower binding energy values are generally associated with stronger ligand affinity and greater complex stability [6].

Two-dimensional interaction analysis revealed that the stability of the BSA–M3Ga complex was primarily supported by the formation of four conventional hydrogen bonds involving residues ARG427, SER428, ARG435, and LEU454. Hydrogen bonding is considered one of the most important non-covalent interactions in protein–ligand recognition because it contributes significantly to ligand orientation, specificity, and stabilization within the binding pocket [14]. The presence of multiple hydrogen bonds suggests that the hydroxyl groups of the anthocyanin structure actively participate in intermolecular interactions with polar amino acid residues of BSA.

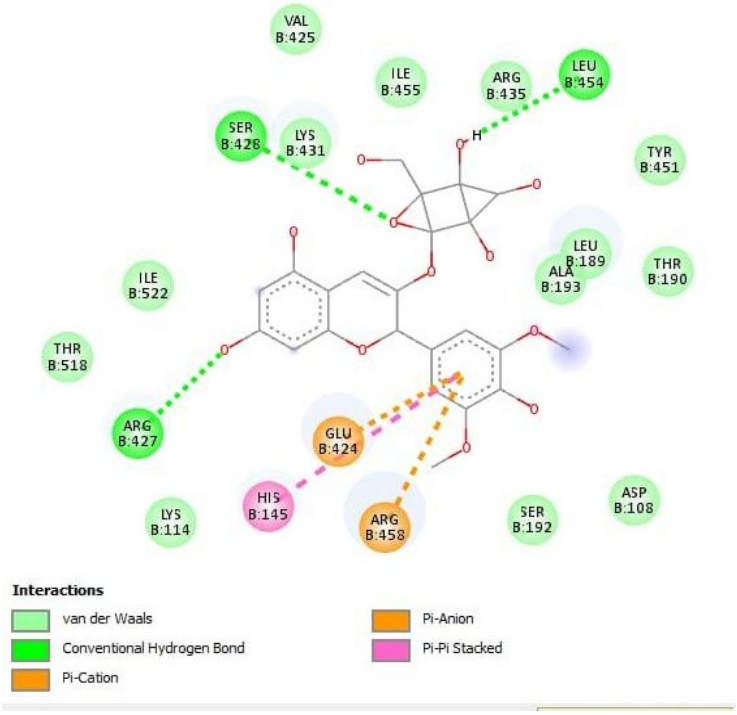

Figure 1. Two-dimensional interaction diagram of Malvidin-3-O-galactoside (M3Ga) with Bovine Serum Albumin (BSA; PDB ID: 4F5S) generated using molecular docking simulation. The ligand formed conventional hydrogen bonds with residues ARG427, SER428, ARG435, and LEU454, as well as Pi–Pi stacked interaction with HIS145. Additional electrostatic interactions, including Pi–Cation and Pi–Anion interactions, were observed with ARG458 and GLU424, respectively. Several surrounding residues, such as VAL425, ILE455, TYR451, LEU189, and THR190, also contributed through van der Waals interactions, indicating stable ligand accommodation within the BSA binding pocket**.**

In addition to hydrogen bonding, the ligand exhibited a Pi–Pi stacked interaction with residue HIS145 through aromatic overlap between the benzopyrylium ring system of the anthocyanin molecule and the aromatic side chain of the amino acid residue. Aromatic stacking interactions are known to enhance conformational stability and contribute to the stabilization of flavonoid–protein complexes [15]. Electrostatic interactions in the form of Pi–

Cation and Pi–Anion interactions were also observed with residues ARG458 and GLU424, respectively. These electrostatic interactions likely contributed to stabilizing charge distribution within the protein–ligand complex and strengthening intermolecular attraction.

Furthermore, several van der Waals interactions were identified with residues VAL425, ILE455, TYR451, THR190, LEU189, ALA193, SER192, ASP108, LYS114, THR518, ILE522, and LYS431. Van der Waals interactions are essential for maintaining steric complementarity between ligands and protein cavities and often contribute cumulatively to overall binding stability [13]. The large number of van der Waals contacts observed in this complex indicates that the M3Ga molecule fits favorably within the geometry of the BSA binding pocket.

Overall, the interaction profile suggests that Malvidin-3-O-galactoside forms a stable complex with BSA through a combination of hydrogen bonding, aromatic stacking, electrostatic interactions, and van der Waals forces. These findings support previous reports indicating that anthocyanin compounds can interact effectively with serum albumin proteins and potentially improve anthocyanin stability through non-covalent complex formation [4].

b. Interaction between Bovine Serum Albumin (BSA) and Malvidin-3-O-arabinoside (M3A)

The molecular docking simulation demonstrated that Malvidin-3-O-arabinoside (M3A) was capable of binding stably within the binding cavity of Bovine Serum Albumin (BSA; PDB ID: 4F5S) with a binding free energy (ΔG) value of −8.2 kcal/mol (Figure 2). The negative ΔG value indicates that the interaction between the ligand and the protein occurred spontaneously and possessed favorable thermodynamic stability. Binding energies within this range are generally associated with moderate to strong protein–ligand affinity stabilized through non-covalent intermolecular interactions [6].

Two-dimensional interaction analysis revealed that the stability of the M3A–BSA complex was primarily supported by conventional hydrogen bonds involving residues THR190, ARG435, and SER428. Hydrogen bonds are essential in maintaining ligand orientation and enhancing molecular recognition between ligands and receptors, thereby contributing significantly to the stability and specificity of protein–ligand complexes [14]. The hydroxyl groups present in the anthocyanin structure likely played an important role as hydrogen bond donors and acceptors during these interactions.

In addition to hydrogen bonding, the M3A–BSA complex exhibited several hydrophobic interactions in the form of Alkyl and Pi–Alkyl interactions involving residues ARG196, LEU189, ALA193, LEU454, and TYR451. Hydrophobic interactions are known to stabilize protein–ligand complexes by reducing unfavorable exposure of non-polar molecular regions to the aqueous environment and improving steric complementarity within the binding cavity [13]. These interactions suggest that the aromatic ring system of M3A was favorably accommodated within the hydrophobic regions of the BSA binding pocket.

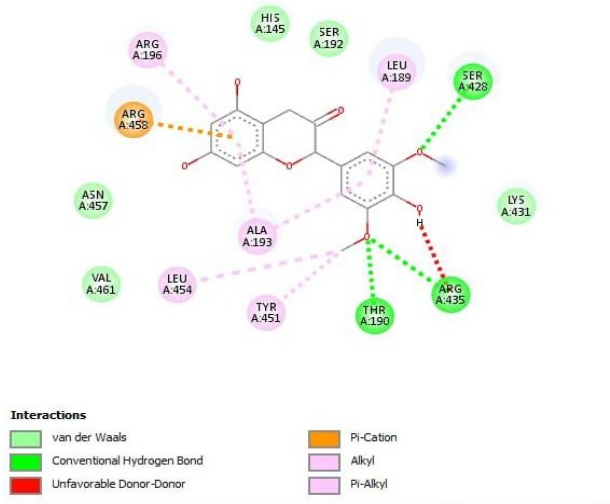

Figure 2. Two-dimensional interaction diagram of Malvidin-3-O-arabinoside (M3A) with Bovine Serum Albumin (BSA; PDB ID: 4F5S) generated using molecular docking simulation. The ligand formed conventional hydrogen bonds with THR190, ARG435, and SER428, as well as hydrophobic Alkyl and Pi–Alkyl interactions with ARG196, LEU189, ALA193, LEU454, and TYR451. A Pi–Cation interaction with ARG458 and extensive van der Waals interactions with surrounding residues contributed to the stabilization of the protein–ligand complex. An unfavorable donor–donor interaction with ARG435 was also observed.

An additional electrostatic Pi–Cation interaction with residue ARG458 was also observed. Pi–Cation interactions are considered important stabilizing forces in protein–ligand complexes because they involve attractive electrostatic interactions between positively charged amino acid residues and aromatic electron clouds of ligands [16]. In anthocyanin compounds, the conjugated aromatic benzopyrylium structure provides an ideal system for such interactions.

Interestingly, an unfavorable donor–donor interaction with residue ARG435, represented by the red dashed line in the interaction diagram, was also detected. This type of interaction may arise from electrostatic repulsion between closely positioned proton donor groups, which can locally reduce interaction stability. However, the destabilizing effect of this unfavorable interaction appeared to be compensated by multiple van der Waals interactions involving residues HIS145, SER192, LYS431, ASN457, VAL461, and ALA193. Van der Waals interactions, although individually weak, collectively contribute substantially to maintaining the structural stability and proper accommodation of ligands within protein binding cavities [14].

Overall, the interaction profile indicates that M3A interacts effectively with BSA through a combination of hydrogen bonding, hydrophobic interactions, electrostatic forces, and van der Waals interactions. The presence of multiple stabilizing interactions suggests that M3A possesses considerable potential to form stable protein–anthocyanin complexes with BSA, which may contribute to improved anthocyanin stability in biological and food systems. These findings are consistent with previous studies reporting that serum albumin proteins can act as carrier and stabilizing agents for anthocyanin compounds through non-covalent complex formation [4].

CONCLUSIONS

This study successfully investigated the protein–ligand interactions between Bovine Serum Albumin (BSA; PDB ID: 4F5S) and two anthocyanin derivatives, namely Malvidin-3-O-arabinoside (M3A) and Malvidin-3-O-galactoside (M3GA), using molecular docking simulations. The docking results demonstrated that both compounds exhibited favorable binding affinity toward BSA, with identical binding free energy (Δ*G*) values of −8.2 kcal/mol, indicating spontaneous and thermodynamically stable complex formation. The interaction analysis further revealed that the stability of the complexes was supported by various non-covalent interactions, including hydrogen bonding, hydrophobic interactions, electrostatic interactions, Pi–Pi stacked interactions, and van der Waals forces.

Among the two ligands, M3GA showed a relatively more extensive hydrogen bonding network and aromatic interactions, while M3A exhibited stronger hydrophobic and electrostatic interaction profiles. These findings suggest that both anthocyanin derivatives possess good compatibility with the BSA binding cavity and may potentially form stable protein–anthocyanin complexes. The results also support the potential role of BSA as a stabilizing carrier protein for anthocyanin compounds, which could be beneficial for improving anthocyanin stability in food and pharmaceutical applications.

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Informed Consent Statement
Not applicable. This study did not involve human participants or patient data, as the research was conducted entirely using *in silico* molecular docking simulations

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During the preparation of this manuscript, the authors used ChatGPT by OpenAI for language refinement, grammar correction, and improvement of scientific writing clarity. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

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