Interaction Mechanism of Mangiferin and Ovalbumin Based on Spectrofluorimetry and Molecular Docking

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Abstract
Mangiferin (MAG) is a kind of polyphenol with many bioactivities. However, its application in medicines and functional foods is restricted because of its poor aqueous solubility and stability. The construction of a MAG/protein complex is an effective way to solve this bottleneck. In this study, the interaction of MAG and ovalbumin (OVA) was systematically investigated by spectrofluorimetry, and their binding mode was clarified based on molecular docking. The results suggested that MAG could cause the static fluorescence quenching of OVA with the quenching constant ($K_q$) of $> 2 \times 10^{10}$ L/(mol·s). Their binding performance increased with increasing temperature, and the binding-site number ($n$) was close to 1. The thermodynamic analysis indicated that the binding was a spontaneous process, which was mainly driven by hydrophobic force. During this process, there was no apparent change in the microenvironment surrounding the tyrosine and tryptophan residues of OVA. The molecular docking results demonstrated the hydrophobic interaction and hydrogen bonding in the complex, which well-confirmed the results of the fluorescence experiments.

Keywords
mangiferin, ovalbumin, fluorescence quenching, synchronous fluorescence, molecular docking

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Introduction
Mangiferin (MAG; Figure 1) is a kind of polyphenol, which is mainly found in the fruits, leaves, and barks of Mangifera indica L., the rhizomes, aboveground parts of Anemarrhena asphodeloides Bge., and the flowers and leaves of Belamcanda chinensis (L.) DC (now Iris domestica [L.] Goldblatt & Mabb).¹ It has many health-promoting functions, such as antioxidant, antidiabetic, antibacterial, antitumor, antihypertensive, neuroprotective, and immunoregulatory activities.² Moreover, it has therapeutic effects on atopic dermatitis, bronchial asthma, and other allergic diseases.³,⁴ The latest research indicates that it can reduce the harmful effects of drugs, heavy metals, and environmental chemicals,⁵ and treat osteoarthritis pain.⁶ However, its poor aqueous solubility and stability not only reduce its bioavailability, but also limit its application in medicines and functional foods.⁷ In order to promote its application, Wu et al⁸ synthesized MAG glucosides through glycosylation catalyzed by maltogenic amylase, and Liu et al⁹ prepared MAG microparticles by using a supercritical antisolvent method.

Proteins and polyphenols tend to bind during the processing, transportation, and storage of plant-derived foods and medicines, thus affecting their structural and functional properties.¹⁰–¹² The obtained protein–polyphenol complex is more stable in both physical and chemical properties, which can significantly enhance the aqueous solubility and stability of polyphenols.¹³,¹⁴ Therefore, the construction of a MAG/protein complex is expected to promote the application of MAG.

Ovalbumin (OVA) is a typical globulin with 385 amino acid residues, which accounts for 54% of the total egg white protein. It can be used as a drug carrier to transport hydrophobic components and achieve efficient delivery.¹⁵ Chang et al¹⁶ prepared a curcumin/OVA complex to reduce the degradation rate of curcumin. Liang et al¹⁷ reported curcumin-loaded OVA nanoparticles, which were used for the treatment of allergies. To the best of our knowledge, there is no research on the interaction between MAG and OVA. Herein, the interaction mechanism of MAG and OVA was systematically investigated by spectrofluorimetry and molecular docking.

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Results and Discussion

Fluorescence Quenching

Fluorescence spectrometry is one of the common methods to investigate the interaction mechanisms between drug molecules and proteins in a physiological environment.\textsuperscript{18–20} Figure 2A shows the effect of MAG on the fluorescence spectrum of OVA at 29 °C. With the addition of MAG, the fluorescence intensity of OVA decreased gradually, indicating that MAG could cause the fluorescence quenching of OVA. When comparing the fluorescence spectra obtained at different binding temperatures (Figure 2A–C), it was found that at the same MAG concentration, the fluorescence intensity of OVA decreased with the rise in temperature, which suggested that the temperature could prompt the interaction in the test range.

Quenching Constant

The mechanisms of fluorescence quenching can be generally categorized into 2 types, dynamic quenching and static quenching.\textsuperscript{21–23} The quencher triggers the dynamic quenching by striking the fluorophores of the excited state molecules, causing them to lose their excitation energy and return to the ground state. The static quenching is due to the formation of a nonfluorescent ground state complex between the fluorescence molecule and the quencher. Both types of fluorescence quenching mechanisms follow the Stern–Volmer equation.\textsuperscript{24–26}

Figure 1. Chemical structure of mangiferin (MAG).

Figure 2. Effect of MAG on fluorescence spectra of OVA at 29 (A), 33 (B), and 37 °C (C); Van’t Hoff plot for the interaction of OVA with MAG (D).

Abbreviations: MAG, mangiferin; OVA, ovalbumin.
summarizes the calculated $K_q$ values between MAG and OVA at different temperatures. All the $K_q$ values were much higher than $2 \times 10^{10}$ L/(mol·s), which was the maximum value for the dynamic quenching of biological macromolecules. It could be concluded that MAG could cause the static fluorescence quenching of OVA by forming a complex with OVA.27,28

### Binding Constant and Binding-Site Number

For static quenching, the binding constant ($K_a$) and the binding-site number ($n$) can be calculated by the double logarithm equation.23,24 The specific binding parameters are given in Table 2. Both values of $K_a$ and $n$ were positively correlated with the temperature. This may be due to the fact that the appropriate temperature increase, on the one hand, increased the molecular velocity, which was conducive to the contact and binding of MAG and OVA. On the other hand, the conformation of OVA changed, and some new binding sites were exposed.29

### Thermodynamic Parameters

Proteins and ligands mainly combine by hydrophobic interactions, electrostatic interactions, hydrogen bonds, and van der Waals forces, which are normally judged by the thermodynamic parameters.22–24 The involved thermodynamic parameters include enthalpy ($\Delta H$), entropy ($\Delta S$), and Gibb’s free energy ($\Delta G$). $\Delta H$ and $\Delta S$ can be calculated by the slope and the intercept of the Van’t Hoff plot, respectively (Figure 2D), and $\Delta G$ can be calculated by Gibb’s free energy equation. The calculated thermodynamic parameters are summarized in Table 3. The negative value for $\Delta G$ indicated that the formation of the MAG/OVA complex was a spontaneous process, while the positive $\Delta H$ and $\Delta S$ values suggested that their binding process was mainly driven by entropy.30–32 According to the report of Ross and Subramanian,33 the positive $\Delta H$ and $\Delta S$ values indicated that their binding process was mainly driven by hydrophobic force.

### Synchronous Fluorescence

Synchronous fluorescence measurement is widely applied to study the conformation of complexes.23,26 It can simplify the

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**Table 1. Stern–Volmer Quenching Constants Between MAG and OVA.**

| Temperature (°C) | Stern–Volmer equation | $R^2$ | $K_q$ (L/(mol·s)) |
|------------------|------------------------|-------|------------------|
| 29               | $F_0/F = 0.6376 \times 10^9[Q] + 1$ | 0.9944 | 1.8604 $\times 10^{12}$ |
| 33               | $F_0/F = 1.9049 \times 10^9[Q] + 1$ | 0.9896 | 1.9049 $\times 10^{12}$ |
| 37               | $F_0/F = 1.9110 \times 10^9[Q] + 1$ | 0.9623 | 1.9110 $\times 10^{12}$ |

Abbreviations: MAG, mangiferin; OVA, ovalbumin.

**Table 2. Binding Parameters Between MAG and OVA.**

| Temperature (°C) | Lineweaver–Burk equation | $R^2$ | $K_a$ (L/mol) | $n$ |
|------------------|--------------------------|-------|---------------|-----|
| 29               | $\text{Lg}(F_0/F) = 1.0749\times[Q] + 4.6214$ | 0.9946 | 0.4182 $\times 10^5$ | |
| 33               | $\text{Lg}(F_0/F) = 1.1626\times[Q] + 5.0458$ | 0.9999 | 1.1112 $\times 10^5$ | |
| 37               | $\text{Lg}(F_0/F) = 1.2457\times[Q] + 5.4393$ | 0.9977 | 2.7497 $\times 12$ | |

Abbreviations: MAG, mangiferin; OVA, ovalbumin.

**Table 3. Thermodynamic Parameters Between MAG and OVA.**

| Temperature (°C) | $\Delta H$ (kJ/mol) | $\Delta G$ (kJ/mol) | $\Delta S$ (J/(mol K)) |
|------------------|---------------------|---------------------|------------------------|
| 29               | 183.473             | $-21.005$           | 676.743                |
| 33               | $-23.712$           |                     |                        |
| 37               | $-26.418$           |                     |                        |

Abbreviations: MAG, mangiferin; OVA, ovalbumin.

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**Figure 3.** Effect of MAG on synchronous fluorescence spectra of OVA at $\Delta \lambda = 15$ nm (A) and $\Delta \lambda = 60$ nm (B). Abbreviations: MAG, mangiferin; OVA, ovalbumin.
fluorescence spectrum and reduce the spectral bandwidth. By setting a specific wavelength difference \( \Delta \lambda = \lambda_{\text{Em}} - \lambda_{\text{Ex}} \), the synchronous fluorescence spectrum can record the polarity change of the microenvironment surrounding a certain chromophore. If the wavelength difference is set at 15 or 60 nm, the synchronous fluorescence spectra will only reflect the spectral characteristics for tyrosine or tryptophan (Tyr or Trp) residues, respectively. If this chromophore is involved in the formation of the complex, its vicinity will change, resulting in a shift in the maximum fluorescence peak.\(^{34,35}\) Figure 3 displays the effect of MAG on synchronous fluorescence spectra of OVA at \( \Delta \lambda = 15 \) and 60 nm, respectively. With the addition of MAG, the intensity of the fluorescence peaks decreased, but their positions did not shift, which confirmed that these amino acid residues did not participate in the formation of the complex.

Figure 4. Binding modes of the MAG/OVA complex ([A] and [B] are panoramic and local pictures, respectively). Abbreviations: MAG, mangiferin; OVA, ovalbumin.

Figure 5. Hydrogen-bonding (A) and hydrophobic (B) interaction diagrams of the MAG/OVA complex. Abbreviations: MAG, mangiferin; OVA, ovalbumin.
**Molecular Docking**

Molecular docking can clarify the binding mechanism of complexes at the molecular level. As shown in Figure 4, the binding mode of the MAG/OVA complex with the lowest interaction energy (−4.15 kcal/mol) was selected from the 10 complex conformations of molecular docking results. MAG could interact with the amino acid residues in hydrophobic pockets by noncovalent bonds, and maintain the conformation of the complex. Figure 5 shows the hydrophobic interaction and H-bonding in the complex. Some residues of OVA (Lys135, Lys105, Asn101, and Glu136) participated in hydrogen bonding. The Glu136, Gly140, Arg139, Thr104, Asn101, and Lys105 were involved in hydrophobic interaction between OVA and MAG, which was consistent with the thermodynamic analysis.

**Conclusions**

In conclusion, MAG could cause the static quenching of OVA by forming a complex, and the interaction force between them increased as the temperature rose. According to the thermodynamic results, the combination of the complex was a spontaneous process driven mainly by entropy. Based on the results of synchronous fluorescence and molecular docking, it was found that the microenvironment surrounding the Tyr and Trp residues of OVA had not been changed during the binding process, and the binding force mainly came from hydrophobic interaction.

**Materials and Methods**

**Chemicals**

OVA (purity ≥90%), MAG (purity ≥95%), NaH2PO4, and Na2HPO4 were purchased from Aladdin. Ultrapure water was from a Thermo Water Purification System. All other chemicals were of analytical grade.

**Sample Preparation**

A 6 μmol/L OVA solution and a 0.2 mmol/L MAG solution were prepared with 20 mmol/L phosphate-buffered saline (PBS; pH = 7.0). Then the MAG solution was diluted with PBS to 40, 60, 80, 100, and 120 μmol/L and stored in the dark.

**Measurement of the Fluorescence spectrum**

A 4 mL of OVA solution was uniformly mixed with 1 mL of MAG solution at different concentrations and reacted for 10 min at the designed temperature (29°C, 33°C, and 37°C). The fluorescence spectrum of the mixture was recorded at room temperature by an Agilent Cary Eclipse fluorescence spectrophotometer equipped with a xenon flash lamp using a 10 mm quartz cell. The scanning voltage was set to 690 V and the excitation wavelength to 280 nm. Both excitation and emission slit widths were set to 5 nm. Each measurement was repeated in triplicate. The obtained data were first corrected by the following formula:

\[
F = F_{\text{Init}} \times 10^{\frac{\Delta H + \Delta S}{RT}}
\]

where \( F \) is the corrected fluorescence intensity, \( F_{\text{Init}} \) is the average value of measured fluorescence intensity, \( A_{\text{ex}} \) is the ultraviolet absorption value of the solution at the excitation wavelength, and \( A_{\text{em}} \) is the ultraviolet absorption value of the solution at the emission wavelength.

The quenching constant (\( K_q \)) was calculated by the Stern-Volmer equation:

\[
\frac{F}{F_0} = K_q \cdot \tau_0 [Q] + 1
\]

where \( F_0 \) is the fluorescence intensity of the reaction system without quenchant, \( F \) is the fluorescence intensity of the reaction system with quenchant; \( \tau_0 \) is the lifetime of proteins; and \( [Q] \) is the concentration of quenchant.

For static quenching, the \( K_q \) and \( n \) of the interaction between MAG and OVA could be obtained based on the double logarithm equation:

\[
\ln \left( \frac{F_0 - F}{F} \right) = \lg K_q + n \ln [Q]
\]

In order to understand the binding force between MAG and OVA, the thermodynamic constants of the interaction were calculated by using Van’t Hoff equation (formula (4)) and Gibbs free energy equation (formula (5)):

\[
\ln K_q = \frac{-\Delta H}{RT} + \frac{\Delta S}{R}
\]

\[
\Delta G = \Delta H - T \Delta S
\]

where \( R \) is the gas molar constant (8.314 J/[mol·K]) and \( T \) is the thermodynamic temperature.

**Measurement of Synchronous Fluorescence spectrum**

The synchronous fluorescence spectrum was obtained on an Agilent Cary Eclipse fluorescence Spectrophotometer with \( \Delta \lambda \) values of 15 and 60 nm. The excitation wavelength range was set at 250 to 350 nm, the scanning speed was 600 nm/min, and the excitation and emission widths were fixed at 5 nm.

**Molecular Docking**

The 3-dimensional structure of MAG was constructed by MOPAC 2016 and optimized through the PM3 method. The crystal structure of OVA (PDB ID: 1OVA) was from the RCSB Protein Data Bank (https://www.rcsb.org). Molecular docking between protein and MAG was performed by Autodock 4.2 software. The grid size was set as 40×40×40
points and the grid space was 0.375 Å. The rigidity of the acceptor was maintained, while the ligand was allowed flexibility. The Lamarckian GA method was applied to search for the possible docking modes and a total of 10 conformations were searched during the docking.

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