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Short communication

# Study of the interaction between monoammonium glycyrrhizinate and bovine serum albumin

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**Abstract**

The interaction between monoammonium glycyrrhizinate (MAG) and bovine serum albumin (BSA) were studied by fluorescence and absorption spectroscopy. The quenching mechanism of fluorescence of bovine serum albumin by monoammonium glycyrrhizinate was discussed. The binding sites number *n* and apparent binding constant *K* were measured by fluorescence quenching method. The thermodynamic parameters $\Delta H^\circ$, $\Delta G^\circ$, $\Delta S^\circ$ at different temperatures were calculated. The distance *r* between donor (bovine serum albumin) and acceptor (monoammonium glycyrrhizinate) was obtained according to Főrster theory of non-radiation energy transfer. The results of synchronous fluorescence spectra and UV–vis absorption spectra show that the conformation of bovine serum albumin has been changed.


*Keywords:* Monoammonium glycyrrhizinate; Bovine serum albumin; Fluorescence quenching; UV–vis spectroscopy; Thermodynamic parameters; Energy transfer

## 1. Introduction

Monoammonium glycyrrhizinate (MAG) (molecular structure: Fig. 1; formula: $C_{42}H_{65}NO_{16}$; molecular weight: 839.96) could be used in many domains because of its numerous properties. Chinese traditional medicine glycyrrhiza root is a source of glycyrrhizin, it can also be called glycyrrhizic acid (3–5% of this is triterpenic saponin), which is 50–60 times sweeter than sucrose [1]. Glycyrrhizin is steroid-like, protective of the liver [2–6], has antioxidant [7,8], antitussive [9], and antivirus [10,11] properties and can be used in toothpaste for treatment of dental plaque [12,13].

Serum albumins are the most abundant proteins in the circulatory system of a wide variety of organisms. Being the major macromolecule contributing to the osmotic blood pressure [14], they can play a dominant role in drug disposition and efficacy [15]. Many drugs and other bioactive small molecules bind reversibly to albumin and other serum components, which then function as carriers. Serum albumin often increases the apparent solubility of hydrophobic drugs in plasma and modulates their delivery to cells in vivo and in vitro. Consequently, it is important to study the interactions of drugs with this protein. The effectiveness of drugs depends on their binding ability.

Fluorescence and UV–vis absorption spectroscopies are powerful tools for the study of the reactivity of chemical and biological systems. The aim of this work was to determine the affinity of monoammonium glycyrrhizinate (MAG) to bovine serum albumin (BSA), and to investigate the thermodynamics of their interaction. We also tried to find the stoichiometry of the MAG–BSA complex. To resolve this problem, the UV and fluorescent properties of MAG as well as BSA were investigated.

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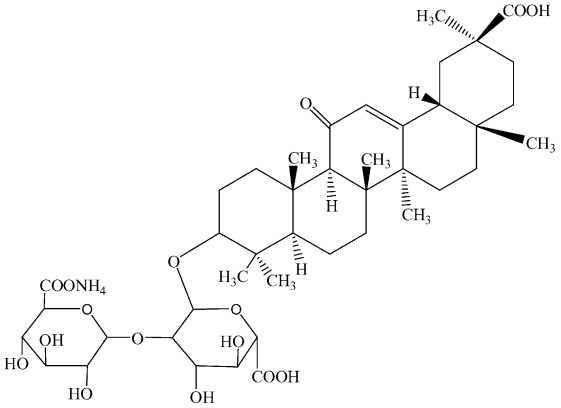


Fig. 1. Molecular structure of monoammonium glycyrrhizinate.

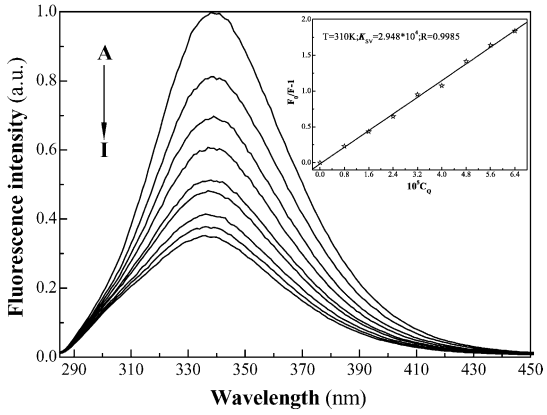


Fig. 2. Emission spectra of BSA in the presence of various concentrations of MAG, $c$ (BSA) = $1.0 \times 10^{-5}$ mol L$^{-1}$; $c$ (MAG)/($10^{-5}$ mol L$^{-1}$), A–I: 0; 0.8; 1.6; 2.4; 3.2; 4.0; 4.8; 5.6; 6.4. ($T$ = 310 K).

## 2. Experimental

### 2.1. Materials

MAG (National Vaccine & Serum Institute, Beijing, China) and BSA (being electrophoresis-grade reagents, was obtained from Sigma) were used. The samples were dissolved in Tris–HCl buffer solution (0.05 mol L$^{-1}$ Tris, 0.10 mol L$^{-1}$ NaCl, pH = 7.4). All reagents were of analytical reagent grade and doubly distilled water was used throughout.

### 2.2. Apparatus

All fluorescence spectra were recorded on F-2500 Spectrofluorimeter in the ratio mode with temperature maintained by circulating bath (Hitachi, Japan); TU-1901 spectrophotometer (Puxi Ltd. of Beijing, China) was used for scanning UV–vis spectra; the mass of the sample was accurately weighed using a microbalance (Sartorius, ME215S) with a resolution of 0.1 mg.

### 2.3. Spectroscopic measurements

The absorption spectra of BSA, MAG and their mixture were performed at room temperature.

The fluorescence measurements were performed at different temperatures (298, 302, 306 and 310 K). Excitation wavelength was 280 nm. The excitation and emission slit widths were set at 2.5 nm. Appropriate blanks corresponding to the buffer were subtracted to correct background fluorescence.

## 3. Results and discussions

### 3.1. Fluorescence characteristics of BSA

The concentrations of BSA were stabilized at $10^{-5}$ mol L$^{-1}$, and the content of MAG varied from 0 to $6.4 \times 10^{-5}$ mol L$^{-1}$ at the step of $0.4 \times 10^{-5}$ mol L$^{-1}$. The effect of MAG on BSA fluorescence intensity is shown in Fig. 2.

The intensity of fluorescence can be decreased by a wide variety of processes. Such decreases in intensity are called quenching. It is apparent from Fig. 2 that the fluorescence intensity of BSA decreased regularly with the increasing of MAG concentration.

The fluorescence quenching data are usually analysed by the Stern–Volmer equation [16]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \quad (1)$$

where, $F_0$ and $F$ are the steady-state fluorescence intensities in the absence and presence of quencher (MAG), respectively. $K_{SV}$ is the Stern–Volmer quenching constant, and [$Q$] the concentration of quencher.

The Stern–Volmer quenching constant $K_{SV}$ of BSA and tryptophan residues fluorescence by MAG at different temperatures are shown in Table 1.

These results indicate that the probable quenching mechanism of fluorescence of BSA by MAG is a dynamic quenching

Table 1
Stern–Volmer quenching constant $K_{SV}$ and relative thermodynamic parameters of MAG–BSA at pH = 7.4

| $T$ (K) | $10^{-4}K_{SV}$ (L mol$^{-1}$) | $R$[a] | S.D.[b] | $\Delta H^\circ$ (kJ mol$^{-1}$) | $\Delta G^\circ$ (kJ mol$^{-1}$) | $\Delta S^\circ$ (J mol$^{-1}$ K$^{-1}$) |
|---|---|---|---|---|---|---|
| 298 | 2.339 | 0.9982 | 0.0290 | 15.0 | −24.9 | 134 |
| 302 | 2.548 | 0.9974 | 0.0382 | | −25.5 | |
| 306 | 2.779 | 0.9989 | 0.0273 | | −26.0 | |
| 310 | 2.948 | 0.9985 | 0.0343 | | −26.5 | |

[a] $R$ is the linear quotient.
[b] S.D. is standard deviation.

procedure, because the $K_{SV}$ increased with the rising temperature [16].

### 3.2. The determination of the force acting between MAG and BSA

The interaction forces between a drug and biomolecule may include hydrophobic force, electrostatic interactions, van der Waals interactions, hydrogen bonds, etc. The slope of a plot of the bimolecular quenching constant versus $1/T$ ($T$, absolute temperature) allows one to calculate the energy change for the quenching process [17]. If the enthalpy change ($\Delta H^\circ$) does not vary significantly over the temperature range studied, then its value and that of entropy change ($\Delta S^\circ$) can be determined from the van't Hoff equation:

$$\ln K = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (2)$$

where $K$ is the Stern–Volmer quenching constant at the corresponding temperature and $R$ is the gas constant. The temperatures used were 298, 302, 306 and 310 K. The enthalpy change ($\Delta H^\circ$) is calculated from the slope of the van't Hoff relationship. The free energy change ($\Delta G^\circ$) is estimated from the following relationship:

$$\Delta G^\circ = \Delta H^\circ - T\,\Delta S^\circ \quad (3)$$

Table 1 shows the values of $\Delta H^\circ$ and $T\,\Delta S^\circ$ obtained for the binding site from the slopes and ordinates at the origin of the fitted lines.

From Table 1, it can be seen that the negative sign for free energy ($\Delta G^\circ$) means that the interaction process is spontaneous. The positive enthalpy ($\Delta H^\circ$) and entropy ($\Delta S^\circ$) values of the interaction of MAG and BSA indicate that the binding is mainly entropy-driven and the enthalpy is unfavorable for it, the hydrophobic forces playing a major role in the reaction [18].

### 3.3. Analysis of binding equilibria

When small molecules bind independently to a set of equivalent sites on a macro-molecule, the equilibrium between free and bound molecules is given by the equation [19]:

$$\lg\left(\frac{F_0 - F}{F}\right) = \lg K + n \lg [Q] \quad (4)$$

where, in the present case, $K$ is the binding constant to a site, and $n$ the number of binding sites per BSA.

According to the Eq. (4), the binding constant $K$ and the number of binding sites $n$ can be obtained as $K = 1.79 \times 10^4\ \mathrm{L\,mol^{-1}}$, $n = 1.01$, respectively.

### 3.4. Energy transfer between MAG and BSA

The Főrster theory of molecular resonance energy transfer [20] points out: in addition to radiation and reabsorption, a transfer of energy could also take place through direct electrodynamic interaction between the primarily excited molecule and its neighbors. According to this theory, the distance $r$ of binding between MAG and BSA could be calculated by the equation [21]:

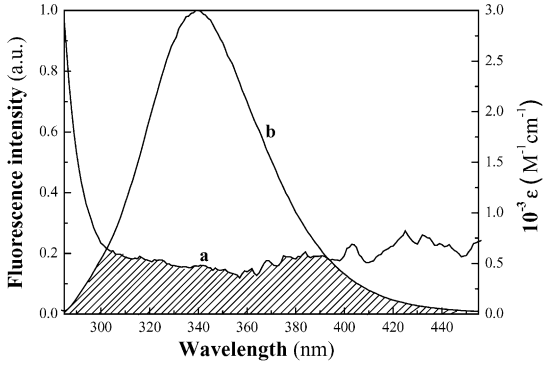


Fig. 3. Spectral overlap of MAG absorption with BSA fluorescence $c$ (BSA) = $c$ (MAG) =$1.0 \times 10^{-5}\ \mathrm{mol\,L^{-1}}$.

$$E = \frac{R_0^6}{R_0^6 + r^6} \quad (5)$$

where $E$ is the efficiency of transfer between the donor and the acceptor, $R_0$ is the critical distance when the efficiency of transfer is 50%.

$$R_0^6 = 8.79 \times 10^{-25} K^2 n^{-4} \phi J \quad (6)$$

In Eq. (6), $K^2$ is the space factor of orientation; $n$ the refracted index of medium; $\phi$ the fluorescence quantum yield of the donor; $J$ the effect of the spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor (Fig. 3), which could be calculated by the equation:

$$J = \frac{\int_0^\infty F(\lambda)\varepsilon(\lambda)\lambda^4 \mathrm{d}\lambda}{\int_0^\infty F(\lambda)\mathrm{d}\lambda} \quad (7)$$

where $F$ ($\lambda$) is the corrected fluorescence intensity of the donor in the wavelength range $\lambda$–($\lambda + \Delta\lambda$); $\varepsilon(\lambda)$ is the extinction coefficient of the acceptor at $\lambda$. The efficiency of transfer ($E$) could be obtained by the equation:

$$E = 1 - \frac{F}{F_0} \quad (8)$$

In the present case, $K^2 = 2/3$, $N = 1.36$, and $\phi = 0.15$ [22]. According to the Eqs. (5)–(8), we could calculate that $J = 1.14 \times 10^{-15}\ \mathrm{cm^3\,L\,mol^{-1}}$; $R_0 = 1.76$ nm; $E = 0.20$ and $r = 2.21$ nm. The average distance $r < 8$ nm [23], and $0.5R_0 < r < 1.5R_0$ [24] indicate that the energy transfer from BSA to MAG occurs with high probability.

### 3.5. Conformation investigation

To explore the structural change of BSA by addition of MAG, we measured UV–vis spectra (Fig. 4) and synchronous

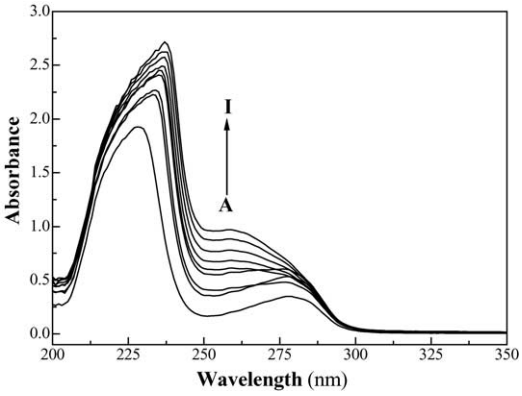


Fig. 4. UV–vis spectra of BSA in the presence of various concentrations of MAG A–I, $c$ (BSA) = $1.0 \times 10^{-5}$ mol L$^{-1}$; $c$ (MAG)/($10^{-5}$ mol L$^{-1}$): 0; 0.8; 1.6; 2.4; 3.2; 4.0; 4.8; 5.6; 6.4.

fluorescence spectra (Fig. 5) of BSA with various amounts of MAG. Fig. 4 displays the UV–vis spectra of BSA at different contents of MAG. It is clear from the figure that in the visible region, the absorption peaks of these solutions showed moderate shifts toward shorter wave lengths indicating that with the addition of MAG, the peptide strands of BSA molecules extended more and the hydrophobicity was decreased.

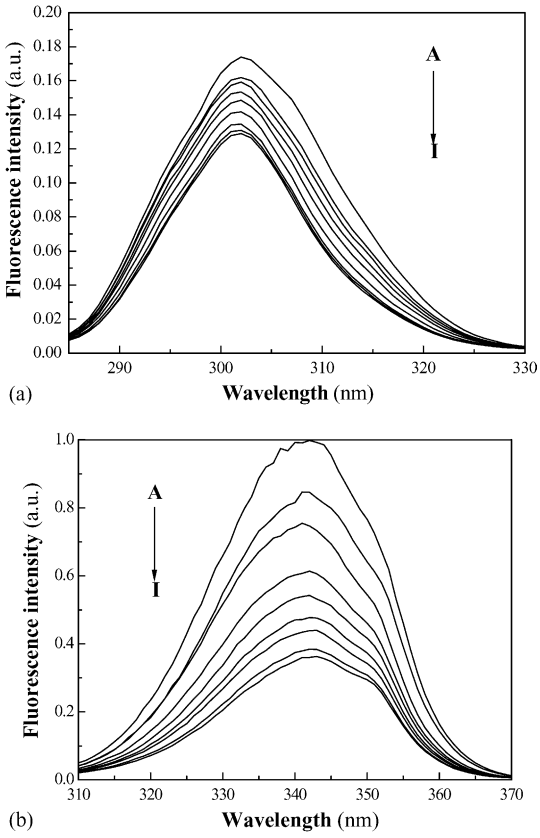


Fig. 5. Synchronous fluorescence spectrum of BSA: (a) $\Delta\lambda$ = 15 nm; (b) $\Delta\lambda$ = 60 nm $c$ (BSA) =$1.0 \times 10^{-5}$ mol L$^{-1}$; $c$ (MAG)/($10^{-5}$ mol L$^{-1}$); A–I: 0; 0.8; 1.6; 2.4; 3.2; 4.0; 4.8; 5.6; 6.4.

The synchronous fluorescence spectra give information about the molecular environment in a vicinity of the chromosphere molecules. In the synchronous spectra, the sensitivity associated with fluorescence is maintained while offering several advantages: spectral simplification, spectral bandwidth reduction, and avoiding different perturbing effects. The authors [25] suggested a useful method to study the environment of amino acid residues by measuring the possible shift in wavelength emission maximum $\lambda_{max}$, the shift in position of emission maximum corresponding to the changes of the polarity around the chromophore molecule. When the $D$-value ($\Delta\lambda$) between excitation wavelength and emission wavelength were stabilized at 15 nm or 60 nm, the synchronous fluorescence gives the characteristic information of tyrosine residues or tryptophan residues [26]. The effect of MAG on BSA synchronous fluorescence spectroscopy is shown in Fig. 5.

It is apparent from Fig. 5 that the maximum emission wavelength moderate shifts towards long wave when $\Delta\lambda$ = 60 nm. The shift effect expresses that the conformation of BSA was changed. It is also indicated that the polarity around the tryptophan residues was increased and the hydrophobicity was decreased [27]. The conclusion agrees with the result of conformational changes by UV–vis spectra.

## 4. Conclusions

In this paper, the interaction of MAG with BSA was studied by Spectroscopic methods including fluorescence spectroscopy and UV–vis absorption spectroscopy. The results of synchronous fluorescence spectroscopy and UV–vis spectra indicate that the secondary structure of BSA molecules is changed dramatically in the presence of MAG. The experimental results also indicate that the probable quenching mechanism of fluorescence of BSA by MAG is a dynamic quenching procedure, the binding reaction is mainly entropy-driven, and hydrophobic interactions played a major role in the reaction.

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