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# 栀子苷和牛血清白蛋白相互作用研究

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**摘　要**:在模拟生理条件下,利用紫外和荧光光谱法研究栀子苷和牛血清白蛋白相互作用。通过 Stern-Volmer 方程和 Lineweaver-Burk 考察栀子苷对牛血清白蛋白内源性荧光的猝灭机制,分别在 298 K、310 K 和 322 K 下利用结合常数和结合位点数计算反应体系的热力学参数。结果表明,当温度为 298 K、302 K 和 322 K 时,栀子苷对牛血清白蛋白的猝灭常数分别为 $4.632\times10^4$、$3.515\times10^4$ 和 $3.575\times10^4$ mol/L,结合常数 $K_A$ 分别为 $1.805\times10^4$、$2.546\times10^4$ 和 $4.165\times10^4$,结合位点数分别为 1.334、1.112 和 0.944,栀子苷对牛血清白蛋白的猝灭方式属于静态猝灭;热力学参数 $\Delta G<0$,$\Delta H<0$,$\Delta S>0$,表明栀子苷与牛血清白蛋白结合作用力为静电引力,根据 Förster 非辐射共振能量转移理论,计算出栀子苷与牛血清白蛋白之间的结合距离为 1.78 nm。

**关键词**:牛血清白蛋白;栀子苷;荧光光谱

**中图分类号**:TQ351　　**文献标识码**:A

# The Interaction between Gardenoside and Bovine Serum Albumin

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**Abstract**: The interaction between gardenoside and bovine serum albumin (BSA) was studied by fluorescence and UV-Vis absorption spectrometry under simulated physiological conditions. The quenching mechanism of the intrinsic fluorescence of BSA by gardenoside was investigated by Stern-Volmer and Lineweaver-Burk equations. It was proved that the quenching mechanism of BSA by gardenoside was a static quenching procedure with quenching constants of $4.632\times10^4$, $3.515\times10^4$ and $3.575\times10^4$ mol/L at 298, 310 and 322K, respectively. Binding constants and number of binding sites at corresponding temperature were $1.805\times10^4$, $2.546\times10^4$ and $4.165\times10^4$ as well as 1.334, 1.112 and 0.944, respectively. The thermodynamic parameters were $\Delta G<0$, $\Delta H<0$, $\Delta S>0$, which proved the electrostatic force played a major role between BSA and gardenoside. The distance was calculated to be 1.78 nm using Fösters resonance energy transfer.

**Key words**: Bovine serum albumin; gardenoside; fluorescence spectrometry

## Introduction

Gardenoside, as shown in Fig1, a terpenoid compound, is one of the major components of the fruit of *Gardenoside jasmnvides* ellis, which belongs to a traditional Chinese herbal medicine. It has been reported to exert a wide range of biological activities in Chinese pharmacopoeia, including lithagogue, cholagogic, liver-protection, antihypertensive, antitumor, anti-inflammatory, hypoglycemic effects, antiatherosclerosis and antithrombotic [1,2], *etc*.

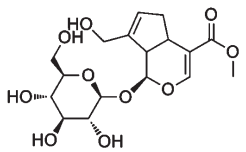

**Fig. 1　Chemical structure of gardenoside**

Human Serum albumin (HSA), the most abundant proteins in the circulatory system of human as well as other mammals, plays a dominant role in the transport and deposition of a variety of endogenous and exogenous compounds such as drugs [3]. It has been one of the most extensively studied proteins. Bovine serum albumin (BSA), due to its structural similarity to HSA, low

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cost and considerable stability, has been used to replace HSA as a model protein in protein-drugs studies. BSA is a globular protein consisting of 583 amino acid residues in three homologous domains (I, II, III). Each domain contains two subdomains (A and B) [4]. BSA has two tryptophan residues, which are embedded in the first sub-domain IB and sub-domain IIA, respectively. It can produce intrinsic fluorescence properties because of the high sensitivity of tryptophan residues to its local environment [5]. Therefore, it is informative to study the interaction of drug with BSA because protein-drug binding plays an important role in pharmacology, pharmacokinetics and pharmacodynamics, *e. g.*, binding studies of drugs with BSA are useful for understanding reaction mechanism, as well as providing guidance for the application and design of new drug [6]. So far, there have been many examples in studying the interaction of drugs and BSA [7]. However, detailed reports about the binding of gardenoside with BSA have not yet to be conducted. In the present report, we have focused on the interaction of gardenoside with BSA employing spectroscopic techniques and have obtained the quenching mechanism, binding constant, number of binding sites, the distance of binding and thermodynamics parameters.

## Materials and Methods

### Materials

BSA was purchased from sigma chemicals (electrophoresis-grade regents). Gardenoside was obtained from Nanjing TC Institute of Chinese Materia Medica, and the purity reached 99%. The gardenoside stock solution was prepared in ethanol. BSA was dissolved in Tris-HCl buffer solution (0.05 mol/L Tris, 0.1 mol/L NaCl, pH = 7.4). All other chemical were of analytical reagent grade and millipore water was used throughout. All stock solutions were stored at 0-4 ℃.

### Apparatus

The UV spectrum was recorded at room temperature on the Shimadzu 2450 UV-Vis spectrophotometer equipped with 1.0 cm quartz cells. The fluorescence spectra were recorded on RF-5301 spectrofluorometer. The widths of both excitation and emission slits were set at 5 nm.

### Procedures

The UV absorbance spectra of BSA, gardenoside and the equal mixture of BSA and gardenoside were recorded at 298K, respectively. The BSA solution (3.0 mL, $1 \times 10^{-6}$ mol/L) was titrated by successive additions of gardenoside about 10 μL with micro-injector manually. The mixture was allowed to incubate for 10 min at 298, 302 and 322 K, respectively, then the fluorescence emission spectra was recorded in the range of 290 to 500 nm with exiting wavelength 280 nm. Appropriate blanks corresponding to the buffer were subtracted to correct background fluorescence.

## Results and Discussion

### Fluorescence quenching spectra

Due to tryptophan, tyrosine and phenylalanine residues, BSA can produce intrinsic fluorescence when irradiated by UV, so it was called phosphor. Each protein possesses different ratio of fluorescence intensity for tryptophan, tyrosine and phenylalanine residues, about 100: 9: 0.5, respectively [8]. Hence, in many cases, protein fluorescence was mainly contributed by tryptophan residues and its fluorescence change were directly corresponding to the ones of tryptophan residues itself and surrounding environment. The fluorescence intensity of a protein decreased by a variety of molecular interactions, such as excited-state reactions, molecular rearrangements, energy transfer, *etc*. Such decreasing in intensity was caused by fluorescence quenching. In the work, the interaction of gardenoside with BSA was evaluated by measuring the intrinsic fluorescence intensity of protein before and after addition of equal mol of gardenoside (Fig 2).

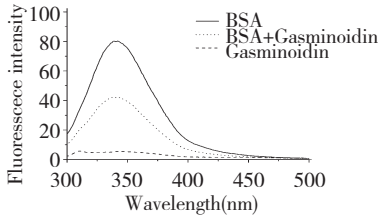


**Fig. 2 Emission spectra of gardenoside and BSA**

$C_{BSA} = C_{Gardenoside} = 1 \times 10^{-6}$ mol/L; pH = 7.40

It is obvious that BSA has a strong fluorescence emission peak at 340 nm after being excited at 280 nm. Different interaction between molecular can lead to different pattern of quenching, and the typical pattern of quenching includes static and dynamic quenching. Static quenching refers to formation of complex between quencher and the fluorophore, while dynamic quenching refers to the collision of the quencher and fluorophore during the excitation process. One way to distinguish dynamic from static quenching is to examine the temperature effects on the interaction of drug to BSA. So a fixed concentration of BSA was titrated with different amounts of gardenoside at different temperatures (298, 310, 322 K, respectively). As shown in Fig 3, fluorescence quenching was dependent on gardenoside concentration at 298 K.

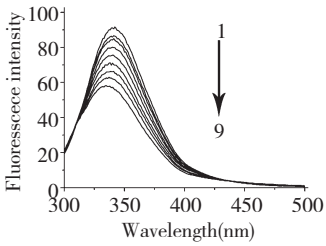


**Fig. 3 Fluorescence spectra of BSA-Gardenoside system at 298K**

$C_{BSA}$: $1 \times 10^{-6}$ mol/L; $C_{Gardenoside}$: $1 \times 10^{-4}$ mol/L; 0, 5, 10, 15, 20, 25, 30, 35 40 μL from curve 1 to 9

Furthermore, there was a slight blue shift at the maximum wavelength of BSA fluorescence emission when the solution of gardenoside was added. This suggested that the chromophore of a protein was placed in a more hydrophobic environment after the addition of gardenoside. The fluorescence quenching data were analyzed using the Stern-Volmer equation as shown below:

$$F_0/F = 1 + Kq\tau_0[Q] = 1 + Ksv[Q] \quad (1)$$

Where $F_0$ and F are the fluorescence intensities before and after the addition of quencher, respectively. Kq is the quenching rate constant, Ksv is the Stern-Volmer dynamic quenching constant, $\tau_0$ is the average lifetime of the biomolecule without quencher, and [Q] is the concentration of quencher.

Fig 4 showed the Stern-Volmer plots of the quenching of BSA by gardenoside at different temperatures. The

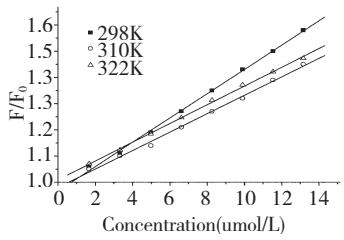


**Fig. 4 Stern-Volmer plots of fluorescence quenching of BSA caused by gardenoside**

curves have linear relationships and the slopes decreased with increasing temperature. The values of Ksv at different temperatures were shown in Table 1.

**Table 1 Stern-Volmer quenching constants and correlation coefficients**

| T /K | Ksv( mol/L) | Kq/( mol. s/L) | R |
|---|---|---|---|
| 298 | $4.632 \times 10^4$ | $4.632 \times 10^{12}$ | 0.99803 |
| 310 | $3.515 \times 10^4$ | $3.515 \times 10^{12}$ | 0.99625 |
| 322 | $3.575 \times 10^4$ | $3.575 \times 10^{12}$ | 0.99813 |

These results indicated that the probable quenching mechanism of BSA fluorescence by gardenoside was a static quenching type. Static quenching indicated that gardenoside-BSA cannot transport across membrane, but by free gardenoside distribution to the body's tissues and other parts during blood circulation. The quenching rate constants, Kq, can be calculated using following equation, $Kq = Ksv/\tau_0$. The values of Kq were listed in Table 1. Meanwhile, Ksv was used to evaluate the protein binding affinity to drugs, always about $10^2$-$10^4$ and $10^{-1}$-10 mol/L for high and low affinity, respectively. Hence, gardenoside-BSA interaction may be considered as a high affinity force, which was not conductive to the full use of drugs. Generally, the maximum scatter collision quenching constant of various kinds of quenchers on biomolecules was $2 \times 10^{10}$ mol · s/L[9]. However, the rate constant for the quenching of BSA was found to be much greater than that. The static quenching equation can be expressed as follow [10]:

$$\lg(F_0/F\text{-}1) = \lg K + n\lg[Q] \quad (2)$$

Where $F_0$ and F denoted BSA fluorescence intensities in the absence and presence of quencher, respectively, Kq is the binding constant of BSA with gardenoside, [Q] is the equilibrium concentration of gardenoside,

and n is the number of binding sites. By ploting lg ($F_0$/F-1) against lg [Q], the curves were shown in Fig 5.

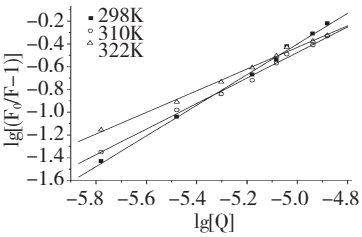


**Fig. 5 A plot of lg ($F_0$/F-1) vs. lg [Q] at 298, 310 and 322 K**

The binding constants (K) and binding sites (n) can be obtained from the intercept and slope of Fig 5 The results were given in Table 2.

**Table 2 The binding constant (K) and value of binding site (n) in BSA-Gardenoside system**

| T /K | n | $K_A$ (L/mol) | r/nm |
|---|---|---|---|
| 298 | 1.334 | $1.805 \times 10^4$ | 1.14 |
| 310 | 1.112 | $2.546 \times 10^4$ | 1.842 |
| 322 | 0.944 | $4.165 \times 10^4$ | 1.78 |

It showed that the binding constant between gardenoside and BSA decreased with the increasing of temperature, resulting in stability reduction of gardenoside-BSA. The binding site value of gardenoside-BSA was 1, showing gardenoside had one binding site to BSA, and synergistic and induction effect did not exist. Binding constants decreased with the increasing of temperature, probably resulting from decreasing stability of gardenoside-BSA. The specific binding site of gardenoside-BSA was dependent on site-directed mutagenesis and other means, *etc*.

## Determination of the interaction force between gardenoside and BSA

There are essentially four types of non-covalent interactions that play a key role in binding of drug to proteins. These are hydrogen bonds, Van der waals forces, electrostatic and hydrophobic interactions. But different drugs have different types of interactions with protein. The thermodynamic parameters dependency to temperature must be obtained in order to elucidate the interaction forces. Enthalpy change ($\Delta H$), entropy change ($\Delta H$) and free energy change ($\Delta G$) can be calculated by the Van't Hoff equation [Eq (3-5)]:

$$\mathrm{Ln}(K_2/K_1) = \Delta H/R(1/T_1 - 1/T_2) \quad (3)$$

$$\Delta G = -RT\mathrm{Ln}K \quad (4)$$

$$\Delta G = \Delta H - T\Delta S \quad (5)$$

Where K is the binding constant obtained from Eq (2) at corresponding temperature, and R is the gas constant. The temperatures were 298, 310 and 322 K, respectively. The valued of $\Delta H$, $\Delta G$ and $\Delta S$ were shown in Table 3.

**Table 3 Thermodynamic parameters for gardenoside-BSA complex**

| T /K | $\Delta H$ (kJ/mol) | $\Delta G$ (kJ/mol) | $\Delta S$ J/(K. mol) |
|---|---|---|---|
| 298 | -16.2 | -27.76 | 35.4 |
| 310 | | -27.18 | |
| 322 | | -28.05 | |

The negative value of $\Delta G$ revealed that the interaction process was spontaneous. According to the report of Ross[a]nd subramanion, the position $\Delta H$ and $\Delta S$ value was associated with hydrogen bonding and Van der Waals interaction. Finally, negative $\Delta H$ and positive $\Delta S$ values were determined to be electrostatic interaction. Thus, the binding of gardenoside to BSA might be electrostatic interactions.

## Fluorescence resonance energy transfer

The fluorescence quenching of BSA by gardenoside revealed the occurrence of energy transfer between the protein and drug. According to the Förster's non-radiative energy transfer theory, if emission fluorescence from a donor could be absorbed by an acceptor, Energy transfer is likely to happen if the following conditions are met: (1) the donor can produce fluorescence light; (2) fluorescence emission spectra of the donor and UV-Vis absorption spectra of an acceptor have more overlap; (3) the distance between the donor and acceptor is less than 7 nm.

The distance between the donor (BSA) and the acceptor (gardenoside) can be calculated according to the Förster's non-radiative energy transfer theory. The efficiency of energy transfer, E, is defined by the following Eq(6):

$$E = R_0^6/(R_0^6 + r^6) = 1 - F/F_0 \quad (6)$$

Where r is the distance from the donor and acceptor; and $R_0$ is the Förster's critical distance, at which 50% of the excitation energy is transferred to the acceptor. $R_0$ can be calculated using the the Förster's formula Eq (7).

$$R_0^6 = 8.8 \times 10^{-25} K^2 N^{-4} \Phi J \quad (7)$$

Where $K^2$ is the spatial orientation factor of dipole, n is the refractive index of the medium, $\Phi$ is the fluorescence quantum yield of the donor, and J is the overlap integral of the fluorescence emission spectrum of the donor with the absorption spectrum of the acceptor (Fig 6).

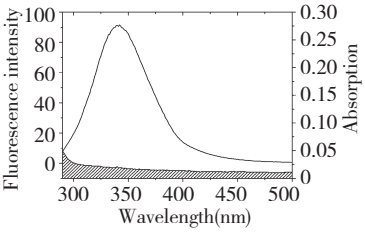


**Fig. 6 Overlapping of BSA fluorescence spectra with Gardenoside UV spectra**

$$J = (\sum F_D(\lambda)\ \varepsilon_A(\lambda)\lambda^4 \Delta\lambda) / \sum F_D(\lambda)\Delta\lambda \quad (8)$$

Where F ($\lambda$) is the fluorescence intensity of fluorescence donor at wavelength $\lambda$, $\varepsilon$ ($\lambda$) is the molar absorption coefficient of an acceptor at wavelength $\lambda$. In the present case, $K^2 = 2/3$, $n = 1.336$ and $\Phi = 0.15$. J was calculated to be $3.65 \times 10^{-14}$ $cm^3 \cdot L/mol$, E = 0.473, $R_0 = 5.62$ nm and r = 1.78 nm, thus r < 7 nm. These data suggested that the energy transfer from BSA to gardenoside can occur with high probability. In accordance with prediction by Förster's non-radiative energy transfer theory, these results indicated again a static quenching interaction between gardenoside and BSA.

## Conclusion

In this work, the interaction between gardenoside and BSA was investigated by fluorescence spectroscopy and UV-Vis absorption spectroscopy. The results indicated that the fluorescence quenching mechanism for BSA with gardenoside is likely a static quenching. In addition, binding reaction of gasminoidion to BSA is spontaneous and largely by electrostatic interactions.

**Reference**

1 Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China (part Ⅳ). Beijing: China Medical Science Press, 2005. 173.

2 Wang GF, Wu SY, Xu W. Ceniposide inhibits high glucose-induced cell adhesion through the NF-Kb signaling pathway inhuman umbilical vein endothelical cells. *Acta Pharmaco Sin*, 2010, 31: 953-962.

3 Sugio S, Kashima A, Mochizuki S, *et al*. Crystal structure of human serum albumin at 2.5Å resolution. *Protein Eng*, 1999, 12: 439-446.

4 Gelamo EL, Tabak M. Spectroscopic studies on the interaction of bovine (BSA) and human (HSA) serum albumins with ionic surfactants. *Spectrochimica Acta Part A*, 2000, 56: 2255-2271.

5 Fu SP, Zhang XZ, Zhang F, *et al*. Study on the interaction between strychnine and bovine serum albumin by capillary electrophoretic frontal analysis. *Chin Chem Lett*, 2005, 16: 1233-1236.

6 Hu YJ, Liu Y, Zhao RM, *et al*. Spectroscopic studies on the interaction between methylene blue and bovine serum albumins. *J Photochem Photobiol A*, 2006, 179: 324-329.

7 Olajire AA, Manosij G, Anita M. Spectrophotometric and thermodynamic studies of the interactions of 4-carboxyl-2, 6-dinitrophenylazohydroxynaphtalenes with bovine serum albumin. *Spectrc Chim Acta A*, 2012, 96: 1038-1046.

8 Feng XQ, Fu GQ, Li XF, *et al*. The interaction of rare earth La (Ⅲ) and Nd (Ⅲ) with BSA. *Chin J Lumin*, 2011, 32 (2): 205-209.

9 Cha DM, Li ST, Yang YF. Study on the interaction of human serum albumin and Mn(Ⅱ), Co(Ⅱ) by fluorescence method. *Spectrosc Spect Anal*, 1999, 19: 788-791.

10 Bian HD, Li M, Yu Q. Studies on the interaction between NaVO3 and bovine serum albumin by fluorescence method. *Chin J Inorg Chem*, 2006, 22: 845-850.