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Studies of the Interaction Between Daidzein and 3'-Daidzein Sulfonic Sodium with Bovine Serum Albumin by Spectroscopic Methods[1]

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Abstract—The interaction between daidzein and 3'-daidzein sulfonic sodium with bovine serum albumin (BSA) in physiological buffer (pH = 7.4) is investigated by fluorescence quenching technique and UV/vis absorption spectra. The results reveal that both daidzein and 3'-daidzein sulfonic sodium could strongly quench the intrinsic fluorescence of BSA. The quenching mechanism of both the daidzein and 3'-daidzein sulfonic sodium for BSA is static quenching procedure. The apparent binding constants K_a and number of binding sites n of daidzein and 3'-daidzein sulfonic sodium with BSA are obtained by fluorescence quenching method. The thermodynamic parameters, enthalpy change ($\Delta_r H^m$), and entropy change ($\Delta_r S^m$), are calculated, respectively, which indicate that the interaction of daidzein with BSA is driven mainly by hydrogen bonding and van der Waals, and 3'-daidzein sulfonic sodium with BSA is driven mainly by hydrophobic forces. The distance r between BSA with daidzein and 3'-daidzein sulfonic sodium are calculated to be 4.02 nm and 3.08 nm, respectively, based on Förster's non-radiative energy transfer theory. The results of synchronous fluorescence spectra show that binding of daidzein and 3'-daidzein sulfonic sodium with BSA cannot induce conformational changes in BSA.

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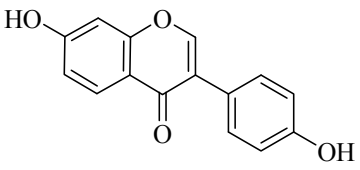
INTRODUCTION

Daidzein belongs to the isoflavone class of flavonoids, and has the typical C^6–C^3–C^6 structure. daidzein is mainly found in vegetables, such as soybeans and chickpeas. Soybeans and soy foods is the major dietary sources of these substances [1]. Daidzein is the second most abundant isoflavones in soybeans and soy foods; daidzein has estrogenic activity, antioxidant activity, antidipsotropic, anticarcinogenic, anti-athero-genic and anti-osteoporotic activity [2–4]. However, daidzein does not dissolve in water. 3'-Daidzein sulfonic sodium is the daidzein structurally modified and is a water-soluble substance [5, 6].

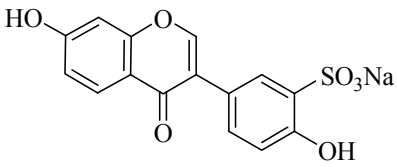
Serum albumins are the most abundant proteins in plasma. As the major soluble protein constituents of the circulatory system, they have many physiological functions. Consequently, it is important to study the interaction of drugs with serum albumin, because the interaction influences the drugs' pharmacology and pharmacodynamics. Among the serum albumins, BSA has a wide range of physiological functions involving binding, transport and delivery of fatty acids, porphyrins, bilirubin and steroids, etc. [7], and the results of all the studies are consistent with the fact that bovine and human serum albumins are homologous proteins [8]. This paper investigates the interaction of daidzein and 3'-daidzein sulfonic sodium with bovine serum albumin (BSA) by spectroscopic methods.

Some techniques are commonly used to detect interaction between drug and serum albumin including the fluorescence spectra [9], UV-spectrophotometry [10], circular dichroism spectra (CD) [11], Raman spectra [12], nuclear magnetic resonance (NMR) [13], electrochemistry [14], equilibrium dialysis [15], and Fourier transform infrared (FT–IR) spectra [16],

[1] The text was submitted by the authors in English.



Daidzein



3'-Daidzein sufonic sodium

Fig. 1. Molecular structure of daidzein and 3'-daidzein sulfonic sodium.

among which fluorescence is a powerful method, because it is highly sensitive, rapid, and simple, and synchronous fluorescence spectra can give information on the changes in the local environment of the fluorophore. By measuring the intrinsic fluorescence quenching of BSA it can reveal accessibility of quenchers to albumin's fluorophores, help to understand albumin binding mechanisms to compounds and provide clues to the nature of the binding phenomenon.

In this work we investigated the binding of BSA with daidzein and 3'-daidzein sulfonic sodium and the relevant energy transfer effect. The effects of temperature on this reaction are also examined by fluorescence quenching method. The results reveal that the daidzein and 3'-daidzein sulfonic sodium for BSA both operate in a static quenching procedure and hydrogen bonding and van der Waals interactions the main role in the binding of daidzein with BSA on the base of the thermodynamic parameters ($\Delta S < 0$, $\Delta H < 0$); the hydrophobic force plays the main role in the binding of 3'-daidzein sulfonic sodium with BSA on the base of the thermodynamic parameters ($\Delta H > 0$, $\Delta S > 0$). Moreover, the effects of the daidzein and 3'-daidzein sulfonic sodium binding on the conformation of BSA are investigated by synchronous fluorescence spectra.

MATERIALS AND METHODS

Materials

Daidzein (analytical grade) was obtained from National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. 3'-Daidzein sulfonic sodium was synthesized in the School of chemistry and materials science of Shaanxi normal university. BSA was obtained from Sino-American Biotechnology Company and used without further purification. The stock solution of daidzein and 3'-daidzein sulfonic sodium (1.0×10^{-3} mol l^{-1}) were prepared in 1% aqueous KOH solution. BSA stock solution (1.0×10^{-3} mol l^{-1}, based on its molecular weight of 65.000) was prepared in 0.1 mol l^{-1} NaCl, 0.05 mol l^{-1} Tris–HCl buffer of pH = 7.4. All other chemicals were of analytical reagent grade and double distilled water was used throughout the experiment. All stock solutions were stored at 0–4°C.

Fluorescence and UV–VIS Absorbance Spectroscopy

Fluorescence measurements were performed with a Shimadzu spectrofluorimeter Model RF-5301 equipped with a 150 W Xenon lamp. BSA is excited at 285 nm and its fluorescence is recorded at 345 nm. The excitation and emission slit widths (each 3 nm) and scan rate (500 nm min^{-1}) were maintained constant for all experiments. Fluorescence quenching of spectra was recorded at 295, 303, 310, and 315 K in the range of 300–500 nm; the absorption spectra were measured on a Hitachi UV-8453 UV/vis spectrophotometer at 295 K. Quartz cells of 1.00 cm were used for the measurements.

RESULTS AND DISCUSSION

Fluorescence Quenching

Fluorescence quenching refers to any process which is a decrease of the fluorescence intensity from a fluorophore due to a variety of molecular interactions. Quenching can occur by different mechanisms, which are usually classified as dynamic quenching and static quenching. During dynamic quenching the fluorescence substance collides with quencher leading to a decrease in the quantum yield and the strength of the fluorescence. The static quenching is initiated from the formation of non-fluorescent complex between quencher and a fluorophore. In general, dynamic and static quenching can be distinguished by their different dependence on temperature and viscosity [17]. For dynamic quenching, the UV–VIS absorption spectra of fluoro-phore is not changed: only fluorescence molecule in the excited state is influenced by quencher, but for static quenching, a compound is formed between the ground state of fluorophore and quencher, therefore, the absorption spectra of fluorophore would be influenced [18].

Both static and dynamic processes can be described by the well known Stern–Volmer equation as Eq. (1):

$$\frac{F_0}{F} = 1 + K_q\tau_0[Q] = 1 + K_{SV}[Q], \quad (1)$$

where F_0 and F are the fluorescence intensities in the absence and the presence of quencher, K_q is the quenching rate constant, τ_0 is the lifetime of the fluorophore in the absence of quencher which equals to 10^{-8} s [19], $[Q]$ is the quencher concentration, and K_{SV} is the Stern–Volmer quenching constant, which can be written as $K_{SV} = K_q\tau_0$ [20]. Within certain concentration, the curve of F_0/F versus $[Q]$ (Stern–Volmer curve) would be linear if the quenching type is single static or dynamic process [21]. If both static and dynamic are involved in the quenching process, the Stern–Volmer plot exhibits an upward curvature, concave towards they-axis at high concentrations of Q [22].

The effect of daidzein and 3'-daidzein sulfonic sodium on BSA fluorescence intensity of different temperature (295, 303, 310, and 315 K) is shown in Fig. 2. It is obvious that BSA has a strong fluorescence emission peaked at 345 nm after being excited at wavelength of 285 nm. When concentration of BSA is fixed, the concentration of daidzein and 3'-daidzein sulfonic sodium increases gradually, then the fluorescence intensity of 340 nm decreases regularly, and we can find a new fluorescence emission peak appeared at the 465 nm whose fluorescence intensity increases regularly when the concentration of daidzein and 3'-daidzein sulfonic sodium increases gradually. These phenomena indicate that daidzein and 3'-daidzein sulfonic sodium could interact with BSA and quench its intrinsic fluorescence.

The Stern–Volmer curves of daidzein and 3'-daidzein sulfonic sodium with BSA of different temperatures are shown in Fig. 3. It can be seen the Stern–Volmer curves is linear, it illustrates that the quenching type is probablely single quenching (static or dynamic quenching) .

The rate constant of BSA quenching procedure of daidzein and 3'-daidzein sulfonic sodium were found to be higher than 10^{13} l mol^{-1} s^{-1} which is greater than 2.0×10^{10} l mol^{-1} s^{-1} value reported for the maximum scatter collision quenching constant of various quenchers with biopolymers [23, 24]. Furthermore, it was observed that the K_{sv} values for the interaction of daidzein and 3'-daidzein sulfonic sodium with BSA are inversely correlated with temperature (Table 1), which indicated that the probable quenching mechanism of BSA–daidzein and BSA–3'-daidzein sulfonic sodium interaction proceeds by a static quenching proce-dure rather than by dynamic collision.

The Numbers of Binding Sites

When small molecules bind independently to a set of equivalent sites on a macromolecule, the binding

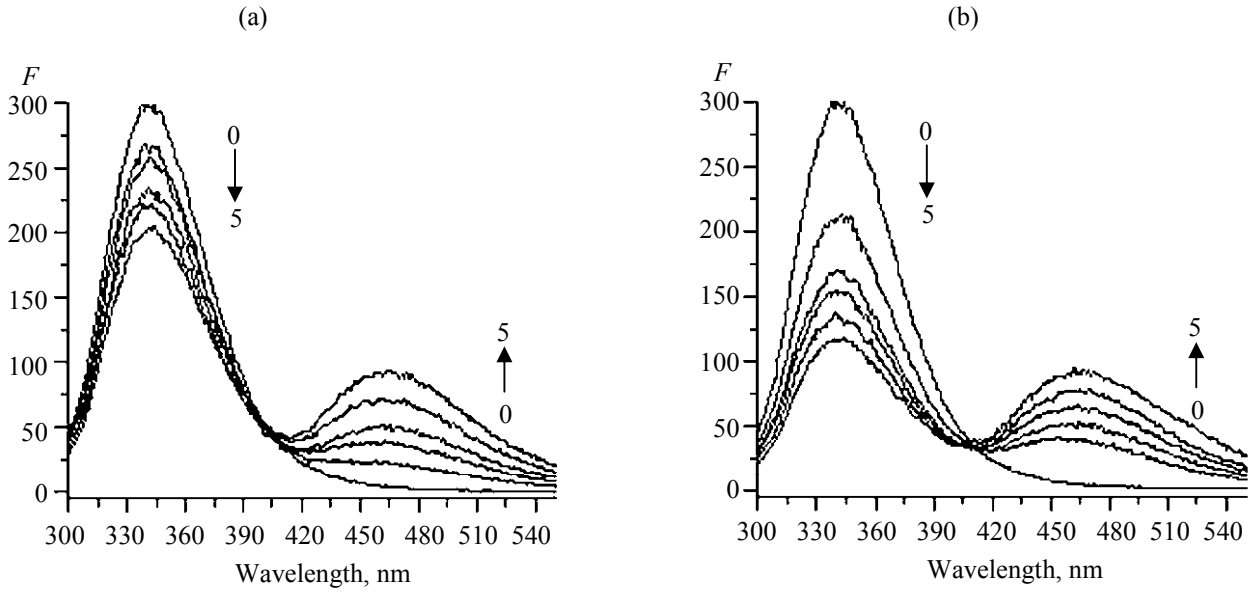


Fig. 2. Fluorescence quenching spectra of (a) daidzein–BSA and (b) 3'-daidzein sulfonic sodium-BSA. T = 310K, $c_{BSA} = 0.1\times10^{-4}$ mol l^{-1}; $c_{Ap}\times10^6$ mol l^{-1}. From 0 to 5: 0, 0.5, 1.0, 1.5, 2.0, 2.5.

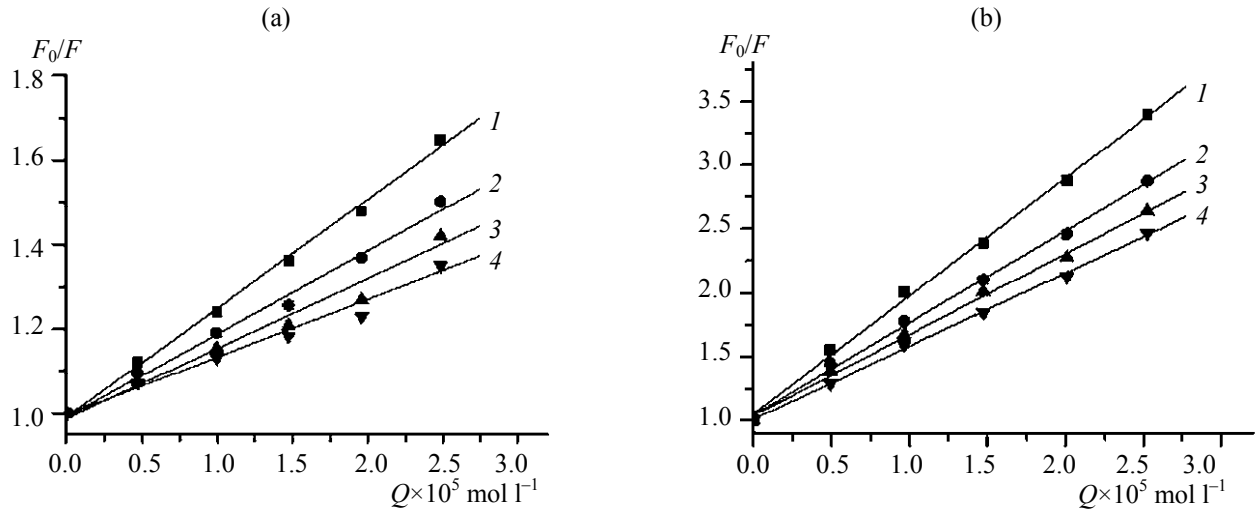


Fig. 3. Stern–Volmer plots at different temperatures: (a) daidzein–BSA and (b) 3'-daidzein sulfonic sodium–BSA. (a, b): (*1*) 295 K, (*2*) 303 K, (*3*) 310 K, and (*4*) 315 K.

constant (K_b) and the numbers of binding sites (n) can be determined by the following equation [25]:

$$\log [(F_0 - F)/F = \log K_b + n\log [Q]. \quad (2)$$

Therefore, K_b and n values can be obtained by the plot (Fig. 4) of log $(F_0 - F)/F$ versus log $[Q]$. The BSA–daidzein and BSA–3'-daidzein sulfonic sodium interaction are initiated by static quenching procedure, the results of K_b and n at different temperatures are presented in Table 2. The linear correlation coefficient is larger than 0.999, and the standard deviation is no more than 0.02, indicating that the assumptions underlying the derivation of Eq. (2) are satisfied. It can be seen from Table 2 that K_b decrease with the increasing temperature, which indicates the forming of an unstable BSA–daidzein and BSA–3'-daidzein sulfonic sodium complex in the binding reaction: the complex would possibly partly decompose when the temperature increases. The values of n at the expeimental temperatures are approximately equal to 1, which indicate that only a single binding site exists in BSA for daidzein and 3'-daidzein sulfonic sodium. In BSA, the tryptophan residues involved in binding could be either Trp134 or Trp212. Trp134 is embedded in the first sub-domain IB and it is more exposed to a hydrophilic environment, whereas Trp212 is embedded in sub-domain IIA and deeply buried in the hydrophobic loop [26]. Therefore, from the value of n, we speculated that daidzein and 3'-daidzein sulfonic sodium most likely bind to the hydrophobic pocket located in sub-domain IIA [27].

Binding Mode Between daidzein and 3'-Daidzein Sulfonic Sodium with BSA

Generally, small molecular substrates bind to protein through four binding forces: Van der Waals

Table 1. Stern–Volmer quenching constant and bimolecular quenching constant at different temperatures

Compound	Temperature, K	Stern–Volmer equation	K_{SV}, l mol^{-1}	K_q, l mol^{-1} s^{-1}	R
Daidzein	295	$F_0/F = 0.1763\times10^5 [Q] + 1$	0.2319×10^5	0.2319×10^5	0.9987
	303	$F_0/F = 0.1763\times10^5 [Q] + 1$	0.1763×10^5	0.1763×10^5	0.9976
	310	$F_0/F = 0.1463\times10^5 [Q] + 1$	0.1463×10^5	0.1463×10^5	0.9970
	315	$F_0/F = 0.1239\times10^5 [Q] + 1$	0.1239×10^5	0.1239×10^5	0.9973
3'-Daidzein sulfonic sodium	295	$F_0/F = 0.9689\times10^5 [Q] + 1$	0.9689×10^5	0.9689×10^5	0.9980
	303	$F_0/F = 0.7311\times10^5 [Q] + 1$	0.7311×10^5	0.7311×10^5	0.9970
	310	$F_0/F = 0.6433\times10^5 [Q] + 1$	0.6433×10^5	0.6433×10^5	0.9968
	315	$F_0/F = 0.5672\times10^5 [Q] + 1$	0.5672×10^5	0.5672×10^5	0.9990

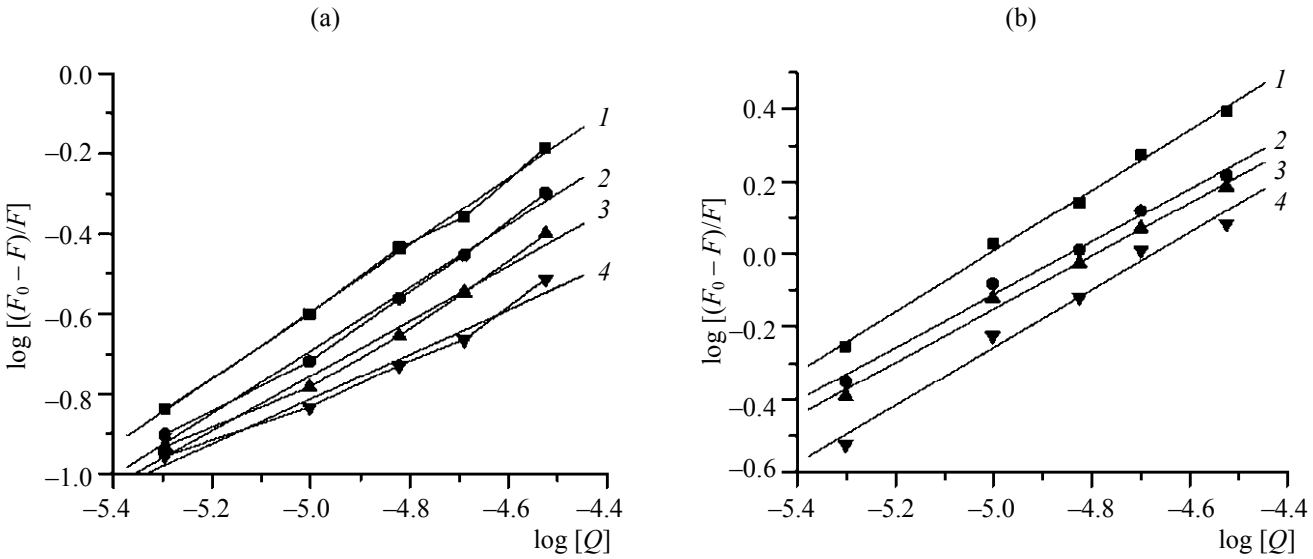


Fig. 4. Double logarithm plot of daidzein and 3'-daidzein sulfonic sodium quenching effect on BSA fluorescence at different temperatures, K. (a) Daidzein: (*1*) 15.5, (*2*) 30, (*3*) 37, and (*4*) 42. (b) 3'-Daidzein sulfonic sodium: (*1*) 15.5, (*2*) 30, (*3*) 37, and (*4*) 42.

interaction, hydrophobic force, electrostatic interaction and hydrogen bond [28]. The thermodynamic parameters, enthalpy change ($\Delta_r H^m$), entropy change ($\Delta_r S^m$) and free energy change ($\Delta_r G^m$) of reaction are the main evidence for confirming the binding mode. The thermodynamic parameters are evaluated using the following equations:

$$\ln (K_2/K_1) = \Delta_r H^m(1/T_1 - 1/T_2)/R, \quad (3)$$

$$\Delta_r G^m = -RT\log K, \quad (4)$$

$$\Delta_r S^m = (\Delta_r H^m - \Delta_r G^m)/T, \quad (5)$$

where R is the gas constant, T is the experimental temperature, and K is the binding constants at the corresponding temperature. If the temperature only changes a little, the enthalpy change ($\Delta_r H^m$) can be regarded as a constant and calculated from the slope of the van't Hoff relationship. According to Eqs. (3), (4), and (5), the values of the thermodynamic parameters ($\Delta_r H^m$, $\Delta_r S^m$, $\Delta_r G^m$) are obtained and presented in Table 3. The negative sign for $\Delta_r S^m$: $\Delta_r H^m$ and $\Delta_r G^m$ of the daidzein indicates that the binding of the daidzein with BSA occurs spontaneously and the binding force is an enthalpy-driven process. A positive sign for $\Delta_r S^m$ and $\Delta_r H^m$, a negative sign for $\Delta_r G^m$ indicate that the binding of 3'-daidzein sulfonic sodium with BSA occurs spontaneously and the binding force is an entropy-driven process [29].

Ross and Subramanian [30] have characterized the sign and magnitude of the thermodynamic parameter associated with various individual kinds of interaction that may take place in protein association process. According to Ross it can be concluded that both

Table 2. The binding constant of Hesperetin and Hesperidin with BSA

Compound	Temperature, K	Double logarithm equation	R	K_b, l mol^{-1}	n
Daidzein	295	$\log [(F_0 - F)/F] = 0.8782\log [Q] + 3.7965$	0.9953	6.26×10^3	0.88
	303	$\log [(F_0 - F)/F] = 0.9421\log [Q] + 3.9725$	0.9976	9.39×10^3	0.94
	310	$\log [(F_0 - F)/F] = 0.7419\log [Q] + 2.9550$	0.9744	9.02×10^2	0.74
	315	$\log [(F_0 - F)/F] = 0.6247\log [Q] + 2.3182$	0.9471	2.08×10^2	0.62
3'-Daidzein sulfonic sodium	295	$\log [(F_0 - F)/F] = 0.8341\log [Q] + 4.1790$	0.9980	1.51×10^4	0.83
	303	$\log [(F_0 - F)/F] = 0.7293\log [Q] + 3.5348$	0.9954	3.43×10^3	0.73
	310	$\log [(F_0 - F)/F] = 0.7336\log [Q] + 3.5129$	0.9961	3.28×10^3	0.73
	315	$\log [(F_0 - F)/F] = 0.7935\log [Q] + 3.7098$	0.9908	5.13×10^3	0.79

Table 3. The thermodynamic parameters of Hesperetin and Hesperidin–BSA binding procedure

Compound	Temperature, K	$\Delta_r H^m$, kJ mol^{-1}	$\Delta_r G^m$, kJ mol^{-1}	$\Delta_r S^m$, kJ mol^{-1}
Daidzein	310	–238.21	–17.54	–711.86
	315		–13.98	
3'-Daidzein sulfonic sodium	310	72.62	–20.86	301.58
	315		–22.37	

hydrogen bonding and van der Waals interaction play a role in the binding of the daidzein with BSA, and hydrophobic force play a role in the binding of 3'-daidzein sulfonic sodium to BSA. Since the daidzein is a polyphenolic compound these interactions could proceed through –OH groups and aromatic rings of flavonoid molecule [31, 32]. Therefore, we think that the hydrophobic interactions also play a role in the binding of the daidzein to BSA.

Energy Transfer from BSA to Daidzein and 3'-Daidzein Sulfonic Sodium

Energy transfer phenomena have wide applications in energy conversion process. According to the Förster's non-radiative energy transfer theory [33], if the emitted fluorescence from a donor could be absorbed by an acceptor, energy may transfer from the donor to the acceptor. The energy transfer effect is related not only to the distance between the acceptor and the donor, but also to the critical energy transfer distance. The distance between the donor (BSA) and the acceptor (Daidzein and 3'-daidzein sulfonic sodium) can be calculated according to the Förster's non-radiative energy transfer theory. The efficiency of energy transfer, E, is given by the following equation:

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

where F and F_0 are the fluorescence intensities of BSA in the presence and absence of daidzein or 3'-daidzein sulfonic sodium, r is the distance between acceptor and donor and R_0 is the critical distance when the transfer efficiency is 50%. R_0^6 is calculated using the equation:

$$R_0^6 = 8.8\times10^{-25}K^2N^{-4}\Phi J, \quad (7)$$

where K^2 is the spatial orientation factor of the dipole, N is the refractive index of the medium, Φ 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, which can be calculated by the equation:

$$J = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda}{\sum F(\lambda)\Delta\lambda}, \quad (8)$$

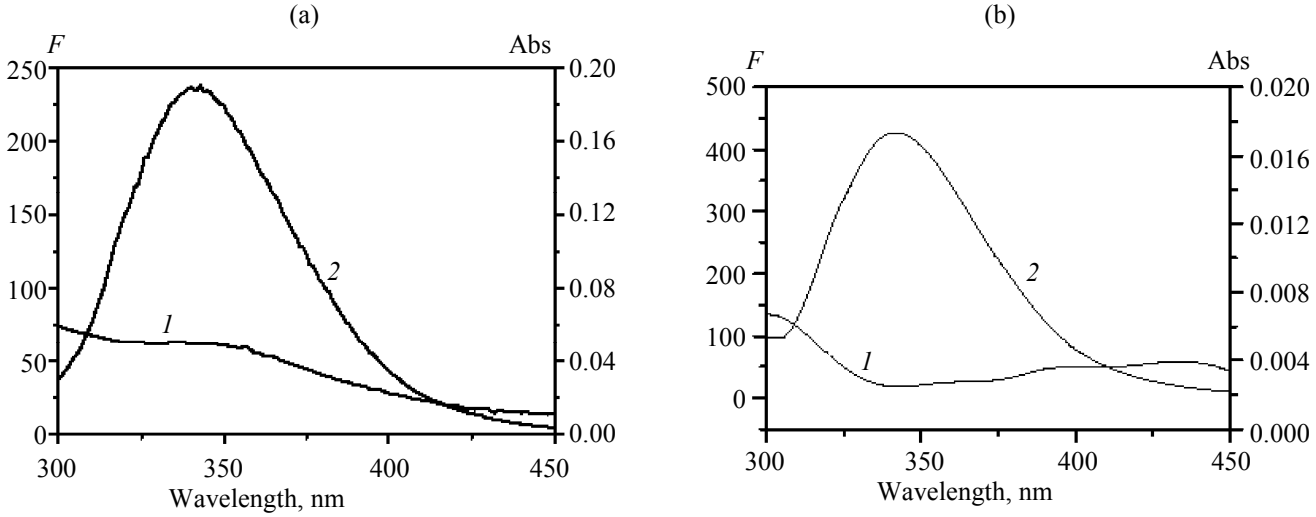


Fig. 5. The overlap of the absorption spectrum of the acceptor and the fluorescence spectrum of donor with the acceptor. (a): (*1*) the absorption spectrum of the daidzein, $c_{\text{Daidzein}} = 1.0\times10^{-5}$ mol l^{-1} and (*2*) the fluorescence spectrum of BSA with daidzein $c_{\text{BSA}} = c_{\text{Daidzein}} = 1.0\times10^{-5}$ mol l^{-1}. (b): (*1*) the absorption spectrum of the 3'-daidzein sulfonic sodium $c_{\text{3'-daidzein sulfonic sodium}} = 1.0\times10^{-5}$ mol l^{-1} and (*2*) the fluorescence spectrum of BSA with 3'-daidzein sulfonic sodium $c_{\text{BSA}} = c_{\text{3'-daidzein sulfonic sodium}} = 1.0\times10^{-5}$ mol l^{-1} ($\lambda_{ex} = 285$ nm) .

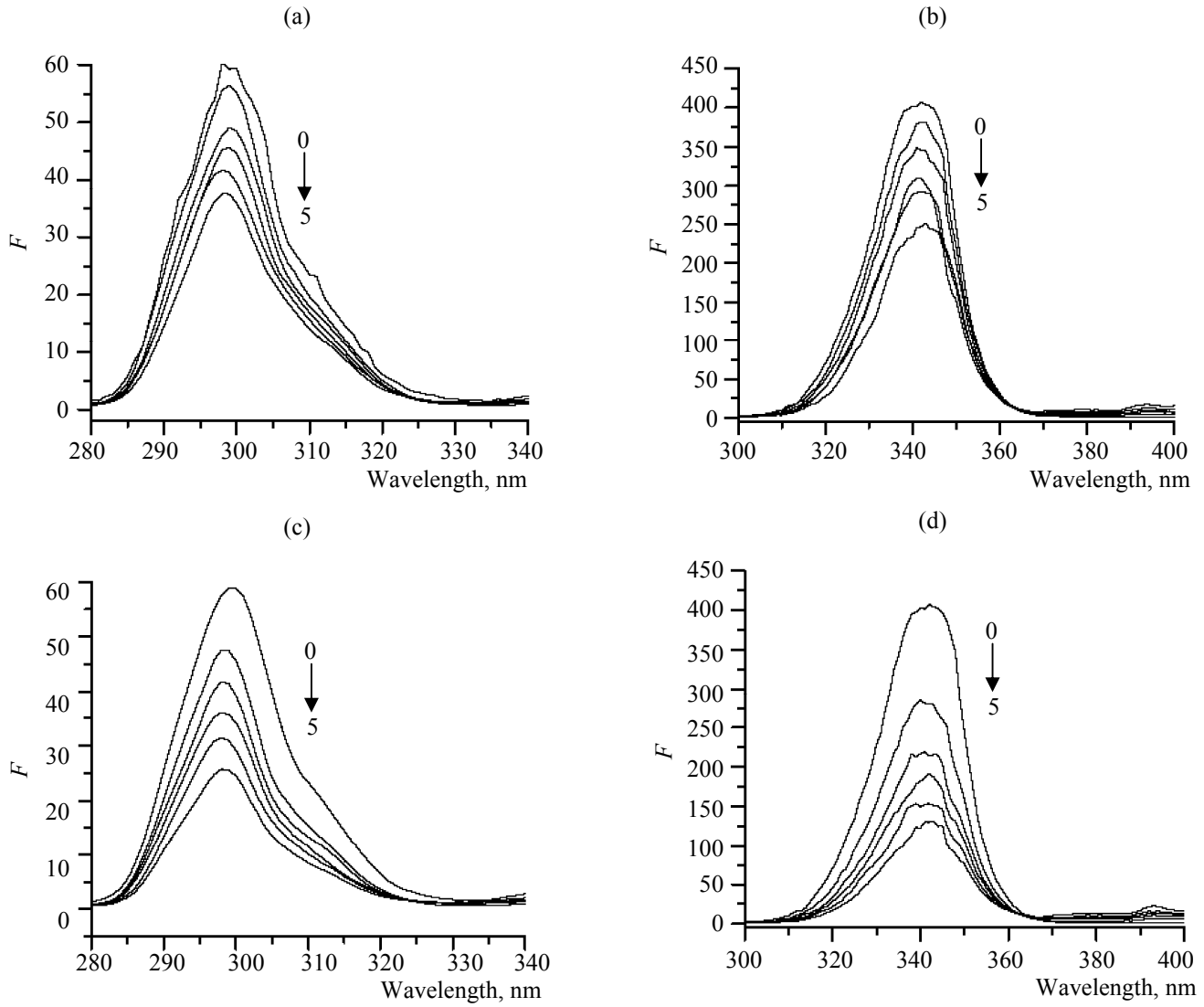


Fig. 6. Synchronous fluorescence spectra (a, b): daidzein and (c, d) 3'-daidzein sulfonic sodium. (a, c) $\Delta\lambda$ = 15 nm, (b, d) $\Delta\lambda$ = 60 nm; $c_{\text{Daidzein}} = c_{\text{3'-daidzein sulfonic sodium}} = 1.0\times10^{-5}$ mol l^{-1}. From 0 to 5: 0, 0.5, 1.0, 1.5, 2.0, 2.5.

where $F(\lambda)$ is the fluorescence intensity of the donor at wavelength range λ, $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at wavelength, nm. The overlap of the absorption spectrum of daidzein or 3'-daidzein sulfonic sodium and the fluorescence emission spectrum of BSA is shown in Fig. 5. The overlap integral, J, can be evaluated by integrating the spectra in Fig. 5 according to Eq. (8). In the present case, K^2 = 2/3, N = 1.36 and Φ = 0.15[34]. According to the Eqs. (6)–(8), we could calculate that for the daidzein–BSA: $J = 4.301\times10^{-15}$ cm^3 mol^{-1} l^{-1}, R_0 = 2.219 nm, E = 0.0283, and r = 4.02 nm, for the 3'-daidzein sulfonic sodium–BSA: $J = 1.091\times$ cm^3 mol^{-1} l^{-1}, R_0 = 1.766 nm, E = 0.0344, and r = 3.08 nm. The donor to acceptor distance are both less than 8 nm, which indicates the energy transfer from BSA to daidzein and 3'-daidzein sulfonic sodium occures with high probability [35]. In this work, daidzein and 3'-daidzein sulfonic sodium is probably bound to Trp-212 residue mainly through the hydrophobic interaction as evident from thermodynamic results. This is in agreement with conditions of Förster's non-radioactive energy transfer theory and indicated the static quenching between daidzein, 3'-daidzein sulfonic sodium and BSA once more [36].

Conformation Investigation

To explore the structural changes of BSA by the addition of daidzein and 3'-daidzein sulfonic sodium,

we measure of the synchronous fluorescence spectra of BSA with the concentrations of daidzein and 3'-daidzein sulfonic sodium used for the fluorescence quenching study. The synchronous fluorescence spectra introduced by Llody [37] has been used to characterize complex mixtures as it can provide fingerprints of complex samples [38]. The synchronous fluorescence spectra can provide the information on the molecular microenvironment, particularly in the vicinity of the fluorophore functional groups [39]. The fluorescence of BSA is due to the presence of tyrosine, tryptophan and phenylalanine residues. Hence spectroscopic methods are usually applied to study the conformation of the serum protein. In synchronous fluorescence spectra, according to Miller [40], the difference between the excitation and emission wavelength ($\Delta\lambda = \lambda_{emi} - \lambda_{exc}$) reflects the spectra of chromophores with the different natures. With larger $\Delta\lambda$ values, such as 60 nm, the synchronous fluorescence of BSA is characteristic of the tryptophan residue, and smaller $\Delta\lambda$ values, such as 15 nm, is characteristic of tyrosine residue [41]. The synchronous fluorescence spectra of BSA with various concentrations of daidzein and 3'-daidzein sulfonic sodium are recorded at $\Delta\lambda$ = 15 nm (Figs. 6a, 6b) and $\Delta\lambda$ = 60 nm (Figs. 6c, 6d), respectively. We find the emission intensity of tryptophan fluorescence and the tyrosine residues decreases regularly, but its emission wavelength is without significant change. It suggests that the interaction of daidzein and 3'-daidzein sulfonic sodium with BSA does not affect the conformation of the tryptophan and the tyrosine micro-region.

CONCLUSIONS

The interaction between daidzein and 3'-daidzein sulfonic sodium with BSA have been studied by UV–VIS absorption, fluorescence and synchronous fluorescence spectra. The results indicate clearly that that both daidzein and 3'-daidzein sulfonic sodium quenches the fluorescence of BSA through static quenching and hydrogen bonding and van der Waals interaction played a main role in the binding of daidzein with BSA on the base of the thermodynamic parameters ($\Delta S < 0$, $\Delta H < 0$); the hydrophobic force played a main role in the binding of 3'-daidzein sulfonic sodium with BSA on the base of the thermodynamic parameters ($\Delta H > 0$, $\Delta S > 0$). Moreover, the results of synchronous fluorescence spectra show that binding of daidzein and 3'-daidzein sulfonic sodium with BSA cannot induce conformational changes in BSA.

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