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Studies of the Interaction Between Hesperidin and Its Aglycone Hesperetin with Bovine Serum Albumin by Spectroscopic Methods

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The interaction between hesperidin and its aglycone hesperetin with bovine serum albumin in physiological buffer (pH = 7.4) is investigated by spectroscopic methods. The results reveal that both hesperetin and hesperidin could strongly quench the intrinsic fluorescence of bovine serum albumin. The quenching mechanism of hesperetin for bovine serum albumin is a static quenching procedure, but hesperidin for bovine serum albumin is a dynamic quenching. The apparent binding constants (Ka) and number of binding sites 'n' of hesperetin and hesperidin with bovine serum albumin are obtained by fluorescence quenching method. The interaction of hesperetin with bovine serum albumin is driven mainly by hydrogen bonding and van't Hoff and hesperidin with bovine serum albumin is driven mainly by hydrophobic. The distance 'r' between bovine serum albumin and hesperetin is calculated to be 3.08 nm. The results of synchronous fluorescence spectra show that binding of hesperidin and hesperetin with bovine serum albumin can't induce conformational changes in bovine serum albumin.

Key Words: Hesperetin, Hesperidin, Bovine serum albumin, Protein-flavonoid interaction, Fluorescence spectra.

INTRODUCTION

Flavonoids are a group of naturally occurring polyphenols that are ubiquitously distributed in various foods and beverages of plant origin[1]. These compounds display many biological and therapeutical properties. They are important components in human diet and received a great deal of attention for their beneficial effects in human health. Most of the therapeutic properties of flavonoids have been ascribed to their antioxidant and enzyme inhibitory activities[2,3]. In recent decades, investigation on flavonoids is mainly focused on their physiological and pharmacological function and many publications suggest that flavonoids possess multiple beneficial biological effects.

Hesperidin (Fig. 1) is a flavanone glycoside and is consisted of the flavone hesperitin bound to the disaccharide rutinose. The flavanone mainly isolated from citrus fruits and the sugar cause of hesperidin is more soluble than hesperitin. Hesperidin is believed to play a role in plant defense. It acts as an antioxidant according to *in vitro* studies[4]. In human nutrition it contributes to the integrity of the blood vessels. Various preliminary studies reveal novel pharmaceutical properties. Hesperidin reduces cholesterol and blood pressure in rats[5]. In a mouse study large doses of the glucoside hesperidin decrease bone density loss[6]. Hesperidin has antiinflammatory effects[7] and it is also a sedative, possibly acting through opioid or adenosine receptors[8-10].

Hesperetin (3',5,7-trihydroxy-4-methoxyflavanone) (Fig. 1) is a bioflavonoid and, to be more specific, a flavanone, it is the aglycone and a metabolite of hesperidin. Hesperetin is a cholesterol lowering flavanoid found in a number of citrus juices. It appears to reduce cholesteryl ester mass, inhibit apoB secretion by up to 80 % and may have antioxidant, antiinflammatory, antiallergic, hypolipidemic, vasoprotective and anticarcinogenic actions.

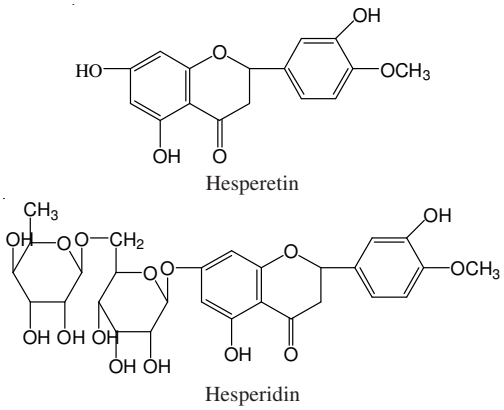

Fig. 1. Molecular structure of hesperetin and hesperidin

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. Bovine serum albumin (BSA) has a wide range of physiological functions involving binding, transport and delivery of fatty acids, porphyrins, bilirubin and steroids, *etc.*[11] and the results of all the studies are consistent with the fact that bovine and human serum albumins are homologous proteins[12]. This paper investigates the interaction of hesperidin and its aglycone hesperetin with bovine serum albumin by spectroscopic methods.

Some techniques are commonly used to detect interaction between drug and serum albumin including the fluorescence spectra[13], UV-spectrophotometry[14], circular dichroism spectra (CD)[15], Raman spectra[16], nuclear magnetic resonance[17], electrochemistry[18], equilibrium dialysis[19] and fourier transform infrared[20]. Fluorescence spectra is a powerful one among all the methods, because it is highly sensitive, rapid and simple and synchronous. Fluorescence spectra can give information about the changes in the local environment of the fluorophore. By measuring the intrinsic fluorescence quenching of bovine serum albumin, 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 investigate the interaction of bovine serum albumin with hesperidin and its aglycone hesperetin. The effects of temperature on this reaction are also examined by fluorescence quenching method. The results reveal that the hesperetin for bovine serum albumin is a static quenching procedure, but hesperidin for bovine serum albumin is a dynamic quenching. And hydrogen bonding and van't Hoff play a main role in the binding of hesperetin with bovine serum albumin on the base of the thermodynamic parameters ($\Delta S < 0$, $\Delta H < 0$). The hydrophobic force plays a main role in the binding of hesperidin with bovine serum albumin on the base of the thermodynamic parameters ($\Delta H > 0$, $\Delta S > 0$). Moreover, the effects of the hesperidin and hesperetin binding on the conformation of bovine serum albumin are investigated by synchronous fluorescence spectra.

EXPERIMENTAL

Hesperidin and hesperetin (analytical grade) are obtained from National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. Bovine serum albumin is obtained from Sino-American Biotechnology Company and used without further purification. The stock solutions of hesperidin and hesperetin (1.0×10^{-3} mol L^{-1}) are prepared in 1 % aqueous KOH solution. Bovine serum albumin stock solution (1.0×10^{-3} mol L^{-1}, based on its molecular weight of 65,000) is prepared in 0.1 mol L^{-1} NaCl, 0.05 mol L^{-1} *Tris*-HCl buffer of pH = 7.4. All other chemicals are of analytical reagent grade and double distilled water is used throughout the experiment. All stock solutions are stored at 0-4 ºC.

Fluorescence and UV-vis absorbance spectroscopy: Fluorescence measurements are performed with a Shimadzu spectrofluorimeter Model RF-5301 equipped with a 150W 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) are constantly maintained for all experiments. Fluorescence quenching spectra is recorded at 295, 303, 310 and 315 K in the range of 300-500 nm. The absorption spectra are measured on a Hitachi UV-8453 UV/VIS spectrophotometer at 295 K. Quartz cells of 1.00 cm are 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 of the quantum yield and strength of the fluorescence. The static quenching is initiated from the formation of non-fluorescent complex between quencher and fluorophore. In general, dynamic and static quenching can be distinguished by their different dependence on temperature and viscosity[21]. For dynamic quenching, the UV/vis absorption spectra of fluorophore is not changed, only excite-state fluorescence molecule is influenced by quencher, but for static quenching, a compound is formed between ground-state of fluorophore and quencher, therefore, the absorption spectra of fluorophore would be influenced[22].

Both static and dynamic processes can be described by the well known Stern-Volmer equation as eqn. 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 presence of quencher, K_q is the quenching rate constant, τ_0 is the average lifetime of the molecule without any quencher and the fluorescence lifetime of the biopolymer is 10^{-8} s[23], [Q] is the quencher concentration and K_{sv} is the Stern-Volmer quenching constant, which can be written as $K_{sv} = K_q\tau_0$[24]. 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[25]. 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[26].

The effect of hesperidin and hesperetin on bovine serum albumin fluorescence intensity under different temperature (295, 303, 310 and 315 K) is shown in Fig. 2. It is obvious that bovine serum albumin has a strong fluorescence emission peak at 345 nm after being excited with a wavelength of 285 nm. When concentration of bovine serum albumin is fixed, the concentration of hesperidin and hesperetin increases gradually, the fluorescence intensity of bovine serum albumin decreases regularly and we can find the maximum emission wavelength of bovine serum albumin fluorescence has a slight red-shift (form 340 nm to 351 nm) with increase of the concentration of hesperetin gradually. These phenomena indicate that hesperidin and hesperetin could interact with bovine serum albumin and quench its intrinsic fluorescence.

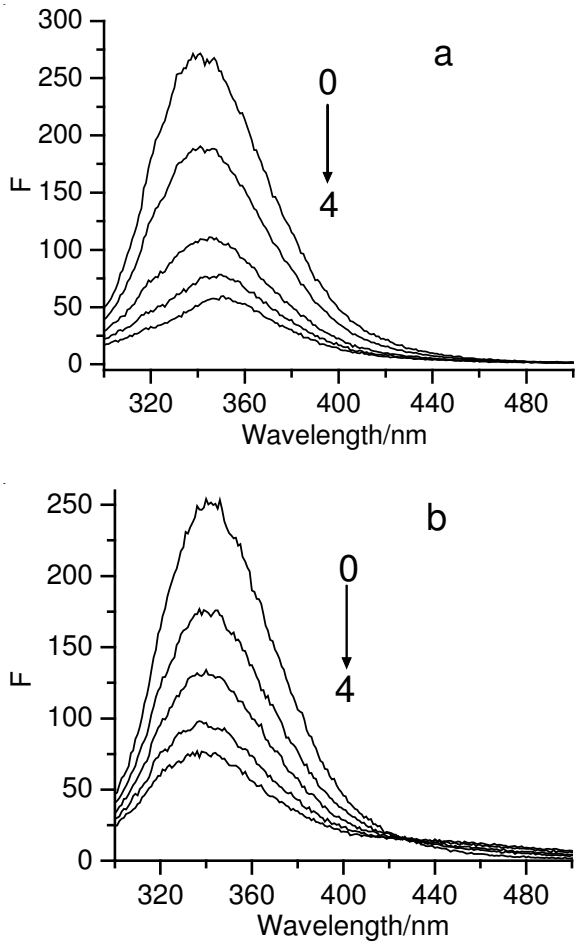

Fig. 2. Fluorescence quenching spectra of hesperetin-bovine serum albumin (BSA) (a) and hesperidin-bovine serum albumin (b) (T = 310 K) $c_{BSA} = 0.1 \times 10^{-4}$ mol L^{-1}; $c_{Hesperetin(Hesperidin)}/10^{-6}$ mol L^{-1} from 0 to 4: 0, 0.5, 1.0, 1.5, 2.0

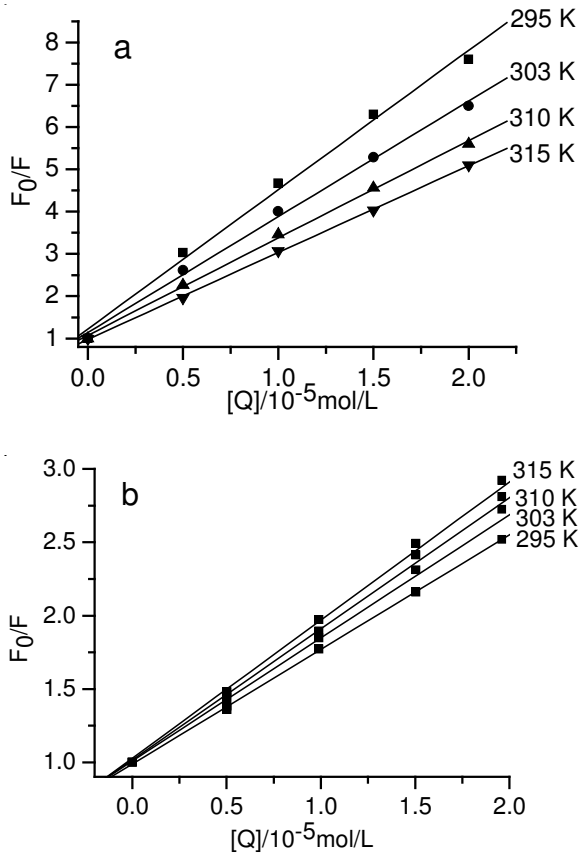

Fig. 3. Stern-Volmer plots at different temperatures hesperetin-bovine serum albumin (a) and hesperidin-bovine serum albumin (b)

The Stern-Volmer curves of hesperidin and hesperetin with bovine serum albumin in different temperatures are shown in Fig. 3. It can be seen that the Stern-Volmer curves are linear. It illustrates that the quenching type is probablely single quenching (static or dynamic quenching).

The analysis of interaction between hesperidin and hesperetin with bovine serum albumin at different temperatures reveal a quenching mechanism for this flavonoid. We study the relationship between the value of K_{sv} and temperatures (295, 303, 310 and 315 K). The K_{sv} of hesperetin with bovine serum albumin are inversely correlated with temperature (Table-1), which indicates that the quenching mechanism of bovine serum albumin-hesperetin interaction is a static quenching procedure rather than dynamic collision. But the K_{sv} of hesperidin with bovine serum albumin are consistent with temperature, which indicates that the probable quenching mechanism of bovine serum albumin-hesperidin interaction is a dynamic quenching[27]. However, the rate constant of quenching procedure of hesperidin and hesperetin are both found to be higher than 10^{12} 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[28]. It seems as if quenching mechanism of hesperetin and hesperidin are both static quenching mechanism. But the ionic strength is an important influencing factor for the rate constant of quenching procedure, the K_q of hesperidin-bovine serum albumin is higher than 2.0×10^{10} L mol^{-1} s^{-1}. This is due to the effects of ionic strength. Moreover, the UV/vis absorption spectra of bovine serum albumin is not changed when the hesperidin is added, which further confirms quenching mechanism of bovine serum albumin-hesperidin interaction is a dynamic quenching rather than static quenching mechanism.

TABLE-1
STERN-VOLMER QUENCHING CONSTANT AND BIMOLECULAR QUENCHING CONSTANT AT DIFFERENT TEMPERATURES

	Temperature (K)	Stern-Volmer equation	K_{SV} (L mol^{-1})	K_q (L mol^{-1} s^{-1})	r
Hesperetin	295	$F_0/F = 1.7447 \times 10^5$ [Q] + 1.1530	1.7447×10^5	1.7447×10^{13}	0.9974
	303	$F_0/F = 1.4149 \times 10^5$ [Q] + 1.1270	1.4149×10^5	1.4149×10^{13}	0.9974
	310	$F_0/F = 1.1712 \times 10^5$ [Q] + 1.1108	1.1712×10^5	1.1712×10^{13}	0.9970
	315	$F_0/F = 0.9931 \times 10^5$ [Q] + 1.0972	0.9931×10^5	0.9931×10^{13}	0.9965
Hesperidin	295	$F_0/F = 0.7781 \times 10^5$ [Q] + 1.0101	0.7781×10^5	0.7781×10^{13}	0.9992
	303	$F_0/F = 0.8376 \times 10^5$ [Q] + 1.0238	0.8376×10^5	0.8376×10^{13}	0.9969
	310	$F_0/F = 0.8721 \times 10^5$ [Q] + 1.0429	0.8721×10^5	0.8721×10^{13}	0.9971
	315	$F_0/F = 0.9239 \times 10^5$ [Q] + 1.0305	0.9239×10^5	0.9239×10^{13}	0.9962

Therefore, the following conclusions have been drawn. The quenching mechanism of bovine serum albumin-hesperetin interaction is a static quenching procedure, but quenching mechanism of bovine serum albumin-hesperidin interaction is a dynamic quenching.

Numbers of binding sites of bovine serum albumin-hesperetin: When small molecules bind independently to a set of equivalent sites on a macromolecule, the binding constant (Kb) and the numbers of binding sites (n) can be determined by the following equation[29]:

$$\log\left[\frac{(F_0 - F)}{F}\right] = \log Kb + n\log[Q] \quad (2)$$

Therefore, Kb and n values can be obtained by the plot of log (F_0-F)/F *versus* log [Q] (Fig. 4). The bovine serum albumin-hesperetin interaction is initiated by a static quenching procedure. The results of Kb and n at different temperatures are presented in Table-2. The linear correlation coefficient is larger than 0.999, indicating that the assumptions underlying the derivation of eqn. 2 are satisfied. It can be seen from Table-2 that Kb decreases with the increasing of temperature, which indicates the forming of an unstable bovine serum albumin-hesperetin complex in the binding reaction, the complex would possibly be partly decomposed when the temperature increases. The values of 'n' at the experimental temperatures are approximately equal to 1, which indicates that only a single binding site exists in bovine serum albumin for hesperetin.

Binding mode between hesperidin and hesperetin with bovine serum albumin: Generally, small molecular substrates bind to protein through four binding forces: van der Waals interaction, hydrophobic force, electrostatic interaction and hydrogen bond[30]. The thermodynamic parameters (enthalpy change (ΔrHm), entropy change (ΔrSm) and free energy change (ΔrGm)) of reaction are the main evidence for confirming the binding mode. The thermodynamic parameters are evaluated using the following equations:

$$\ln(K_2/K_1) = \Delta rHm\,(1/T_1 - 1/T_2)/R \quad (3)$$

$$\Delta rGm = -RT\log K \quad (4)$$

$$\Delta rSm = (\Delta rHm - \Delta rGm)/T \quad (5)$$

where R is the gas constant, T is the experimental temperature and K is the binding constants at corresponding temperature. If the temperature only changes a little, the enthalpy change (ΔrHm) can be regarded as a constant and calculated from the slope of the van't Hoff relationship. According to eqns. 3, 4 and 5, the values of the thermodynamic parameters (ΔrHm, ΔrSm, ΔrGm) are obtained and presented in Table-3. The negative sign for ΔrSm,ΔrHm and ΔrGm of the hesperetin, indicates

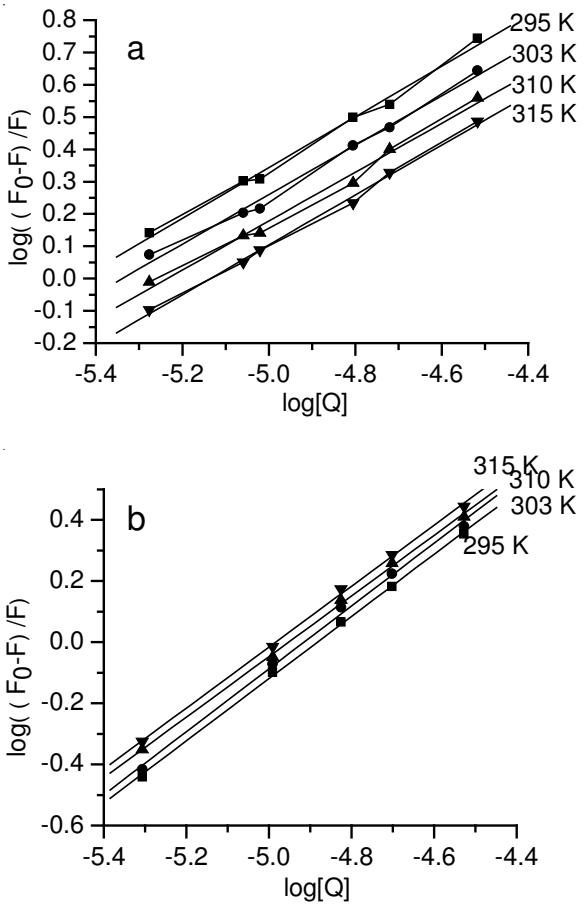

Fig. 4. Double logarithm plot of hesperetin and hesperidin quenching effect on bovine serum albumin fluorescence at different temperatures (a) hesperetin, (b) hesperidin

that the binding of the hesperetin with bovine serum albumin occurs spontaneously and is an enthalpy-driven process. A positive sign for ΔrSm, ΔrHm and a negative sign for ΔrGm of the hesperidin, indicate that the binding of hesperidin with bovine serum albumin occurs spontaneously and is an entropy-driven process[31].

Ross and Subramanian[32] 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 them, it can be concluded that both hydrogen bonding and van't Hoff play a role in the binding of the hesperetin with bovine serum albumin and hydrophobic force plays a role in the binding of hesperidin to bovine serum albumin. But because the hesperetin is a polyphenolic compound and these interactions could proceed through -OH groups and aromatic rings of flavonoids

TABLE-2
BINDING CONSTANT OF HESPERETIN AND HESPERIDIN WITH BOVINE SERUM ALBUMIN

	Temperature (K)	Double logarithm equation	r	K_b/(L mol^{-1})	n
Hesperetin	295	log[(F_0-F)/F] = 0.8314 log[Q] + 4.4794	0.9994	3.02×10^4	0.83
	303	log[(F_0-F)/F] = 0.8348 log[Q] + 4.4050	0.9998	2.54×10^4	0.83
	310	log[(F_0-F)/F] = 0.8172 log[Q] + 4.2417	0.9990	1.74×10^4	0.82
	315	log[(F_0-F)/F] = 0.8047 log[Q] + 4.1122	0.9978	1.29×10^4	0.80
Hesperidin	295	log[(F_0-F)/F] = 1.0210 log[Q] + 4.9953	0.9942	9.89×10^4	1.02
	303	log[(F_0-F)/F] = 0.9753 log[Q] + 4.7886	0.9870	6.15×10^4	0.98
	310	log[(F_0-F)/F] = 0.9316 log[Q] + 4.6075	0.9915	4.05×10^4	0.93
	315	log[(F_0-F)/F] = 0.9725 log[Q] + 4.7967	0.9891	6.26×10^4	0.97

TABLE-3
THERMODYNAMIC PARAMETERS OF HESPERETIN AND HESPERIDIN-BOVINE SERUM ALBUMIN BINDING PROCEDURE

	Temp. (K)	ΔrHm (kJ mol^{-1})	ΔrGm (kJ mol^{-1})	ΔrSm (J mol^{-1})
Hesperetin	310	-60.54	-25.16	-114.12
	315		-24.71	
Hesperidin	310	70.71	-28.92	316.29
	315		-27.34	

molecule[33,34]. Therefore, it is presumed that the hydrogen bonding and hydrophobic interactions, van't Hoff all play a role in the binding of the hesperetin to bovine serum albumin.

Energy transfer from bovine serum albumin to hesperetin: Energy transfer phenomena have wide applications in energy conversion process. According to the Förster's non-radiative energy transfer theory[35], 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 them, but also to the critical energy transfer distance. The distance between the donor (bovine serum albumin) and the acceptor (hesperetin) 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 bovine serum albumin in the presence and absence of hesperetin, '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:

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

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:

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

where F(λ) is the fluorescence intensity of the donor at wavelength range λ, ε(λ) is the molar absorption coefficient of the acceptor at wavelength range λ. The overlap of the absorption spectrum of hesperetin and the fluorescence emission spectrum of bovine serum albumin is shown in Fig. 5. The overlap integral, J, can be evaluated by integrating the spectra in Fig. 5 according to eqn. 8. In the present case, K^2 = 2/3, N = 1.36 and F = 0.15[36]. According to the eqns. 6-8, we could calculate the results: J = 1.091 × 10^{-15} cm^3/mol L^{-1}, R_0 = 1.766 nm, E = 0.0344 and r = 3.08 nm. The donor to acceptor distance is less than 8 nm, which indicates the energy transfer from bovine serum albumin to hesperetin occures with high probability[37]. In this work, hesperetin is probably bound to Trp-212 residue mainly through the hydrophobic interaction from thermodynamic results. This accords with conditions of Förster's non-radioactive energy transfer theory and indicates static quenching between hesperetin and bovine serum albumin again[38].

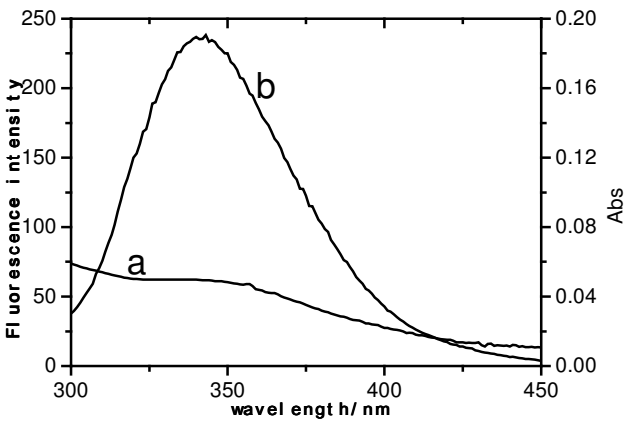

Fig. 5. Overlap of the absorption spectrum of hesperetin (a) $C_{Hesperetin}$ = 1.0 × 10^{-5} mol L^{-1} and the fluorescence spectrum of bovine serum albumin with hesperetin (b) $C_{BSA} = C_{Hesperetin}$ = 1.0 × 10^{-5} mol L^{-1} (λ_{ex} = 285 nm)

Conformation investigation: To explore the structural changes of bovine serum albumin by the addition of hesperidin and hesperetin, we measure the synchronous fluorescence spectra of bovine serum albumin with the concentration change of hesperidin and hesperetin. The synchronous fluorescence spectra introduced by Llody[39] has been used to characterize complex mixtures as it can provide fingerprints of complex samples[40]. It can also provide the information on the molecular microenvironment, particularly in the vicinity of the fluorophore functional groups[41]. The fluorescence of bovine serum albumin is due to the presence of tyrosine, tryptophan and phenylalanine residues. According to Miller[42], the difference between the excitation and emission wavelength (Δλ = λemi – λexc) reflects the spectra of chromophores with the different natures. With larger Δλ values, such as 60 nm, the synchronous fluorescence of bovine serum albumin is characteristic of the tryptophan residue and smaller Δλ values, such as 15 nm, is characteristic of tyrosine residue[43]. The synchronous fluorescence spectra of bovine serum albumin with various concentrations of hesperidin and hesperetin are recorded at Δλ = 15 nm (Fig. 6(a)) and Δλ = 60 nm [Fig. 6(b)], respectively. We find the emission intensity of tryptophan fluorescence and the tyrosine residues decreases both regularly, but its emission wavelength without significant change. It suggests that the interaction of hesperidin and hesperetin with bovine serum albumin does not affect the conformation of the tryptophan and the tyrosine micro-region.

Conclusion

The interaction between hesperidin and its aglycone hesperetin with bovine serum albumin has been studied by UV/vis absorption, fluorescence and synchronous fluorescence spectra. The results indicate clearly that hesperetin quenches the fluorescence of bovine serum albumin through static quenching, but for the hesperidin dynamic quenching is involved in the quenching process. The hydrogen bonding and van't Hoff play a main role in the binding of hesperetin with bovine serum albumin on the base of the thermodynamic parameters (ΔS < 0,ΔH < 0), the hydrophobic force plays a main role in the binding of hesperidin with bovine serum albumin on the base of the thermodynamic parameters (ΔH > 0, ΔS > 0).

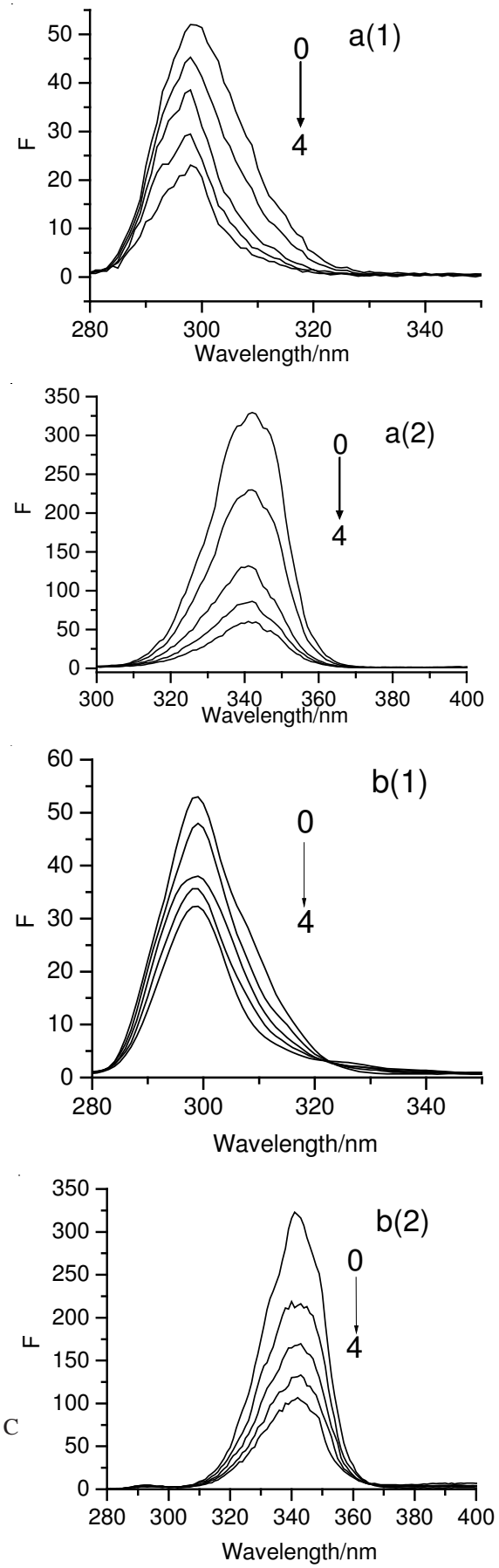

Fig. 6. Synchronous fluorescence spectra (a): Hesperetin (b) Hesperidin (1): $\Delta\lambda$ = 15 nm (2) $\Delta\lambda$ = 60 nm; $c_{Hesperetin} = c_{Hesperidin}/10^{-5}$ mol L^{-1}, from 0 to 4: 0, 0.5, 1.0, 1.5, 2.0

Moreover, the results of synchronous fluorescence spectra show that binding of hesperidin and hesperetin with bovine serum albumin can't induce conformational changes in bovine serum albumin.

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REFERENCES

1. E. Middleton, C. Kandaswami and T.C. Theoharides, *Pharmacol. Rev.*, **52**, 673 (2000).
2. B.H. Havsteen, *Pharmacol. Ther.*, **96**, 67 (2002).
3. M.R. Webb and S.E. Ebeler, *Biochem. J.*, **384**, 527 (2004).
4. Y. Murakami, M. Shoji, Y. Kadoma and S. Fujisawa, *Anticancer Res.*, **25**, 3367 (2005).
5. K. Ohtsuki, A. Abe and H. Mitsuzumi, *J. Nutr. Sci. Vitaminol.*, **49**, 447 (2003).
6. K. Kawaguchi, S. Kikuchi, R. Hasunuma, H. Maruyama, T. Yoshikawa and Y. Kumazawa, *Biol. Pharm. Bull.*, **27**, 679 (2004).
7. J.A. Emim, A.B. Oliveira and A.J. Lapa, *J. Pharm. Pharmacol.*, **46**, 118 (1994).
8. L.M. Loscalzo, C. Wasowski, A.C. Paladini and M. Marder, *Eur. J. Pharmacol.*, **580**, 306 (2008).
9. S.L. Guzmán-Gutiérrez and A. Navarrete, *Planta Med.*, **75**, 295 (2009).
10. K.A. Youdim, M.S. Dobbie, G. Kuhnle, A.R. Proteggente, N.J. Abbott and C. Rice-Evans, *J. Neurochem.*, **85**, 180 (2003).
11. S. Ashoka, J. Seetharamappa, P.B. Kandagal and S.M.T. Shaikh, *J. Lumin.*, **121**, 179 (2006).
12. J. Guharay, B. Sengupta and P.K. Sengupta, *Proteins*, **43**, 75 (2001).
13. P.B. Kandagal, S. Ashoka, J. Seetharamappa, S.M.T. Shaikh, Y. Jadegoud and O.B. Ijare, *J. Pharm. Biomed. Anal.*, **41**, 393 (2006).
14. C.D. Kanakis, P.A. Tarantilis, M.G. Polissiou, S. Diamantoglou and H.A. Tajmir-Riahi, *J. Mol. Struct.*, **798**, 69 (2006).
15. P.B. Kandagal, S.M.T. Shaikh, D.H. Manjunatha, J. Seetharamappa and B.S. Nagaralli, *J. Photochem. Photobiol.*, **189A**, 121 (2007).
16. X.C. Shen, H. Liang, J.H. Guo, C. Song, X.W. He and Y.Z. Yuan, *J. Inorg. Biochem.*, **95**, 124 (2003).
17. A. Sulkowska, B. Bojko, J. Rownicka, P. Rezner and W.W. Sulkowski, *J. Mol. Struct.*, **744**, 781 (2005).
18. M. Hepel, *Electroanalysis*, **17**, 1401 (2005).
19. I. Girard and S. Ferry, *J. Pharmaceut. Biomed.*, **14**, 583 (1996).
20. M.C. Millot, S. Servagent-Noinville, N.L. Taleb, M.H. Baron, M. Revault and B. Sebille, *J. Chromatogr. B: Biomed. Sci. Appl.*, **753**, 101 (2001).
21. J.R. Lakowicz, Plenum Press, New York, pp. 237-265 (1999).
22. X.W. Zhang, F.L. Zhao and K.A. Li, *J. Chem. Chin. Univ.*, **20**, 1063 (1999).
23. Y. Wang, H. Zhang, G. Zhang, W. Tao, Z. Fei and Z. Liu, *J. Pharm. Biomed. Anal.*, **43**, 1869 (2007).
24. J. Min, X. Meng-Xia, Z. Dong, L. Yuan, L. Xiao-Yu and C. Xing, *J. Mol. Struct.*, **692**, 71 (2004).
25. T. Spector, W.W. Hall and T.A. Krenitsky, *Biochem. Pharmacol.*, **35**, 3109 (1986).
26. A. Gong, X. Zhu, Y.Y. Hu and S. Yu, *Talanta*, **73**, 668 (2007).
27. Y.-J. Hua, H.-G. Yua, J.-X. Donga and X. Yanga, *Spectrochim. Acta*, **65A**, 988 (2006).
28. S. Bi, L. Ding, Y. Tian, D. Song, X. Zhou, X. Liu and H. Zhang, *J. Mol. Struct.*, **703**, 37 (2004).

29. Y.-J. Hu, Y. Liu, J.-B. Wang and X.-H. Xiao, *J. Pharm. Biomed. Anal.*, **36**, 915 (2004).
30. D. Leckband, *Ann. Rev. Biophys. Biomol. Struct.*, **29**, 1 (2000).
31. J.-C. Li, N. Li, Q.-H. Wu, Z. Wang, J.-J. Ma and C. Wang, *J. Mol. Struct.*, **833**, 184 (2007).
32. P.D. Ross and S. Subramanian, *Biochemistry*, **20**, 3096 (1981).
33. J. Tian, J. Liu, Z. Hu and X. Chen, *Bioorg. Med. Chem.*, **13**, 4124 (2005).
34. F. Zsila, Z. Bikádi and M. Simonyi, *Biochem. Pharmacol.*, **65**, 447 (2003).
35. T. Förster and O. Sinanoglu, Modern Quantum Chemistry, Academic Press, New York, Vol. 3, pp. 93-96 (1996).
36. F.-L. Cui, J. Fan, J.-P. Li and Z.-D. Hu, *Bioorg. Med. Chem.*, **12**, 151 (2004).
37. Y.J. Hu, Y. Liu and L.X. Zhang, *J. Mol. Struct.*, **750**, 174 (2005).
38. Y.-P. Wang, Y.-L. Wei and C. Dong, *J. Photochem. Photobiol.*, **17**, 6 (2006).
39. J.B.F. Lloyd, *Nat. Phys. Sci.*, **231**, 64 (1971).
40. G.Z. Chen, X.Z. Huang, J.G. Xu, Z.Z. Zheng and Z.B. Wang, Methods of Fluorescence Analysis, Science Press, Beijing, edn. 2, pp. 81-105 (1990).
41. B. Apicella, A. Ciajolo and A. Tregrossi, *Anal. Chem.*, **76**, 2138 (2004).
42. J.N. Miller, *Proc. Anal. Div. Chem. Soc.*, **16**, 203 (1979).
43. J.H. Tang, F. Luan and X.G. Chen, *Bioorg. Med. Chem.*, **14**, 3210 (2006).