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Characterize the interaction between naringenin and bovine serum albumin using spectroscopic approach

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ABSTRACT

Naringenin, a flavanone compound highly enriched in grapefruits, has been identified as a possible inhibitor of cell proliferation; and thus has the potential to act as an antitumorigenic agent. In this study, the binding of naringenin to bovine serum albumin (BSA) was studied at the physiological conditions (pH=7.40) by fluorescence and UV–vis spectroscopy. Naringenin strongly quenches the intrinsic fluorescence of BSA, and a decrease in the fluorescence quenching constant was observed together with an increase in temperature, which indicates that the fluorescence quenching of BSA by naringenin is a result of the formation of naringenin–BSA complex. Binding parameters calculating from Stern-Volmer method and Scatchard method showed that naringenin bind to BSA with the binding affinities of the order 10^4 L mol^{-1}. Thermodynamic parameters such as ΔG, ΔH and ΔS, were calculated at different temperatures, showing that electrostatic interactions were mostly responsible for the binding of naringenin to BSA. Site marker competitive displacement experiments demonstrating that naringenin bind with high affinity to site I (subdomain IIA) of BSA. Furthermore, the effect of metal ions to naringenin–BSA system was studied, and the specific binding distance r (3.30 nm) between donor (Trp-212) and acceptor (naringenin) was obtained according to fluorescence resonance energy transfer (FRET).

1. Introduction

Naringenin (4′,5,7-trihydroxyflavonone; molecular structure: inset of Fig. 1), a plant bioflavonoid found in grape fruit, tomato and citrus fruits, is a frequent component of the human diet and has gained increasing interest because of their positive health effects [1]. As nature's tender drugs, naringenin possess various biological/pharmacological activities including antioxidant, antiinflammatory, antimicrobial, antiviral, antimutagenic, antiestrogenic, antiatherogenic, hepatoprotective, nephroprotective, and anticarcinogenic activities, and to reduce plasma lipids and cholesterol [2–9].

In spite of these broad pharmacological uses of naringenin mentioned above, its effects on plasma protein and the mechanism of action is poorly understood. It is widely accepted in the pharmaceutical industry that the overall distribution, metabolism, and efficacy of many drugs can be altered based on their affinity to serum albumin [9–13]. Serum albumin has long been the center of attention of the pharmaceutical industry due to its ability to bind various drug molecules and alter their pharmacokinetic properties [13–16]; BSA has two tryptophan residues that possess intrinsic fluorescence. Trp-134 in the first domain and Trp-212 in the second domain. Trp-212 is located within a hydrophobic binding pocket of the protein, and Trp-134 is located on the surface of the molecule [17,18]. The crystal structure analyses indicate that the principal regions of ligand binding sites in albumin are located in hydrophobic cavities in subdomains IIA and IIIA [19,20]. According to Sudlow's nomenclature, two primary sites (I and II) have been identified for ligand binding to serum albumin [21,22]. Warfarin, an anticoagulant drug, and ibuprofen, a nonsteroidal antiinflammatory agent, have been considered as stereotypical ligands for Sudlow's site I and II, respectively. Warfarin, as the other bulky heterocyclic anions, binds to Sudlow's site I located in subdomain IIA, whereas ibuprofen, as other aromatic carboxylates with an extended conformation, prefers Sudlow's site II, located in subdomain IIIA [21–24]. Even though the binding sites of fatty acids on BSA have been located, the interaction of naringenin with BSA was poorly investigated. Therefore, it was of interest to study the binding of naringenin with BSA.

In this article, we present a spectroscopic approach to investigate the high affinity binding of naringenin to BSA under the physiological conditions. The interaction information

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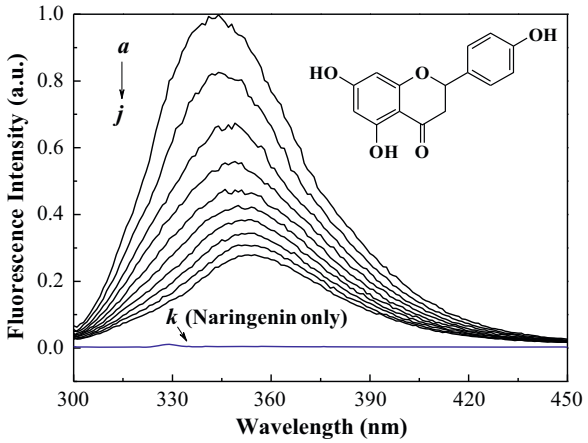

Fig. 1. Emission spectra of BSA in the presence of various concentrations of naringenin. c (BSA)$=1.0\times10^{-5}$ mol L^{-1}; c (naringenin)/(10^{-5}), a–j: from 0.0 to 3.6 mol L^{-1} at increments of 0.40 mol L^{-1}; curve k (blue line) shows the emission spectrum of naringenin only. ($T=292$ K, $\lambda_{ex}=295$ nm). The inset corresponds to the molecular structure of naringenin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

regarding quenching mechanisms, binding parameters, thermodynamic parameters, binding modes, high-affinity binding site, effect of metal ions, and intermolecular distances are all our investigation aims.

2. Materials and methods

2.1. Materials

BSA (defatted BSA, approx. 99%) and Warfarin were obtained from Sigma-Aldrich (St. Louis, MO, USA); Naringenin (CAS Number 480-41-1) was obtained from National institute for control of pharmaceutical and biological products (Beijing, China); Ibuprofen was obtained from Hubei biocause pharmaceutical Co., Ltd (Hubei, China; the purity no less than 99.7%); the buffer Tris had a purity of no less than 99.5% and NaCl, HCl, metal ions (metal chloride), etc. were all of analytical purity. All samples were dissolved in Tris–HCl buffer solution (0.05 mol L^{-1} Tris, 0.15 mol L^{-1} NaCl, pH 7.4). Appropriate blanks, run under the same conditions, were subtracted from the sample spectra. Sample masses were accurately weighted on a microbalance (Sartorius, ME215S) with a resolution of 0.1 mg.

2.2. Equipments and spectral measurements

The UV spectrum was recorded at room temperature on a U-3010 Spectrophotometer (Hitachi, Japan) equipped with 1.0 cm quartz cells. All fluorescence spectra were recorded on F-4500 Spectrofluorimeter (Hitachi, Japan) equipped with 1.0 cm quartz cells and a thermostat bath. The widths of both the excitation slit and the emission slit were set to 2.5 nm. An excitation wavelength of 295 nm was chosen since it is exclusive due to the intrinsic tryptophan (Trp) fluorophore.

All BSA solutions (1.0×10^{-5} mol L^{-1}) were dissolved in the pH 7.40 Tris–HCl buffer to maintain the pH, and NaCl solution was used to maintain the ionic strength at 0.15.

Fluorescence titration experiments: 2.0 mL 1.0×10^{-5} mol L^{-1} BSA was titrated manually by successive additions of a 4.0×10^{-3} mol L^{-1} naringenin (to give a final concentration of 0.4 $\times10^{-5}$ mol L^{-1}–3.6×10^{-5} mol L^{-1}) with trace syringes, and the fluorescence intensity was measured. All experiments were measured after 15 min at three temperatures (292, 301, and 310 K). The temperature of the sample was maintained by recycled water throughout the experiment. The maximal volume of naringenin solution added in BSA (2.0 mL) was 18 μL (when final concentration was 3.6×10^{-5} mol L^{-1}), which indicates that the change of the total volumes could be negligible with the naringenin solution addition, then the final BSA concentration will be left as 1.0×10^{-5} mol L^{-1}.

3. Results and discussions

3.1. BSA fluorescence characteristics and quenching mechanism

In this work, the concentrations of BSA solution were stabilized at 1.0×10^{-5} mol L^{-1}, and the concentrations of naringenin varied from 0 to 3.6×10^{-5} mol L^{-1} at increments of 0.4×10^{-5} mol L^{-1}. The effect of naringenin on BSA fluorescence intensity at 292 K is shown in Fig. 1. It was observed from Fig. 1 that a progressive decrease in the fluorescence intensity was caused by quenching, accompanied by an increase of wavelength emission maximum λ_{max} (a red shift, from 341 to 355 nm) in the albumin spectrum, a shift that can reasonably be attributed to an increased polarity (or a decreased hydrophobicity) of the region surrounding the tryptophan site [25]. In Fig. 1, curve k (blue line) shows only the emission spectrum of naringenin, which indicates that naringenin does not possess significant fluorescence features; thus, the effect of naringenin absorbs at the excitation wavelength of tryptophan (295 nm) would be negligible.

For fluorescence quenching, the decrease in intensity is usually described by the well-known Stern-Volmer equation [26]:

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

where F_0 and F denotes the steady-state fluorescence intensities in the absence and in the presence of quencher (naringenin), respectively, K_{SV} is the Stern-Volmer quenching constant, and [Q] is the concentration of the quencher. Hence, Eq. (1) was applied to determine K_{SV} by linear regression of a plot of F_0/F against [Q].

The quenching mechanisms are usually classified as either dynamic quenching or static quenching. Dynamic and static quenching can be distinguished by their differing dependence on temperature and viscosity [26]. Higher temperatures result in faster diffusion and hence larger amounts of dynamic quenching. Higher temperatures will typically result in the dissociation of weakly bound complexes, and hence smaller amounts of static quenching.

Fig. 2 displays the Stern-Volmer plots of the quenching of BSA tryptophan residues fluorescence by naringenin at different temperatures, the corresponding results at different temperatures are shown in Table 1. The results demonstrated the effect on fluorescence quenching by naringenin at each temperature (292, 301, and 310 K) studied, the result shows that the Stern-Volmer quenching constant K_{SV} is inversely correlated with temperature,

Table 1
Stern-Volmer quenching constants for the interaction of naringenin with BSA at various temperatures.

pH	T (K)	$10^{-4}K_{SV}$ (L mol^{-1})	R[a]	S.D.[b]
7.4	292	6.796	0.999 0	0.048 5
	301	5.554	0.999 1	0.039 0
	310	4.492	0.998 6	0.035 9

[a] R is the correlation coefficient.
[b] S.D. is standard deviation.