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Research Article
Spectral Properties of the Interaction Between Hesperidin of Tangerine Peel's Active Ingredient with Protein

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Abstract

Background and Objective: Tangerine peel has high medical value due to its physiological active including volatile components, flavonoids, alkaloids etc. In this paper, the aim of this work was to study the spectral properties of tangerine peel and the binding interaction between hesperidin of its effective components and bovine serum albumin (BSA). **Methodology:** The fluorescence spectroscopy was used with the different excitation wavelength under simulative physiological conditions. The dynamic quenching mechanism could be described by the Stern-Volmer equation. The binding parameters between hesperidin and BSA was calculated by double logarithmic equation. **Results:** The results show the fluorescence peak of tangerine peel is about 448 nm and there a certain red shift occur with the increase of the excitation. The investigation of between BSA and hesperidin show that hesperidin could intact with BSA and the hesperidin-BSA complex was formed. The binding constants between hesperidin and BSA are 3.26, 2.71 and 1.98×10^4 mol^{-1} L at 298, 305 and 310 K, respectively, indicating the binding capacity of hesperidin to BSA was weakened with the increasing temperature. **Conclusion:** It is concluded that, the peak wavelength of tangerine peel is about 448 nm, the hesperidin could interact with BSA, the fluorescence quenching of BSA caused by hesperidin is a static quenching.

Key words: Tangerine peel, serum albumin, fluorescence spectroscopy, fluorescence quenching, binding interaction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tangerine peel derived from Shen Nong Ben Cao Jing, is a Chinese herbal medicine and tastes bitter and acrid. It is a dry pericarp of citrus reticulate blanco or cultivar, whose alias is red tangerine dahongpao and chuan tangerine etc[1,2]. Its major components include flavonoids, volatile oil, limonin, alkaloids and trace elements (such as calcium, potassium, magnesium, iron, etc.) and that flavonoids are the major compounds among all physiological activities ingredients. Due to its properties of regulating and strengthening spleen, tangerine peel has been applied widely to treat the full abdominal distention[3], the acid[4], nausea[5] and constipation or diarrhea[6], also used in the treatment of digestive and respiratory diseases[7].

Many research have carried out to investigate the tangerine peel's characteristics and pharmacological effects for wider range of application. Liu TY *et al*.[8] studied that tangerine peel could reduce side effects of maprotiline and enhance maprotiline antidepressant effect. He and Xiao[9] shown that tangerine peel could provide as glazing layer on fish preservation during super-chilling storage. Ho and Kuo[10] investigated that the anti-inflammatory capacity of tangerine peel and its corresponding active compounds for treating neurodegenerative diseases. To the best of knowledge, most studies focused on its active compounds and application, seldom reports are about the spectral characteristic of Tangerine peel and its interaction with proteins, which is important for understanding the pharmacological effect in the body. Hesperidin is the main component of flavonoids in dried tangerine peel, so the hesperidin is selected as the research object.

Serum albumin plays a role in storage and transport and is the most abundant carrier protein in plasma, which could be combined with many endogenous and exogenous compounds[11]. Investigating the interaction between serum albumin and drugs is very significance to understand the existence, transportation, absorption, metabolism and pharmacological effects of the drug in the body[12]. The BSA is selected as the protein model in this work due to its advantage of the more stable nature, the abundant source and relatively cheap price, as well as its structural homology with human serum albumin[13-15]. Therefore, BSA has been widely applied in the fields of chemistry, life sciences and medical sciences. The BSA is a major carrier of plasma and could combine with many water-soluble substances, for instance, drugs, steroid hormones and long chain fatty acids *et al*. It contain 580 amino acid residues of a single polypeptide, two tryptophan residues, eight tyrosine residues, nine double rings formed by 17 disulfide bonds, a free sulfhydryl group in the 34th position of the peptide chain, not contain a component of sugar[16,17].

In recent years, many optical technique have been carried out to study the properties of proteins in structure and conformation. For instance, Fourier transform infrared spectroscopy, fluorescence spectroscopy, circular dichromatic spectroscopy, three-dimensional fluorescence spectroscopy and so on[18,19]. Of all these methods, fluorescence spectroscopy is a very effective method to investigate the conformation and structure of protein molecules, which may could provide many physical parameters including emission and excitation spectra, fluorescence life, quantum yield, fluorescence intensity, fluorescence polarization etc, such as these parameters may reflect the molecular structure and bonding from different aspects.

The aim of research was to study the spectral properties of tangerine peel and the binding interaction between hesperidin of its effective components and BSA. The fluorescence spectroscopy of tangerine peel under the different excitation wavelength was also determined.

MATERIALS AND METHODS

Reagents: Tangerine peel material was collected between November and December, 2017, from Jiangmen Jihui Food Co., Ltd. (Guangdong, China). The hesperidin was collected at December, 2017, from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). The tangerine peel and hesperidin stock solution was prepared and measured within 3 days in Changzhou City, Jiangsu Province of China. The preparation of tangerine peel stock solution: Tangerine peel 6 g was accurately weigh and immersed in distilled water 300 mL, heated and slightly boiled 15 min, cooled and filtered, then diluted to a certain concentration (6 mg mL^{-1}) and used appropriate dilution. Hesperidin solution (6.0×10^{-4} mol L^{-1}) was prepared in pH 7.4 phosphate buffer solution. The BSA was purchased from Beijing Boer West Technology Co., Ltd. (Beijing, China). The BSA stock solution (6.0×10^{-4}mol L^{-1}) was prepared in pH 7.4 phosphate buffer solution containing 0.1 mol L^{-1} NaCl.

Equipment: The fluorescence spectroscopy was measured by RF-5301PC spectrofluorophotometer (Shimadzu, Japan).

Procedure: The fluorescence spectroscopy of tangerine peel: A 3 mL tangerine peel solution (2 mg mL^{-1}) was measured at room temperature under the different excited wavelength.

The binding of hesperidin to BSA: A 3 mL solution BSA (1.0×10^{-6} mol L^{-1}), was added by different concentration hesperidin (to give a final concentration of 5×10^{-6} mol L^{-1}). After each added, shaken up, the reaction was maintained on 2 min.

Dynamic quenching mechanism analysis: As stated in the Eq. 1, the dynamic quenching mechanism could be described by the Stern-Volmer equation[20]:

$$F_0/F = 1+k_q\tau_0 [Q] = 1+K_{SV} [Q] \quad (1)$$

$$K_q = K_{SV}/\tau_0 \quad (2)$$

where, F_0 is the fluorescence intensities without the quencher, F is the fluorescence intensities with the different concentration quencher, k_q (as shown Eq. 2) represents the quenching rate constant (bimolecular), K_{SV} represents the dynamic quenching constant, [Q] is the concentration of the quencher and τ_0 is the average lifetime of the molecular without quencher ($\tau_0 = 10^{-8}$ sec)[21].

The binding parameters calculations: Then the relationship between bound molecules and free molecules could be expressed as the following Eq. 3:

$$\log(F_0-F) = \log K_A+n\log[Q] \quad (3)$$

where, K_A represents the binding constant and n is the number of binding sites.

RESULTS AND DISCUSSION

Fluorescence spectroscopy of tangerine peel excited by different wavelength: Figure 1a shows the fluorescence spectroscopy of tangerine peel under the excitation wavelength from 300-360 nm at every 20 nm interval. It can be seen that the maximum fluorescence intensity was excited at 360 nm and corresponding fluorescence peak was about 448 nm. When excited at 300, 320 and 340 nm, the corresponding fluorescence peak wavelengths were 446, 448 and 448 nm. It can be concluded that the fluorescence peak wavelengths were almost unchanged but the fluorescence intensity increases with the increasing excitation wavelength. From Fig. 1a, the fluorescence wavelength of tangerine peel was always greater than the excitation wavelength, the main reason of fluorescence emission is that the excited electron could jump from the lowest vibration and rotational energy of the excited single state to ground state[22]. The ground level contains different vibration energy level, so the fluorescence wavelength emitted when the excited electron returns to the ground state is different. Then the fluorescence spectra of tangerine peel have a certain width, which is the reason that there is a wide spectrum peak at 350-360 nm in Fig. 1a and b.

Figure 1b shows the fluorescence spectroscopy of tangerine peel under the excitation wavelength from 370-410 nm at every 10 nm interval. The maximum fluorescence intensity was excited at 370 nm and corresponding fluorescence peak was about 452 nm. When excited at 380, 390 and 400 nm, the corresponding fluorescence peak wavelengths were 452, 457, 467 and 461 nm. The fluorescence peak wavelength has an obvious red shift (452-461 nm) with the increasing excitation wavelength, moreover, the fluorescence intensity decreases with the increasing excitation wavelength. The red shift of the fluorescence peak of tangerine peel is mainly due to the n electron in the ground state absorbing the different energies excitation photon. The level interval of electronic transition is greater when absorbing the larger photon

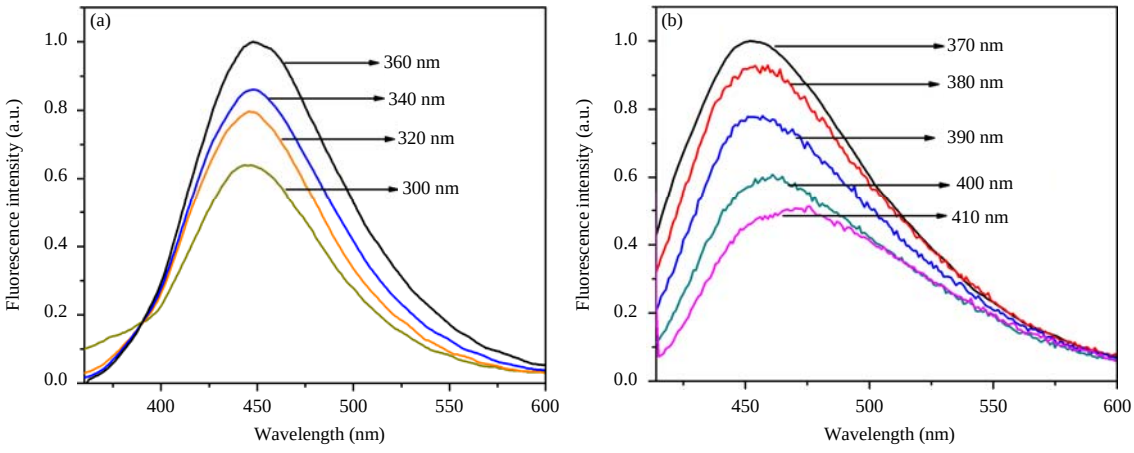

Fig. 1(a-b): Fluorescence spectroscopy of tangerine peel extraction under different wavelength, (a) 300-360 nm and (b) 370-410 nm

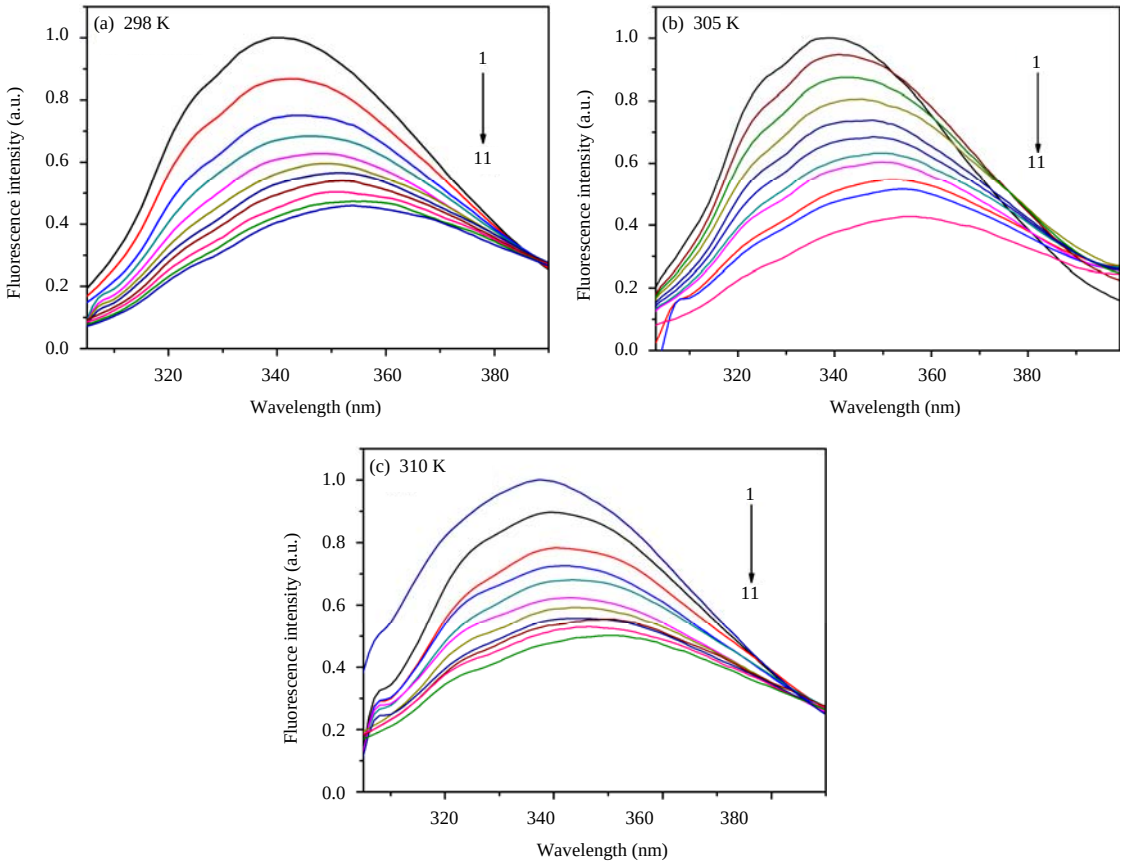

Fig. 2(a-c): Fluorescence spectra of the interaction between hesperidin and BSA. The concentration of hesperidin is 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0×10^{-6} mol L^{-1} from (1) to (11); C_{BSA} = 1.0×10-6 mol L^{-1}; λ_{ex} =285 nm; pH = 7.40; T = 298, 305 and 310 K, respectively.

energy[23]. That is, the red shift phenomenon occurs due to its excitation wavelength become longer.

Fluorescence spectroscopy of BSA-hesperidin interaction system: The binding parameters of the hesperidin-BSA system including binding constant, binding site and binding distance could be obtained from the fluorescence spectroscopy. Figure 2 represents the fluorescence spectroscopy of hesperidin-BSA interaction system under the excitation wavelength of 285 nm. From Fig. 2, BSA has a strong fluorescence emission peak at 340, 339 and 337 nm with the 298, 305 and 310 K, respectively. Obviously, the maximum fluorescence intensity of BSA decreased with the increasing concentration of hesperidin. The main reason of reduction of the maximum emission intensity was due to a complex formed between hesperidin and BSA under the present conditions. On the other time, the maximum emission wavelength of BSA has a red shift with the addition of hesperidin at 10 nm from 337 nm to 347 nm at 298 K, 17 nm from 339 nm to 356 nm and 13 nm from 340 nm to 353 nm, respectively. These results indicate that hesperidin changed the conformation of BSA[24].

The binding mechanism of hesperidin to BSA: The interaction mechanism could be classified as the static quenching and dynamic quenching mechanism, which mainly depend on the relationship between the quenching rate constants and different temperature. For static quenching, the quenching rate constants increase with the increasing temperature and the inverse effect is considered as the dynamic quenching.

Figure 3 represents the Stern-Volmer curves for BSA-hesperidin interaction system. Based on the Eq. 1 and 2, the K_{SV} and the correlation coefficient R could be calculated as 2.34, 2.09 and 1.82×10^{6} mol^{-1} L, 0.9925, 0.9947 and 0.9913 at 298, 305 and 310 K, respectively. The k_q at the different temperature could be obtained as 2.34, 2.09 and 1.82×10^{14} L mol^{-1} sec^{-1} at 298, 305 and 310 K, respectively. It could be noticed that the dynamic quenching constant K_{SV} decreased with increasing temperature, implying the

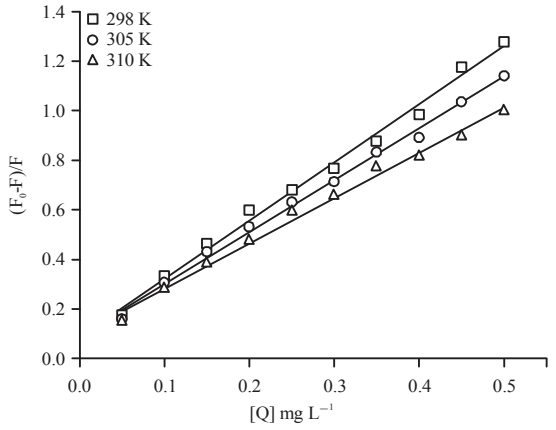

Fig. 3: The Stern-Volmer plots for the quenching of BSA by hesperidin at different temperatures

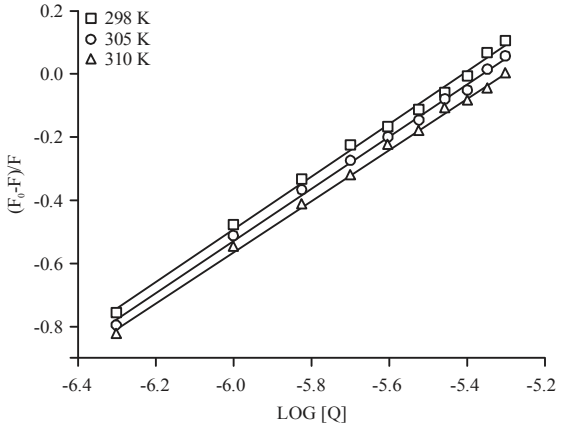

Fig. 4: The curves of log[(F_0-F)/F] versus log[Q]

Table 1: The binding parameters for hesperidin-BSA interaction system at different temperatures

T (K)	K_A (mol^{-1} L)	n	R[b]
298	3.26×10^4	0.8345	0.9957
305	2.71×10^4	0.8269	0.9973
310	1.98×10^4	0.8104	0.9978

[b]R is the correlation coefficient for the K_A values

fluorescence quenching mechanism of BSA caused by hesperidin may be a static quenching process rather than dynamic type. Moreover, the quenching rate constants k_q in the BSA-hesperidin system were larger than the limiting diffusion rate constant of the biomolecule (2.0×10^{10} L mol^{-1} sec^{-1})[25], indicating that the binding ability of BSA to hesperidin is better.

The binding parameters in BSA-hesperidin system: It is assumed that there are identical and independent sites in biological molecules for static quenching mechanism[26].

Figure 4 shows the plots of log[(F_0-F)/F] versus log[Q], the corresponding binding parameters could be calculated as given in Table 1. It was found that the K_A was in the order of 10^{-4} mol^{-1} L and decreased with the increasing temperature, which revealed that the binding capacity of hesperidin to BSA was weakened with the increasing temperature, that is, the stability of the hesperidin-BSA complex may be affected. The binding site was about 1 implying one binding site in hesperidin-BSA interaction system.

In this section, the binding mechanism is explored by fluorescence spectroscopy and some binding parameters were obtained. Thus, the binding mechanism may need many methods to further be confirmed, such as time-resolved fluorescence, absorption and infrared spectroscopy etc.

CONCLUSIONS

The fluorescence spectroscopy of tangerine peel and the binding of hesperidin to BSA were studied by fluorescence technology under simulative physiological conditions. Results show that the maximum fluorescence peak of tangerine peel was about 448 nm with the excitation wavelength of 360 nm. The fluorescence peak wavelength has an obvious red shift (452-461 nm) with the increasing excitation wavelength. The binding site was about 1 and the binding constants were obtained to be 3.26, 2.71 and 1.98×10^4 mol^{-1} L at 298, 305 and 310 K, respectively. The fluorescence quenching of BSA caused by hesperidin is a static quenching.

SIGNIFICANCE STATEMENTS

This study investigated the spectral properties of tangerine peel and the binding interaction between hesperidin of its effective components and BSA, which may be beneficial for researchers in understanding the physiological activity of tangerine peel and binding mechanisms at a molecular level. Thus the best theory on it may be arrived at.

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