Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 103 (2013) 114–119

Contents lists available at SciVerse ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

A comparison study on the binding of hesperetin and luteolin to bovine serum albumin by spectroscopy

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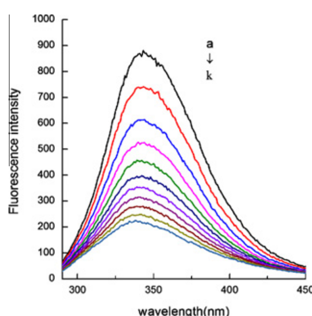
HIGHLIGHTS

- ▶ Binding of hesperetin to BSA is typical static quenching.
- ▶ Luteolin binds to BSA as a combined quenching and static quenching is prevailing.
- ▶ A SV plot linear interval of luteolin binding to BSA ranges from 0.5 to 1.25.
- ▶ 4′-Hydroxide group is more helpful for flavone binding to BSA than 4′-methoxyl group.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 May 2011
Received in revised form 22 October 2012
Accepted 27 October 2012
Available online 7 November 2012

Keywords:
Hesperetin
Luteolin
Bovine serum albumin
Fluorescence spectroscopy

ABSTRACT

Binding mechanism of luteolin (LUT) and hesperetin (HES) to bovine serum albumin (BSA) was investigated at 288,298,310 K and pH = 7.40 by UV absorption spectroscopy, fluorescence quenching and synchronous fluorescence spectroscopy. Under simulated physiological conditions, the fluorescence data indicated that hesperetin binding to BSA mainly occurs through a static mechanism. In contrast, binding of luteolin to BSA is a combined quenching process while static quenching is prevailing. Linear interval of the Stern–Volmer plot of LUT–BSA for the concentration ratio of LUT to BSA ranged from 0.5 to 1.25 was obtained. The thermodynamic parameters obtained from the Van't Hoff equation indicated that electrostatic force was the predominant force in the LUT–BSA and HES–BSA complex. The inner filter effect was eliminated to get accurate data. The conformational changes of BSA caused by LUT and HES were observed in the UV absorption. Results of fluorescence quenching and synchronous fluorescence showed that degree of luteolin–BSA quenching was higher than hesperetin–BSA quenching, which indicated that the 4′-hydroxide radical was more helpful to the ligand binding to proteins than 4′-methoxyl group for flavones.

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Introduction

Flavonoids are widespread in plants as secondary metabolites. Flavonoids are the active ingredient in many traditional Chinese herbal medicines and functional foods [1,2]. As important bioactive compounds in Chinese traditional medicine such as *Pericarpium citri reticulatae and Lonicera japonica*, hesperetin (5, 7, 3′-trihydroxyl-4′methoxyl-flavanone, LUT) and luteolin (5, 7, 3′, 4′-tetrahydroxyflavone, HES) (Fig. 1) have been received much attention because of their specific healthy benefits. Both luteolin and hesperetin belong to flavonoids. Luteolin abundantly exists in the honeysuckle and peanut shells, which has been reported to exert a widely anti-oxidant, inhibit development of solid tumor, as well as radical scavenge properties [3–5]. Hesperetin are widely present in many plants, especially abundant in citrus fruits, which has been reported to show many pharmacological properties including anti-oxidant, effects on blood-brain barrier, inhibition of cancer

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http://dx.doi.org/10.1016/j.saa.2012.10.068

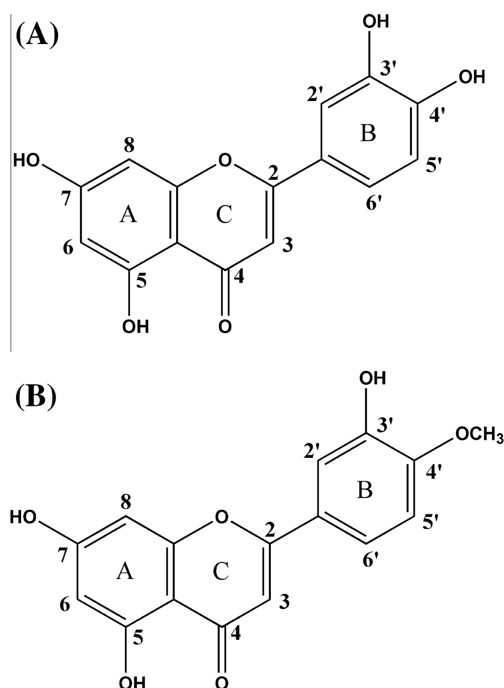

Fig. 1. Structures of (A) luteolin and (B) hesperetin.

development [6–8]. Their bioactive effects show both similarities and differences. The only difference in structure between luteolin and hesperetin is the 4′-substitutional group in ring B. Maybe the slight difference in structure leads to a great of differences in function. It has been reported that the number and location of the hydroxyl in the B ring of flavonoids have a significant impact on the functional properties of the flavonoids [9]. Differences in biological activity are generally explored through clinical trials or animal experiments. The active ingredient and serum albumin binding in vitro experiments has been widely used in recent years [10].

Serum albumin is the most abundant protein in blood plasma for animals including human beings. It acts as the most important carrier in transportation and distribution for endogenous and exogenous substances [11]. Active ingredients should combine with serum albumin before reaching the target sites to play the pharmacodynamic role. Drug-protein interaction experiment has great significance in discovering pharmacokinetic and pharmacodynamics implications [12], which is an essential step for a new drug design. Apart from that, the interaction experiment is helpful for revealing the transportation and distribution of the flavonoid drugs in vivo, explaining the toxicity at the molecular level [13]. Human serum albumin (HSA) has been usually used as a model protein to research on the interaction between bioactive component or drugs and protein. On the other hand, BSA is similar to HSA in tertiary structure with high sequence homology of 76.52% [14]. BSA is usually employed as the model protein because of its low cost and availability.

The binding experiments of HES or LUT to serum albumin were reported in recent years. Xie et al. studied hesperetin binding to HSA with multi-spectral [15]. Yang et al. [16] and Jurasekova et al. [17] studied the interaction of LUT–BSA and LUT–HSA, respectively. However, the comparative study of LUT and HES binding to serum albumin has not been reported.

In this work, we selected BSA as the model protein and luteolin as well as hesperetin as the model functional ingredient to investigate the ingredient-serum albumin binding interaction under physiological conditions. The UV absorption and synchronous fluorescence were employed to investigate the secondary structure changes of BSA caused by LUT and HES. Fluorescence quenching method was used to quantitatively compare the effect of luteolin and hesperetin on the intrinsic fluorescence of bovine serum albumin. The overall objective of the experiment is to compare the different effects of 4′-hydroxy-substituted with 4′-methoxy-substituted for binding of flavonoids to serum albumin.

Materials and methods

Materials

Bovine serum albumin (fatty acid free, >99%) was purchased from Roche. Hesperetin and luteolin (>99%) were purchased from Sigma Chemical Co. Tris (>99%) was purchased from Amresco. Other chemicals were of analytical reagent grade. All reagents were used as supplied without further purification. Ultra purity water was used throughout. NaCl (0.1 mol/L) solution was used to maintain the ionic strength. Tris buffer was 0.05 mol/L (containing 0.1 mol/L NaCl), the pH of which was adjusted to 7.4 by 0.1 mol/L HCl monitored by a pHS-3D digital pH-meter (Leici, Shanghai). BSA was dissolved in Tris buffer to prepare into 1.0×10^{-5} mol/L stock solution, which would be diluted to the desired concentration with Tris buffer. Hesperetin and luteolin were dissolved in ethanol to prepare into 1.0×10^{-3} mol/L stock solution. All the stock solutions were stored at 4 °C.

Equipment

A TU-1810PC UV–vis spectrometer (Puxi, Beijing, China) equipped with a 1.0 cm × 1.0 cm × 4.0 cm quartz cell was used to record the UV–vis spectra. Fluorescence spectra was recorded on a Varian Cary Eclipse fluorescence spectrometer equipped with a 1.0 cm × 1.0 cm × 4.0 cm quartz cell. An electric thermostat water-bath (Eyela, Japan) was used to control the temperature throughout the work. A micropipettor (Thermo) was used in UV and fluorescence titration, which can provide accurate volume from 1 μL to 25 μL.

Procedures

Ultra-violet spectroscopy

A 3.00 ml solution in quartz cuvette, containing appropriate 5.0×10^{-7} mol/L BSA solution, was titrated by succession additions of 1.0×10^{-3} mol/L hesperetin or luteolin. Titrations were operated manually by using a micropipettor. UV–vis spectra were recorded in the wavelength range of 190–500 nm after mixing the solution tenderly.

Fluorescence quenching and synchronous fluorescence

A 3.00 ml solution in quartz cuvette, containing appropriate 2.0×10^{-6} mol/L BSA solution, was titrated by succession additions of 1.0×10^{-3} mol/L HES/LUT. Titrations and mix were operated as previously mentioned. In fluorescence quenching experiment, fluorescence emission spectra were recorded in the wavelength range of 290–450 nm by exciting BSA at 295 nm. In synchronous fluorescence experiment, when Δ = 15 nm, excitation wavelength was set from 250 to 330 nm. When Δ = 60 nm, excitation wavelength was set from 200 to 330 nm. Slit width were all 5/5 nm. Experiments were carried out at three temperature 288 K, 298 K, 310 K and every experiment was repeated three times to average.

Principle of fluorescence quenching

The fluorescence quenching intensity of the fluophore can be decreased by many inter-molecular interactions including

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