Journal of Luminescence 130 (2010) 637–644

Contents lists available at ScienceDirect

Journal of Luminescence

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

Studies on interaction between flavonoids and bovine serum albumin by spectral methods

Xiaolei Shi, Xuwen Li, Mingyu Gui, Hongyu Zhou, Ruijie Yang, Hanqi Zhang, Yongri Jin*

College of Chemistry, Jilin University, ChangChun 130012, China

ARTICLE INFO

Article history:
Received 14 May 2009
Received in revised form
3 November 2009
Accepted 5 November 2009
Available online 12 November 2009

Keywords:
Flavonoids
Bovine serum albumin
Three-dimensional fluorescence spectra
Synchronous fluorescence spectra
Circular dichroism spectrum

ABSTRACT

The interaction between three kinds of flavonoids and bovine serum albumin (BSA) was investigated by fluorescence and UV–vis absorption spectrometry. The results indicated that flavonoids have strong ability to quench the intrinsic fluorescence of BSA by forming complexes. The binding constants, number of binding sites, thermodynamic parameters and energy transfer mechanisms were also investigated. Conformation change of BSA was observed from synchronous, three-dimensional fluorescence and circular dichroism spectrum.

1. Introduction

Flavanoids can be found in many plants and one of the most important characters of these compounds is the activity of pharmacodynamics and pharmacokinetics [1]. In recent years, there have been a number of studies on the bioactivities of flavonoids. It was reported that flavonoids are related to protective effects against cardiovascular disease and certain forms of cancer [2–4]. They were also used widely as anti-inflamatory drugs [5] and the antioxidatant activity in vitro has been studied for many years [6–8].

Therefore, the interaction between flavonoids and serum albumin has absorbed much of attention. It is known that the interaction between drugs and serum albumin plays an important role in the distribution and metabolism of drugs [9,10]. The studies on the interaction can provide information on the therapeutic effectiveness of drugs and other information, such as the information of storage, and transportation of drugs. Generally we used BSA as a model protein, because BSA is a small protein with a single polypeptide chain containing 585 amino acid residues, which is cross-linked by 17 difusulfide bonds. BSA is made up of three linearly arranged structurally distinct, homologus domains (I–III), which were divided into nine loops (L_1–L_9) and each domain contains two sub-domains (A and B). The specific hydrophobic cavities in the sites existing on BSA are sites I and II, which are located in IIA and IIIA sub domains [11]. BSA has two tryptophan residues that possess intrinsic fluorescence [12].

Three kinds of flavonoids obtained from the leaves of *Actinidia kolomikta* (*Rupr. et Maxim.*) *Planch*, including kaempferol-3-O-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→6)-β-D-galacto-pyranoside (drug 1), kaempfol-7-O-rhamnosyl-3-O-rutinoside (drug 2) and kaempferide-7-O-(4″-O-acetylrhamnosyl)-3-O-rutinoside (drug 3), are studied and their structures are shown in Fig. 1. The fluorescence spectrometry can be applied to the study of interaction between drug and BSA. In order to obtain further information on the interaction, three dimensional fluorescence, synchronous fluorescence and circular dichroism spectra were obtained.

2. Materials and methods

2.1. Reagents

Bovine serum albumin (BSA) was purchased from sigma (America). Tris was purchased from Amresco (America). Sodium chloride (NaCl Beijing Chemical company) was used to keep the ionic strength in 0.05 mol L^{-1}. Tris–HCl buffer solution of pH 7.4 was used to prepare all solutions.

All other reagents were of analytical reagent grade. Water used in the experiment was double distilled. Flavonoids were extracted and isolated from the leaves of *Actinidia kolomikta* (*Rupr. et*

* Corresponding author. Tel./fax: +86 431 85167996.
E-mail address: jinyr@jlu.edu.cn (Y. Jin).

0022-2313/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jlumin.2009.11.008

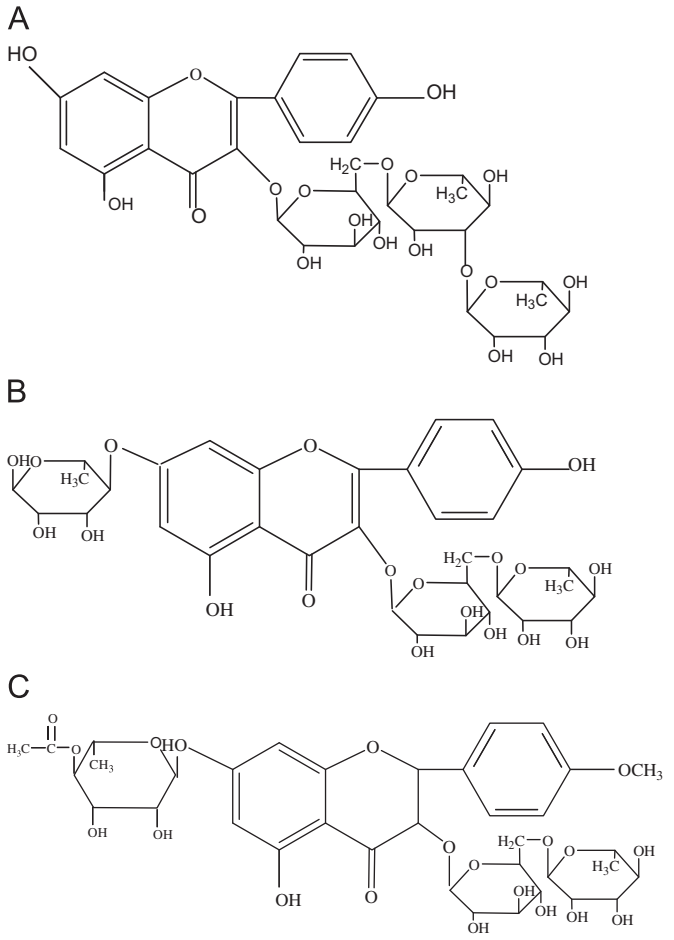

Fig. 1. Structures of (A) kaempferol-3-O-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (drug 1), (B) kaempfol-7-O-rhamnosyl-3-O-rutinoside (drug 2) and (C) kaempferide-7-O-(4″-O-acetylrhamnosyl)-3-O-rutinoside (drug 3).

Maxim.) *Planch* in our laboratory. The leaves of *Actinidia kolomikta* (*Rupr. et Maxim.*) were refluxed with 90% ethanol three times. Silica gel was used as packed material for column chromatography (CC) to obtain the simifinished drug 1 and drug 2 from the extract. Then preparative HPLC was used to purify the simifinished products. Drug 1 and drug 2 were obtained and their purity was higher than 99%. To obtain drug 3, the extract was dissolved in water and extracted by ethyl acetate. Then the layer of water was extracted with *n*-butanol. The obtained *n*-butanol phase was introduced into the silica gel column and the elute was purified by CC using C_{18}-bonded silica gel as packing material. Drug 3 was obtained and its purity was higher than 99%.

2.2. Measurements of spectra

All solutions are prepared in 0.05 mol L^{-1} Tris–HCl buffer of pH 7.40 containing 0.1 mol L^{-1} NaCl. The final concentration of BSA was 1.0×10^{-6} mol L^{-1}. All solutions were kept in dark at 0–4 °C.

A Varian spectrofluorimeter (Varian, Australia) was used for all the fluorescence measurements. The fluorescence spectra were recorded under the following conditions: both excitation and emission slits were 5.0 nm, excitation wavelength was 280 nm and emission wavelengths were 290–500 nm. 1 cm of quartz cell was used. The synchronous scan spectra were obtained when the differences between excitation and emission were equal to 15 and 60 nm, respectively. The three-dimensional fluorescence spectra were obtained under the following conditions: the excitation wavelength was 200 nm, the emission wavelengths were between 350 and 800 nm, scanning number was 30 and increment was 5 nm. The other parameters were the same as applied in fluorescence spectra.

Circular dichroism (CD) measurements were made on a JASCD J-810 spectropolarimeter at room temperature and recorded in the range of 200–270 nm. The solutions contains 7.29×10^{-5} mol L^{-1} BSA and flavonoids; 1.07×10^{-4}, 1.44×10^{-4} and 1.27×10^{-4} mol L^{-1} solutions were used to obtain CD spectra.

The UV–vis absorption spectra were recorded on a UV 1700 spectrophotometer (Shimadzu, Japan). Tris–HCl buffer (pH 7.4) was used as the blank.

3. Results and discussion

3.1. Calculating method

Absorption of flavonoids at the emission and excitation wavelength of fluorophore has an important effect on the fluorescence spectra, so the fluorescence intensity must be corrected. When the absorbance of drugs was lower than 0.3, the following equation can be used to correct the inner filter effects [13]:

$$F_{corr} = F_{obs} \times e^{(A_1+A_2)/2} \quad (1)$$

where F_{corr} is the corrected fluorescnce intensity, F_{obs} is the observed intensity, and A_1 and A_2 are the absorbance of drugs at emission and excitation wavelength, respectively.

3.2. Fluorescence quenching

The fluorescence intensity of a fluorophore can be decreased by molecular interaction, excited-state reaction, molecular rearrangements, energy transfer, ground state complex formation and collisional quenching [14]. In this paper, the interactions between flavonoids and BSA were investigated by fluorescence quenching. BSA was used as the fluorophore and the flavonoids were used as the quencher.

The interaction between flavonoids and BSA was investigated by evaluating florescence intensity of the BSA before and after addition of flavonoids. It can be seen clearly in Fig. 2 that the intrinsic fluorescence of BSA was quenched obviously in the presence of flavonoids, which indicates that there may be the binding interactions between flavonoids and BSA.

Commonly, fluorescence quenching can be described by the following Stern–Volmer equation:

$$\frac{F_0}{F} = 1 + K_{SV}c_q = 1 + k_q\tau_0 c_q \quad (2)$$

where F_0 and F represent the corrected fluorescence intensities in the absence and presence of flavonoids, K_{SV} is the Stern–Volmer quenching constant with the unit L mol^{-1}, c_q is the free concentration of the flavonoids, k_q is the quenching rate constant, τ_0 is the average lifetime of the fluorophore without any quencher and is usually equal to 10^{-8} s [15]. For static quenching, with the increase in temperature, the reduction in the stability of the complex results in decrease of binding constant. In contrast, in dynamic quenching, high temperatures can cause collision to increase, so the quenching constant is expected to increase [16]. The Stern–Volmer plots for drug 1 are shown in Fig. 3 and are similar for other drugs. The values of K_{SV} obtained from Stern–Volmer plots demonstrated that the temperatures have a