Journal of Luminescence 132 (2012) 132–140

Contents lists available at SciVerse ScienceDirect

Journal of Luminescence

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

Investigation of three flavonoids binding to bovine serum albumin using molecular fluorescence technique

Shuyun Bi*, Lili Yan, Bo Pang, Yu Wang

College of Chemistry, Changchun Normal University, Changchun 130032, PR China

ARTICLE INFO

Article history:
Received 28 March 2011
Received in revised form
13 July 2011
Accepted 8 August 2011
Available online 12 August 2011

Keywords:
Flavonoids
Bovine serum albumin
Competitive binding
Fluorescence
Synchronous fluorescence

ABSTRACT

The three flavonoids including naringenin, hesperetin and apigenin binding to bovine serum albumin (BSA) at pH 7.4 was studied by fluorescence quenching, synchronous fluorescence and UV–vis absorption spectroscopic techniques. The results obtained revealed that naringenin, hesperetin and apigenin strongly quenched the intrinsic fluorescence of BSA. The Stern–Volmer curves suggested that these quenching processes were all static quenching processes. At 291 K, the value and the order of the binding constant were $K_{A\ (naringenin)}=4.08\times10^4<K_{A\ (hesperetin)}=5.40\times10^4\approx K_{A\ (apigenin)}=5.32\times10^4\ L\ mol^{-1}$. The main binding force between the flavonoid and BSA was hydrophobic and electrostatic force. According to the Förster theory of non-radiation energy transfer, the binding distances (r_0) were obtained as 3.36, 3.47 and 3.30 nm for naringenin–BSA, hesperetin–BSA and apigenin–BSA, respectively. The effect of some common ions such as Fe^{3+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+} and Ca^{2+} on the binding was also studied in detail. The competition binding was also performed. The apparent binding constant (K'_A) obtained suggested that one flavonoid had an obvious effect on the binding of another flavonoid to protein when they coexisted in BSA solution.

1. Introduction

Flavonoid is any member of a class of widely distributed biological natural products containing aromatic heterocyclic skeleton of flavan (2-Phenylbenzopyran) but no nitrogen in plants. Flavonoids exhibit a wide range of biological properties including antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities. According to the chemical structure, flavonoids are usually classified into 6 main subgroups: flavonols, flavones, flavanones, isoflavones, flavononones, anthocyanidin [1]. Naringenin (4′,5,7-trihydroxyflavanone) and hesperetin (3′,5,7-trihydroxy-4′-methoxyflavanone) belong to flavononones (derivation by reduction of the 2(3) C=C bond), and apigenin (4′,5,7-trihydroxyflavone) belongs to flavones (skeleton: 2-phenylchromen-4-one). Their structures are shown in Fig. 1. Naringenin and hesperetin are two of the citrus bioflavonoids, which naturally occur in citrus fruits, such as lemons, oranges and grapefruit [2]. Apigenin abundantly exists in ordinary fruits and vegetables [3], and it can reduce the levels of plasma cholesterol in rats that feed on a cholesterol-enriched diet [4]. Flavonoids have aroused considerable interest because of their potential beneficial effects on human health [5–8].

Serum albumin, the most abundant protein in the mammalian blood circulation, is prominent for its ability to bind reversibly many endogenous and exogenous compounds [9,10]. Bovine serum albumin (BSA) is usually employed as a model protein because of its low cost, availability and structural similarity with human serum albumin (HSA) [11,12]. It is known that drug–protein interaction greatly influences the absorption, distribution, metabolism and excretion of drugs in organism [13]. Naringenin, hesperetin and apigenin are easily obtained from fruits, vegetables and traditional Chinese medicine, so the understanding of the binding features with albumin is meaningful. BSA has intrinsic fluorescence that arises from the two tryptophans, Trp−134 and Trp−212, whose intensity varies upon ligand binding. So, the fluorescence technique was used as a central powerful tool in this study.

Three ligands were studied under the same conditions in this work, which can help us make a comparison between one ligand and another ligand. Further, because of the similarity of their structures, it can be inferred that the binding site of the three flavonoids might be the same site on the protein. The extent of binding of one ligand to protein may be reduced when another ligand coexists in the protein solution. The competition occurs between them. More importantly, the studies of competitive binding can help us better understand the nature of protein binding. There was a viewpoint on the binding of two ligands (L_1 and L_2) to the protein [14,15], i.e. there were two types interaction:

$$L_1 \xrightarrow{+BSA} L_1-BSA \xrightarrow{+L_2} L_2-BSA+L_1 \quad (1)$$

$$L_1 \xrightarrow{+BSA} L_1-BSA \xrightarrow{+L_2} L_2+BSA-L_1 \quad (2)$$

Eqs. (1) and (2) denoted the competitive binding and non-competitive binding, respectively. Eq. (1) suggested that when L_2 was added to the L_1-BSA solution, it completely displaced L_1 and

* Corresponding author. Tel.: +86 431 86168098; fax: +86 431 86168096.
E-mail address: sy_bi@sina.com (S. Bi).

0022-2313/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jlumin.2011.08.014

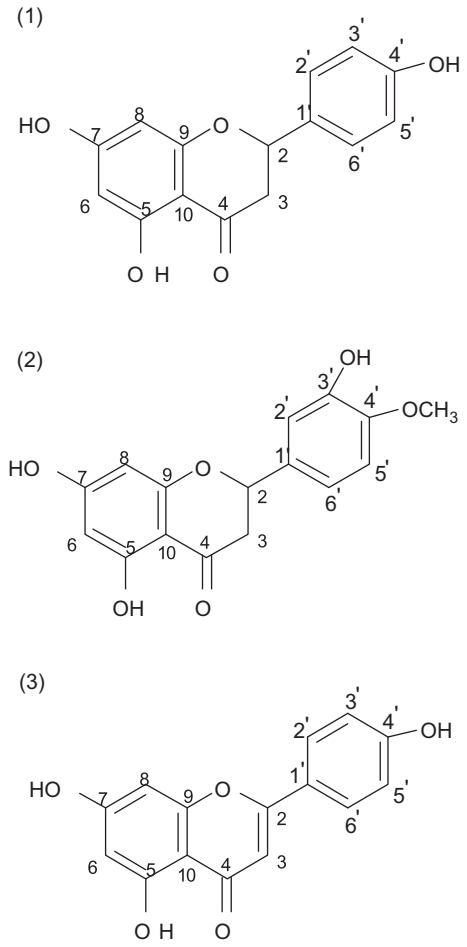

Fig. 1. Structures of (1) naringenin, (2) hesperetin and (3) apigenin.

formed the complex of L_2–BSA. We think it is a one-sided view. If there was a great difference in binding constant between the two ligands, this view might be correct. But if this was not the case, this view might be incorrect; probably another type occurs as follows:

$$L_1 \xrightarrow{+BSA} L_1-BSA \xrightarrow{+L_2} L_1-BSA+L_2-BSA+L_1 \quad (3)$$

Eq. (3) was put forward in this work. As shown in Eq. (3), the complete displacement (denoted by Eq. (1)) could not occur when the difference between the binding constant of L_1 to BSA and that of L_2 to BSA was close. Based on this observation, the assay of competitive binding was conducted in this study by calculating the apparent binding constant K_A' of one ligand with BSA in the presence of another ligand. The proposed method may be useful for further studies on the competitive binding.

2. Experimental

2.1. Reagents

BSA (purity > 95%) was purchased from Changchun Dingguo Biotechnology Company, Sigma packaging. Naringenin, hesperetin and apigenin were purchased from Chinese Drug Biological Products Qualifying Institute. Tris–HCl buffer (0.05 mol L^{-1}, pH 7.40) containing 0.1 mol L^{-1} NaCl was used to keep pH value and the ionic strength. $FeCl_3$, $CuCl_2$, $MgCl_2$, $MnCl_2$, $ZnCl_2$ and $CaCl_2$ were prepared to 1 g L^{-1} stock solution and diluted before being used. All other chemicals used were of analytical reagent grade and deionized water was used throughout.

2.2. Apparatus

Fluorescence measurements were made on a RF-5301PC fluorophotometer equipped with a xenon lamp source, a 1.0 cm × 1.0 cm × 4.0 cm quartz cell and a thermostatic controller (Shimadzu, Japan). A TU-1901 UV-Spectrometer (Beijing Purkinje General Instrument Co., Ltd.) was used to measure the UV spectrum.

2.3. Procedures

2.3.1. Flavonoid–BSA interactions

A series of assay solutions were prepared by adding 125 μL 1×10^{-4} mol L^{-1} BSA and appropriate amount of flavonoids into each mark tube and the total volume was made to 2.5 mL with 0.05 mol L^{-1} Tris–HCl buffer of pH 7.4 containing 0.1 mol L^{-1} sodium chloride. So, there were a series of tubes containing different amounts of flavonoids. For each tube, the total concentration of BSA was constant (5.0×10^{-6} mol L^{-1}) and the concentration of flavonoids was different (from 0 to 6×10^{-6} mol L^{-1}). The excitation wavelength was 280 nm and both the excitation and emission slit widths were 3 nm. The fluorescence spectra of BSA were recorded in the range of 290–500 nm at three temperatures, 291, 301 and 311 K.

2.3.2. Synchronous fluorescence measurements

Synchronous fluorescence spectra of BSA with various concentrations of the flavonoid were obtained from 300 to 400 nm at $\Delta\lambda = 60$ nm and $\Delta\lambda = 15$ nm.

2.3.3. UV–vis absorption spectra

The measurements of UV spectra of the flavonoids were performed under the conditions: the scan rate is middle (8 nm/s), scan range is 290–500 nm.

2.3.4. Effect of some common ions

The fluorescence spectra of the flvonoids binding to BSA were recorded in the presence of metal ions (Fe^{3+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+} and Ca^{2+}) from 290 to 500 nm upon excitation at 280 nm.

2.3.5. Binding of one flavonoid to protein in the presence of another flavonoid

The effect of one flavonoid on the binding constant of another one with BSA was studied. When the concentration of one flavonoid (the interference) in a series of test tubes containing BSA was kept constant, the concentration of another flavonoid (the target) was successively changed; the apparent binding constant K_A' of the target–BSA was obtained. Such a determination process was performed in various concentrations of the interference.

3. Results and discussion

3.1. Binding characteristics of the flavonoids to BSA

Fluorescence technique can easily be used as a powerful tool in the study of the interactions, provided that one of the interacting species exhibits an intrinsic fluorescence whose intensity varies upon ligand binding. When a 5×10^{-6} mol L^{-1} BSA solution was excited at 280 nm, the fluorescence peak occurred at 340 nm (shown in Fig. 2). This peak originated from two Trp residues: Trp − 134 and Trp − 212. In fact, in addition to Trp, there are two kinds of intrinsic fluorescence probes in serum albumin: tyrosine