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Spectroscopic studies on the interaction of bovine serum albumin with ginkgolic acid: Binding characteristics and structural analysis

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ABSTRACT

The interaction between ginkgolic acid (GA, C15:0) and bovine serum albumin (BSA) is investigated by several spectroscopic methodologies. At first, the binding characteristics of GA and BSA are determined by fluorescence emission spectra. It is showed that GA quenches the fluorescence of BSA and the static quenching constant K_{LB} is 11.7891×10^4 L mol^{-1} s^{-1} at 297 K. GA and BSA form a 1:1 complex with a binding constant of 9.12×10^5 L mol^{-1}. GA binds to the Sudlow's drug binding site II in BSA, and the binding distance between them is calculated as 1.63 nm based on the Förster theory. The thermodynamic parameters indicate that the interaction between BSA and GA is driven mainly by hydrophobic forces. On the other hand, structural analysis indicates that GA binding results in an increased hydrophobicity around the tryptophan residues of BSA as revealed by the synchronous fluorescence spectra, and a decrease in α-helix as revealed by the far-UV CD spectra. In addition, ANS, UV–vis and RLS experiments confirmed that GA binding causes a certain structural changes in BSA. These findings provide the binding information between BSA and GA, and may be helpful for pharmacokinetics and the design of dosage forms of GA.

1. Introduction

Ginkgolic acids (GAs) extensively exist in leaves, nuts and external seed coat of *Ginkgo biloba* L. [1]. Both negative and positive properties have been attributed to ginkgolic acids. GAs have ever been recognized as hazardous compounds with suspected cytotoxic, allergenic, mutagenic and carcinogenic properties, but recent studies have identified novel functions of GAs, including physiological and pharmacological activities directed against tumors, fungi and bacterium [2–4]. Especially, GAs can significantly inhibit the growth of many tumorigenic cell lines, such as HeLa, S180, U937 and so on [2]. These researches make it possible to treat kinds of diseases using ginkgolic acids.

Recently, many optical techniques have been carried out to investigate the interaction of proteins with drugs [5–7]. Drug–protein interaction has significance in the biological activity and toxicity of a drug [8,9]. An important aspect of a drug's biodisposition profile is the extent to which it binds to plasma proteins [8,10]. Serum albumins, the most abundant proteins in blood, have many important physiological functions, such as regulating the osmotic blood pressure and blood pH [6]. Another important physiological role of serum albumins involves the binding, transport, and delivery of numerous drugs to their target organs [6,11]. The studies on the binding of drugs to albumins may also provide the information on the structure and the therapeutic effectivity of a drug [6,7,10,12]. Moreover, the interaction may change the volume of distribution, clearance and elimination of a drug [11]. Therefore, the investigation of GAs with respect to serum albumin binding is of great importance.

Bovine serum albumin (BSA, NCBI accession number: CAA76847) is used in the present study, for its medical importance, low cost, ready availability, and high similarity to its homolog human serum albumin (HSA) [6,13]. BSA is a single-chain globular protein, and it is composed of three domains (I–III) and each of them is the product of two subdomains (A and B) [5,6,14]. Two tryptophan residues Trp-134 and Trp-214 embed in the subdomain IB and subdomain IIA, respectively, and the tyrosine residues located in subdomain IA (Tyr-30, Tyr-84), IB (Tyr-140, Tyr-148, Tyr-150, Tyr-156 and Tyr-157) and IIA (Tyr-263) [8]. There are two famous binding sites of BSA for ligands, namely Sudlow's binding sites I (in subdomains IIA) and II (in subdomains IIIA) [8,15]. For example, dibazol and piracetam bind in site I [5,6], and flufenamic acid and ibuprofen bind in site II [16,17], but the binding sites for FU in BSA are proved to be non-specific [8]. Some

Abbreviations: BSA, bovine serum albumin; GA, ginkgolic acid; RLS, resonance light scattering; ANS, 8-anilino-1-naphthalenesulfonic acid; CD, circular dichroism; λ_{max}, the maximum wavelength; λ_{ex}, the excitation wavelength; λ_{em}, the emission wavelength; ΔH, enthalpy change; ΔG, free energy; ΔS, entropy change

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researchers also find that digitoxin binds to another site (so-called site III) in BSA, but it is less important than the two Sudlow's sites [8,16].

Normally, GAs are mixtures of a series of GA homologs containing 13–17 side chain carbon atoms at site 6 and 0–3 side chain double bonds, for example, C13:0, C15:0, C15:1, C17:1 and C17:2 [2,4]. Usually, one or two monomers of GAs are used as representative compounds of GAs for researches [1,18], and it is showed that the structures, activities and safety of GAs are close to each other [4,19]. GA (C15:0, Formula: $C_{22}H_{36}O_3$, molecular weight: 348.5) is one of the most important ginkgolic acids [20]. Recent researches showed that GA (C15:0) had unique functions, such as preventing fruit infestation at very low concentration, while other GAs such as C13:0, C15:1 and C17:1, had no such effect [21]. In the present work, the binding characteristics between GA (C15:0) and BSA are analyzed for the first time using spectroscopic methods, such as intrinsic fluorescence emission, synchronous fluorescence, ANS fluorescence, resonance light scattering (RLS) and far-UV circular dichroism (CD) spectroscopies. The studies can provide the binding information between GA and BSA and reflect the conformational changes of BSA induced by GA. It may be also helpful for pharmacokinetics and the design of dosage forms of GA.

2. Material and methods

2.1. Materials

BSA was purchased from Lizhudongfeng Biochemical Technology Co., Ltd. (Shanghai, China). BSA solution (3×10^{-6} mol L^{-1}) was prepared in PBS (pH 7.4) and was kept at 4 °C. GA (C15:0) was a product of Shanghai Tauto Biotech Co., Ltd. 8-anilino-1-naphthalenesulfonic acid (ANS) was obtained from Sigma-Aldrich. The stock solution of ANS was prepared by dissolving it in methanol. Bilirubin and Chlorpheniramine maleate were dissolved in DMSO–H_2O (1:9) solution. Na_2HPO_4, NaH_2PO_4, NaCl and other reagents were of analytical grade. Solutions were prepared with Milli-Q water. The assays in the presented work were carried out at pH 7.4.

2.2. The measurements of UV–visible absorption spectra

UV–visible absorption spectra for samples were recorded in a quartz cuvet with 1 cm path length on a UV-2550 UV/vis spectrophotometer (Shimadzu, Japan). The absorption spectra for BSA in the absence or in the presence of different concentrations of GA were recorded in the range 200–700 nm at the indicated temperatures [6]. The UV–visible absorption spectra were mainly used for the correction of the fluorescence intensity as described by Eq. (1). The UV absorption spectra of GA, BSA and the GA–BSA system with the molar ratio of 1:1 were measured and plotted, respectively.

2.3. The measurements of fluorescence emission spectra

Fluorescence measurements were performed on a Hitachi F-4500 fluorescence spectrophotometer (Hitachi, Kyoto, Japan) equipped with a xenon lamp source and a water bath as described before [22]. 2 mL sample of BSA (3×10^{-6} mol L^{-1}) in PBS (10 mmol L^{-1}, pH 7.4) was titrated with μL additions of GA stock solution (1×10^{-3} mol L^{-1}) at the indicated temperatures. The mixture was incubated in a quartz cuvet with 1 cm path length for 5 min before the measurements were carried out. Excitation slit and emission slit were set as 5 nm. The fluorescence emission spectra were recorded in the range 300–400 nm at the excitation wavelength (λ_{ex}) of 280 and 295 nm. Every spectrum was the mean of at least three scans. The fluorescence intensities were corrected to avoid the inner filter effect using the relationship [9,23]:

$$F_{cor} = F_{obs} \times e^{(Aex+Aem)/2} \quad (1)$$

where F_{cor} and F_{obs} are the corrected fluorescence intensity and the observed fluorescence intensity, respectively, and A_{ex} and A_{em} are the absorption of the system at the excitation wavelength (λ_{ex}) and the emission wavelength (λ_{em}), respectively.

2.4. The measurements of synchronous fluorescence spectra

The samples were the same as described in section 2.3 (the measurements of fluorescence emission spectra). With $\Delta\lambda$ (the constant wavelength interval between the emission wavelength and the excitation wavelength, $\Delta\lambda=\lambda_{em}-\lambda_{ex}$) of 15 and 60 nm, the synchronous fluorescence spectra were recorded from 270 to 320 nm and from 250 to 300 nm, respectively [24,25]. Every spectrum was the mean of three scans, and data were corrected for the signal of the buffer solution. The fluorescence intensities were corrected using Eq. (1).

2.5. The measurements of RLS spectra

The samples were the same as described in section 2.3 (the measurements of fluorescence emission spectra). RLS spectra were obtained in the range 200–600 nm by synchronous scanning with the same excitation and emission wavelengths [22,26]. Every spectrum was the mean of three scans, and data were corrected for the signal of the buffer solution. The fluorescence intensities were corrected using Eq. (1).

2.6. The measurements of ANS fluorescence spectra

The solution of BSA (3×10^{-6} mol L^{-1}) and ANS (3×10^{-6} mol L^{-1}) was prepared in PBS (pH 7.4). After GA stock solution (1×10^{-3} mol L^{-1}) was added, the fluorescence spectra for BSA–ANS system were recorded in the range 300–600 nm with the excitation wavelength of 380 nm [8,27]. Every spectrum was the mean of three scans, and data were corrected for the signal of the buffer solution. The fluorescence intensities were corrected using Eq. (1).

2.7. The measurements of the far-UV CD spectra

The far-UV CD spectra for BSA (2×10^{-6} mol L^{-1}, pH 7.4) in the absence or presence of GA were measured in a quartz cuvet with 2 mm path length on an AVIV Model 400 circular dichroism spectrophotometer [22,28]. The far-UV CD spectra were recorded in the range 190–260 nm at 20 °C. GA stock solution was titrated into the BSA solution, and the molar ratio of GA to BSA was varied as 0:1, 1:1 and 5:1. Every spectrum was the mean of three scans, and data were corrected for the signal of the buffer solution. The results were calculated as molar ellipticity [θ] (degrees cm^2 $dmol^{-1}$). The contents of the secondary structures were estimated by CDNN software [29].

2.8. Displacement experiments

The displacement experiments were performed using the site probes bilirubin (binding to site IIA) [30,31] and chlorpheniramine maleate (binding to site IIIA) [32,33]. The concentrations of BSA and GA were stabilized at 3×10^{-6} mol L^{-1}, and bilirubin or chlorpheniramine maleate (3×10^{-6} mol L^{-1}) was then gradually added to the BSA–GA mixtures [5,34]. The fluorescence spectra