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Multi-spectroscopic studies on the interaction of human serum albumin with astilbin: Binding characteristics and structural analysis

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ABSTRACT

Five spectroscopic techniques were used to investigate the interaction of astilbin (ASN) with human serum albumin (HSA). UV–vis absorption measurements prove that ASN–HSA complex can be formed. The analysis of fluorescence spectra reveal that in the presence of ASN, quenching mechanism of HSA is considered as static quenching. The quenching rate constant k_q, K_{SV} and the binding constant K were estimated. According to the van't Hoff equation, the thermodynamic parameters enthalpy change (ΔH) and entropy change (ΔS) were calculated to be -12.94 kJ mol^{-1} and 35.92 J mol^{-1} K^{-1}, respectively. These indicate that the hydrophobic interaction is the major forces between ASN and HSA, but the hydrogen bond interaction cannot be excluded. The changes in the secondary structure of HSA which was induced by ASN were determined by circular dichroism (CD), Fourier transform infrared spectroscopy (FT-IR) and Raman spectroscopy.

1. Introduction

Astilbin (ASN Scheme 1), 3, 3', 4', 5, 7-pentahydroxyflavanone 3-(6-deoxy-2- mannopyranoside), is a glycosyl flavonoid which can be isolated from the plant [1]. ASN has been proven to show some important bioactivities such as aldose reductase inhibitory effects [2], coenzyme A reductase-inhibiting, hepatoprotective, insecticidal, anti-oedematogenic, and antioxidative activities [1,3].

Serum albumin (SA) is the most abundant protein in plasma, which plays important role in transporting, distribution, storing and metabolism of many exogenous ligands, such as drugs, fatty acids, and amino acids [4,5]. Human serum albumin (HSA) molecule contains a single peptide chain of 585 amino acids, which can bind with kinds of drugs and various small bioactive molecules, such as metal cations, fatty acids [6,7] and so on. As a consequence, the studies on the interaction between HSA and drugs can offer available information of drug action which can help understanding the distribution and absorption of the drugs [7–9].

In the present work, the interaction of HSA with ASN was studied systematically under simulated physiological conditions. The binding mechanism, the thermodynamic parameters were characterized by fluorescence approach. The conformational and microenvironment changes of HSA in the presence of ASN were estimated by CD, FT-IR and Raman spectroscopic methods.

2. Materials and methods

2.1. Materials

ASN (99%, obtained from ICAMA) was initially dissolved in 10% of ethanol and then diluted with double distilled water (ethanol/water mixture i.e. 10/90%) to prepare a stock solution of 1×10^{-3} mol L^{-1}; HSA was purchased from Sigma (molecular weight 66500). HSA (1×10^{-3} mol·L^{-1}) was dissolved in a 0.05 mol L^{-1} phosphate buffer solution (PBS) of pH=7. 43, contained 0.1 mol L^{-1} NaCl and stored at 4 °C. The other chemicals such as sodium chloride and ethanol were all of analytical purity. Double distilled water was used in all experiments.

2.2. UV–vis absorption spectrum

The UV absorption spectra were recorded on a Cary 100 UV–vis Spectrophotometer (Varian) equipped a 1 cm cuvette. Each time, a certain amount of 1×10^{-3} mol L^{-1} ASN was added into 1×10^{-5} mol L^{-1} HSA. The concentration of ASN was varied from 0 to 9×10^{-5} mol L^{-1} with a step of 1×10^{-5} mol L^{-1}. The UV absorption difference spectra of HSA were measured in the range of 190–350 nm in the absence and presence of ASN.

2.3. Fluorescence spectroscopy measurements

The fluorescence spectra were measured on an RF-5301 fluorescence spectrophotometer (Japan Shimadzu Company). Each time, a certain amount of 1×10^{-3} mol L^{-1} ASN was added into 6×10^{-6}

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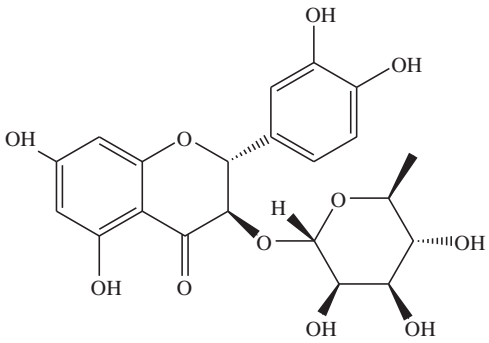

Scheme 1. The chemical structure of ASN.

mol L^{-1} HSA. The concentration of ASN was varied from 0 to 5.4×10^{-5} mol L^{-1} with a step of 6×10^{-6} mol L^{-1}.The fluorescence spectra were recorded in the wavelength range of 300–450 nm. The wavelength of 295 nm was used as an excitation wavelength. The excitation and emission bandwidths were 5 nm.

2.4. Circular dichroism measurement

The circular dichroism spectra were measured on a Jasco-810 Circular dichroism spectrometer (Japan, Jasco company), using a rectangular quartz cuvette of path length 1.0 cm absorption cell. All circular dichroism spectra were taken in a wavelength range 190–260 nm under nitrogen atmosphere. The buffer solution and ASN solution were deducted.

2.5. FT-IR characterization

All infrared spectra were carried out at room temperature on a Perkin Elmer instruments Spectrum One FT-IR spectrometer. KBr was used as the window material, for each spectrum, a 64-scan interferogram was recorded at a resolution of 4 cm^{-1}. The FT-IR spectra of HSA (0.5 mM) in the presence and absence of ASN were collected in the range of 4000-400 cm^{-1}. The molar ratio of HSA to ASN was maintained at 1:1. The spectrum of free HSA was obtained by subtracting the absorption of the buffer solution from the spectrum of the HSA solution, while the difference spectrum of HSA was acquired by subtracting the spectrum of ASN form from that of HSA–ASN form. The deconvolved spectra were accomplished on OMNIC 8.0. The curve fitting of these difference spectra could be used to calculate the secondary structure compositions of free HSA and HSA–ASN system.

2.6. Raman spectrum

The Raman spectra were measured on a Renishaw Invia+Plus FT-Raman spectrometer using an Ar^{+} laser excitation with a wavelength of 514 nm. The laser power was 20 mW. The Raman spectra were recorded in wavelength ranges of 250–2,000 cm^{-1} with a resolution of 1 cm^{-1}. Under the temperature of 25 °C, record the Raman spectra of 0.5 mM HSA and the same concentration of HSA–ASN system. The curve fitting procedure (Peak Analyzer module of Origin 8.0, Microcal Origin, USA) using Gaussian curves analyzed the curve fitting of Raman spectral regions.

3. Results and discussion

3.1. UV–vis absorption spectrum

UV–vis absorption measurement, a very simple way, is used to explore the structural change and the compound formation [10].

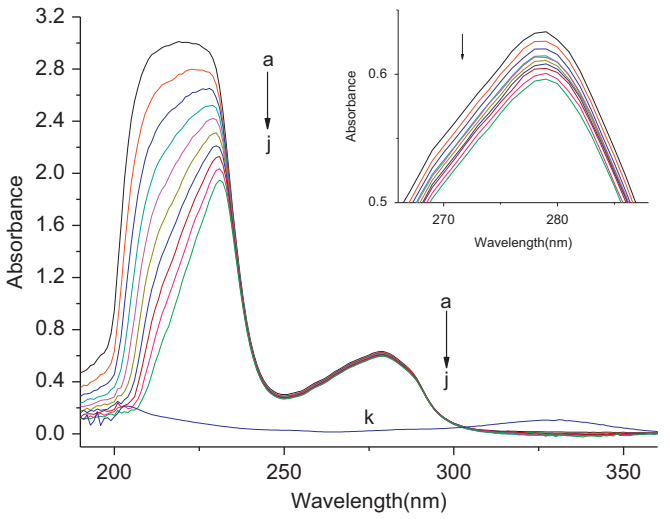

Fig. 1. The UV–visible absorption spectra of HSA in the absence and presence of different concentration of ASN. From a–j, the concentration of ASN were varied from 0 to 9.0×10^{-5} mol L^{-1} at increments of 1.0×10^{-5} mol L^{-1}. [HSA]=1.0×10^{-5} mol L^{-1}. k: ASN only, 1.0×10^{-5} mol·L^{-1}. Inset: the absorption spectra of HSA varied between 260 and 290 nm.

In the UV–vis absorption spectra at about 210 nm represents the α-helical structure of HSA. The absorption spectra of HSA show two maximum absorption wavelength (λ_{max1}=219 nm and λ_{max2}=279 nm), respectively (Fig. 1). The absorbance (219 nm) intensity of HSA decreased obviously with increasing concentration of ASN, meanwhile an apparent red shift (from 219 to 231 nm) was observed. However, the absorbance intensity of 279 nm decreased 9.42% and the position was not obviously changed. The decrease and red shift of the absorption indicate that the bind of drug induce the loosening and unfolding of the protein skeleton and decreasing the hydrophobicity of the microenvironment of the aromatic amino acid residue.

3.2. Analysis of fluorescence spectrum of HSA

HSA contains three intrinsic fluorophores, tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) residues. The reasons for tryptophan as the intrinsic fluorophores of HSA are that the fluorescence of Tyr residue is almost totally quenched when it is close to a carboxyl group, an amino group or ionized and the Phe residue has a very low quantum yield [11]. In order to obtain more information about a drug–protein interaction system, the fluorescence quenching spectra of protein were measured [12]. When the excitation wavelength is setting as 280 nm, both tyrosine and tryptophan residues in protein have fluorescence emission. However, when the excitation wavelength is 295 nm, only tryptophan has fluorescence emission [7]. The fluorescence quenching spectra of HSA were performed with various concentrations of ASN. When the excitation wavelength was 280 nm, a strong fluorescence emission peak at 340 nm was observed (Fig. S1). It can be found that the fluorescence intensity of HSA decreases slowly with unnoticeable change in the position with the addition of ASN. While when the excitation wavelength was 295 nm, a strong fluorescence emission peak at 356 nm was observed (Fig. 2). The fluorescence intensity of HSA decreases regularly with the addition of ASN, and a red shift (from 356 to 370 nm) also can be detected. This proved that the drug induce the microenvironment changes of tryptophan residues, which led to increase hydrophilicity of the tryptophan environment but not affect that of tyrosine residues [13,14].

The fluorescence quenching mechanism can be divided into static quenching and collisional quenching [15]. The collision