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Investigation on the interaction between bovine serum albumin and 2,2-diphenyl-1-picrylhydrazyl

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ABSTRACT

Albumin represents a very abundant and important circulating antioxidant in plasma. In this paper, the ability of bovine serum albumin (BSA) to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical has been investigated using UV–vis absorption spectra. The result shows that the antioxidant activity of BSA against DPPH radical is similar to glutathione and the value of IC_{50} is 5.153×10^{-5} mol L^{-1}. The interaction between BSA and DPPH has been investigated without or with the eight popular antioxidants (L-ascorbic acid, α-tocopherol, glutathione, melatonin, (+)-catechin hydrate, procyanidine B3, β-carotene and astaxanthin) by means of fluorescence spectroscopy and circular dichroism (CD) spectroscopy. The fluorescence experiments show that DPPH quenches the fluorescence intensity of BSA through a static mechanism. The quenching process of DPPH with BSA is easily affected by the eight antioxidants, however, they cannot change the quenching mechanism of DPPH with BSA. Additionally, as shown by synchronous fluorescence spectroscopy and CD, DPPH may induce conformational and microenvironmental changes of BSA.

1. Introduction

Serum albumin is the most abundant protein in blood plasma (~60%), which has many physiological functions, such as maintaining the osmotic pressure and pH of blood and scavenging free radicals as an antioxidant [1]. It is an attractive macromolecular carrier, the lack of toxicity and immunogenicity make it an ideal candidate for drug delivery. In addition, serum albumin is the most multifunctional transport protein and plays an important role in the transport and deposition of a variety of endogenous and exogenous substances in blood [1]. Bovine serum albumin (BSA) has been one of the most extensively studied of this group of proteins, not only because of its medical importance, abundance, low cost, ease of purification, ready availability, unusual ligand-binding properties and it is widely accepted in the pharmaceutical industry [2–4], but also because of its structural homology with human serum albumin (HSA) [5–7]. The BSA molecule is made up of three homologous domains (I, II, III) that are divided into nine loops (L1–L9) by 17 disulfide bonds. The loops in each domain are made up of a sequence of large–small–large loops forming a triplet. Each domain in turn is the product of two subdomains (IA, IB, etc.) [5]. 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]. Up to now, many literatures have reported the interaction between BSA and the small molecules such as drugs, amino acids, fatty acids, metabolites, metal ions, dye, lipids, and even some toxins [5–7,9–14].

DPPH radical is a stable free radical having an absorption maximum at 517 nm with $\varepsilon = 1.09 \times 10^4$ dm^3 mol^{-1} cm^{-1} [15]. It has been widely used for measuring the efficiency of antioxidants [16]. The capacity of biological reagent to scavenge the DPPH radical can be expressed as its magnitude of antioxidant capacity [17]. The DPPH alcohol solution is deep purple in color, which disappears with the presence of the radical scavenger in the reactive system and when the odd electron of the nitrogen in the DPPH is paired [18]. Spectrophotometric determination of the color change quantitates the antioxidant-induced DPPH radical quenching, allowing calculation of the total radical scavenging power of the samples. Compared with other methods, the DPPH assay has many advantages, such as good stability, credible sensitivity, simplicity and feasibility [17]. To the best of our knowledge, the interaction of DPPH with BSA has not been reported.

Antioxidant capacity of an antioxidant is usually expressed in two ways from a quantitative view: one is the scavenging activity of the antioxidant, it was characterized by IC_{50} values, i.e. the concentration of an antioxidant that reduces the initial concentration of free radical

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to 50% [19]. The other is the affinity between free radical and the antioxidant. It was characterized by the binding constant and the stoichiometry (the number of radical molecules reduced by one molecule of the antioxidant) of the reaction between the free radical and the antioxidant [19,20]. In this paper, the ability of BSA to scavenge DPPH radical was investigated using UV–vis absorption spectra. The interaction between BSA and DPPH was investigated in the absence and presence of eight popular antioxidants using fluorescence spectroscopy and CD spectroscopy. The first purpose of this study is to determine the IC_{50} value of BSA and the binding constant and the stoichiometry of the reaction between the DPPH and BSA in order to understand the antioxidant activity of BSA from the angle of quantification. The second aim of this study is to probe the binding mechanism of DPPH with BSA, and then investigate the influence of the eight antioxidants including L-ascorbic acid, α-tocopherol, glutathione, melatonin, (+)-catechin hydrate, procyanidine B3, β-carotene and astaxanthin on DPPH binding with BSA. The study provides an accurate and full basic data for clarifying the antioxidant capacity of BSA and the binding mechanism of DPPH with BSA in the absence and presence of the eight antioxidants and is helpful for understanding the antioxidant activity of albumin in vivo.

2. Materials and methods

2.1. Materials

BSA, DPPH, eight antioxidants including L-ascorbic acid, α-tocopherol (density: 0.950 g mL^{-1} at 20 °C), glutathione, melatonin, (+)-catechin hydrate, procyanidine B3, β-carotene and astaxanthin were purchased from Sigma-Aldrich Chemicals Company (USA). L-Ascorbic acid, glutathione, (+)-catechin hydrate, procyanidine B3 were directly dissolved in phosphate buffer solution of pH 7.40 (0.01 mol L^{-1} PBS). α-Tocopherol was diluted with 99.5% (v/v) ethanol before use. Melatonin was dissolved in 99.5% (v/v) ethanol and then diluted with phosphate buffer solution of pH 7.40 (0.01 mol L^{-1} PBS). β-Carotene and astaxanthin were dissolved in 99.5% acetone and then diluted with phosphate buffer solution of pH 7.40 (0.01 mol L^{-1} PBS). The stock solutions of the eight antioxidants were prepared and used immediately because of oxidation under light and air. Double distilled water was used to prepare solutions. The BSA was dissolved in a phosphate buffer solution of pH 7.40 (0.01 mol L^{-1} PBS). The concentration of the BSA was determined on a TU-1810 spectrophotometer (Puxi Analytic Instrument Ltd., Beijing, China) using the extinction coefficient $\varepsilon_{280}=44720\ mol^{-1}\ L\ cm^{-1}$ [21]. The pH was determined on a pHS-2C pH-meter (Shanghai DaPu Instruments Co., Ltd., Shanghai, China) at ambient temperature. Sample masses were accurately weighed on a microbalance (Sartorius, BP211D) with a resolution of 0.01 mg. All other reagents were all of analytical reagent grade and were used as purchased without further purification.

2.2. DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined as described by Bersuder et al. [22] with some modifications. 4 mL solution of 3×10^{-4} mol L^{-1} DPPH dissolved in 99.5% ethanol was mixed with different volume stock solution of antioxidants and added double distilled water which be used to make the reaction mixture attaining 10 mL. The mixture was then kept at 298 K in dark for 30 min, and the reduction of DPPH radical was measured at 517 nm against each blank with a TU-1810 spectrophotometer. Appropriate blanks, run under the same conditions, were subtracted from the sample spectra. A lower absorbance of the reacted mixture indicated a higher DPPH radical-scavenging activity. The DPPH radical-scavenging activity was calculated as follows:

$$\text{Radical}-\text{scavenging activity}\ (I\%)=\left[(A_0-A)/A_0\right]100 \quad (1)$$

where A_0 and A are the absorbance of DPPH in the absence and presence of antioxidants, respectively. The test was carried out in triplicates.

The absorption data were analyzed using the following equation [23] to estimate the binding constant K between DPPH and antioxidants.

$$\frac{A_0}{A-A_0}=\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}+\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}\frac{1}{K[Q]} \quad (2)$$

where A_0 and A are the absorbance of DPPH in the absence and presence of antioxidants, ε_G and ε_{H-G} are the absorption coefficients of DPPH and its complex with antioxidants, respectively. [Q] is the concentration of antioxidants, K is the analogous to the binding constants at the corresponding temperature.

2.3. Fluorescence measurements

The fluorescence measurements were performed on Cary Eclipse fluorescence spectrophotometer (VARIAN, USA) equipped with a 1.0 cm quartz cell and a thermostat bath. Appropriate amounts of BSA solution and antioxidants were added to 10 mL volumetric flsks, respectively. After 30 min, appropriate amounts of 2.0×10^{-4} mol L^{-1} DPPH were added and then diluted to 10 mL with PBS buffer. The final concentrations of BSA and antioxidants were 2.0×10^{-6} mol L^{-1} and 2.0×10^{-5} mol L^{-1} respectively. The resultant mixtures were then incubated at 298 K for 2.0 h. After 2.0 h of incubation, the fluorescence emissions spectra were scanned. The excitation and emission slit widths were fixed at 5 nm. The excitation wavelength was set at 280 nm (excitation of the Trp and Tyr), and the emission spectra were read at 300–450 nm at a scan rate of 100 nm min^{-1}. The synchronous fluorescence spectra were scanned from 280 to 330 nm ($\Delta\lambda=15$ nm) and from 310 to 380 nm ($\Delta\lambda=60$ nm), respectively.

he fluorescence measurements are hindered by the inner-filter effect, which is that small ligands absorb the light at the excitation and emission wavelengths of proteins and leads to unreliable results [24]. Thus it is very important to subtract such an effect from the raw quenching data. The extent of this effect can be roughly estimated with the following equation [25]:

$$F_{cor}=F_{obsd}10^{(A_{ex}+A_{em})/2} \quad (3)$$

where F_{cor} and F_{obsd} are the corrected and observed fluorescence intensities, respectively, whereas A_{ex} and A_{em} are the sum of the absorbance of protein and ligand at the excitation and emission wavelengths, respectively. The fluorescence intensity utilized in this study is the corrected intensity.

2.4. Circular dichroism (CD) measurements

The CD measurements were carried out on a Jasco J-715 spectropolarimeter under constant nitrogen flush. For measurements in the far-UV region (190–260 nm), a quartz cell with a path length of 0.2 cm was used. Three scans were accumulated with continuous scan mode and a scan speed of 200 nm min^{-1} with data being collected at 0.2 nm and response time of 2 s. The sample temperature was maintained at 298 K. The protein concentration was fixed to 2.0×10^{-6} mol L^{-1}, the molar ratio of DPPH to BSA was maintained at 1:1, and results were taken as CD ellipticity in mdeg.