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Characterization of interaction between C.I. Acid Green 1 and human serum albumin: Spectroscopic and molecular modeling method

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ABSTRACT

The binding characteristics of human serum albumin with C.I. Acid Green 1 were studied by employing fluorescence, resonance light scattering, ultraviolet–visible, circular dichroism, Fourier transform infrared techniques and molecular modeling. Spectroscopic analysis has revealed that quenching of human serum albumin by C.I. Acid Green operates by a static quenching mechanism. The results by Fourier transform infrared, circular dichroism and ultraviolet–visible absorption spectra experiment indicated that the secondary structures of protein were changed in the presence of C.I. Acid Green 1. Molecular modeling revealed that a dye–protein complex was stabilized by hydrophobic forces, Van-der-Waals force and hydrogen bonding, via amino acid residues. Furthermore, influences of coexisting substances on the binding constant of C.I. Acid Green 1-human serum albumin complexes were investigated.

1. Introduction

C.I. Acid Green 1 (Fig. 1) is an anionic dye. It has been widely used as a biological stain and diagnostic aid. On the other hand, C.I. Acid Green 1 has excellent redox characteristics, thus it can be used as a mediator for electrocatalysis of biological compounds [1,2]. It was reported that some dyes would stain certain tissues, the dyes would selectively stain, combine with and destroy pathogenic organisms without causing appreciable harm to the host [3]. Nowadays, much research on the binding of drugs to HSA has been carried out [4–10], but seldom report on the binding interaction of proteins with the dye. To have a full understanding of the modes of dye–protein interaction is important for understanding the biological effects and functions of dyes in the body.

Human serum albumin (HSA) is the major soluble protein with a high concentration in blood plasma (40 kg/m^3 or 0.6×10^{-3} mol/L). The structure of HSA has been established and it consists of three structurally homologous, predominantly helical domains (domains I, II and III), with each domain containing of two subdomains (A and B). X-ray measurements have revealed that ligands binding to HSA are located in hydrophobic cavities in subdomains IIA and IIIA referred to as Sudlow sites I and II [11,12]. Except from maintaining colloid osmotic pressure, HSA also plays a role in transport of a number of ligands including hormones, hemin, bilirubin and fatty acids, as well as transition metals such as copper, cobalt, nickel, and cadmium [13,14]. Dye-protein interaction can affect the duration and intensity of pharmacological effect [15]. Investigation on the interaction of HSA with a dye can help us better understand the absorption and distribution of dye, and hence become an important research field in chemistry, life sciences and clinical medicine.

The interaction of HSA with C.I. Acid Green 1 was demonstrated via fluorescence spectroscopy, ultraviolet–visible (UV–vis), resonance light scattering (RLS), Fourier transformed infrared (FT-IR), and circular dichroism (CD) approaches. The binding constants were obtained at different temperatures in the medium of HCl–Tris (pH 7.40) buffer solution. Information regarding the binding sites and main types of binding force of the reaction was provided. The conformational changes of HSA were discussed on the basis of synchronous fluorescence spectra, CD and FT-IR. In addition, the partial binding parameters of the reaction were calculated through SGI FUEL work stations.

2. Materials and methods

2.1. Materials

Human serum albumin (fatty acid free $< 0.05\%$) was purchased from Sigma Chemical Co. and used without further purification.

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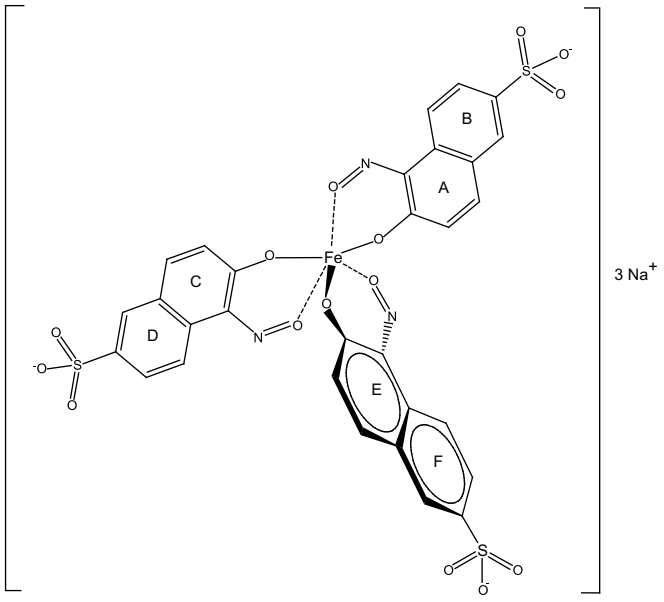

Fig. 1. Chemical structure of C.I. Acid Green 1.

C.I. Acid Green 1 (analytical grade) was obtained from Xi-an huaxue shijichang (China), and the stock solution was prepared in water. All HSA solution was prepared in the pH 7.40 buffer solution, and HSA stock solution was kept in the dark at 4 °C. Buffer (pH 7.40) consists of Tris (0.2 mol/L) and HCl (0.1 mol/L), and the pH was adjusted to 7.40 by 0.5 mol/L NaOH when the experiment temperature was higher than 296 K. The pH was checked with a suitably standardized pH meter. All reagents were of analytical reagent grade and double distilled water was used throughout the experiment.

2.2. Spectroscopic measurements

Fluorescence measurement and the spectrum of resonance light scattering (RLS) were obtained with an RF-5301PC spetrofluorophotometer (Shimadzu). The fluorescence emission spectra were recorded from 290 to 500 nm (excitation wavelength 280 nm) using 5/5 nm slit widths. Synchronous fluorescence spectra of HSA in the absence and presence of increasing amount of C.I. Acid Green 1 were recorded. UV–vis absorption measurements were performed on Shimadzu UV-240 spectrophotometer with a 1.0 cm quartz cell at room temperature. An electronic thermoregulating water bath (NTT-2100, EYELA, Japan) was used to control the temperature of the samples.

FT-IR measurements were carried out at a constant room temperature (296 K) on a Nicolet Nexus 670 FT-IR spectrometer (America) equipped with a germanium attenuated total reflection (ATR) accessory, a DTGS KBr detector and a KBr beam splitter. All spectra were taken via the attenuated total reflection (ATR) method with resolution of 4 cm^{-1} and 60 scans. Spectra of buffer solution were collected at the same condition, then, the absorbance of buffer solution was subtracted from the spectra of sample solution to get the FT-IR difference spectra of proteins. The subtraction criterion was that the original spectrum of protein solution between 2200 and 1800 cm^{-1} was featureless [16].

The CD spectra were gained by Olis DSM1000CD (USA) with a 0.1 cm quartz cell at room temperature, the speed of scanning was 30 nm min^{-1}, the slit width was set at 5 nm. The induced ellipticity was obtained by the ellipticity of the dye–HSA mixture subtracting the ellipticity of dye at the same wavelength and is expressed in degrees. The results were expressed as mean residue ellipticity (MRE) in deg cm^2/dmol, which is defined as [MRE θ_{obs} (mdeg)/$10nlC_p$]. The θ_{obs} represents the CD in millidegree, n is the number of amino acid residues (585), l the path length of the cell and C_p is the mole fraction [17]. The α-helical content of HSA was calculated from the MRE value at 208 nm using (Eq. (1)) as described by Khan et al [18].

$$\alpha - helical\% = [(MRE\ 208 - 4000)/33,000 - 4000] \times 100 \quad (1)$$

2.3. Molecule modeling

A docking study of the binding mode between drug and HSA was performed on SGI Fuel workstation. The crystal structure of HSA in complex with *R*-warfarin was downloaded from the Brookhaven Protein Data Bank (entry codes 1h9z) [19]. The potential of the 3-D structures of HSA was assigned according to the Amber 4.0 force field with Kollman-all-atom charges. The initial structures of all the molecules were generated by molecular modeling software Sybyl 6.9 [20]. The geometry of the molecule was subsequently optimized to minimal energy using the Tripos force field with Gasteiger–Marsili charges with a gradient of 0.005 kcal/mol. FlexX program was applied to calculate the interaction mode between dye and HSA. During the docking process, a maximum of 30 conformers was considered for this compound. The conformer with the lowest binding free energy was used for further analysis.

3. Result and discussion

3.1. The binding mechanism analysis of C.I. Acid Green 1 and HSA

Fluorescence quenching is the decrease of quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions with a quencher molecule. The application of fluorescence measurements can reveal the reactivity of chemical and biological systems since it allows non-intrusive measurements of substances in low concentration under physiological conditions. Fig. 2 shows the fluorescence spectrum of HSA in pH = 7.40 buffer solution at $\lambda_{ex} = 280$ nm. Addition of different concentrations of C.I. Acid Green 1 caused a noticeable decrease in HSA fluorescence intensity, and an increase in the fluorescence intensity at 450 nm was assigned to C.I. Acid Green 1. Moreover, the occurrence of an isobestic point at 410 nm might also indicate the existence of bound

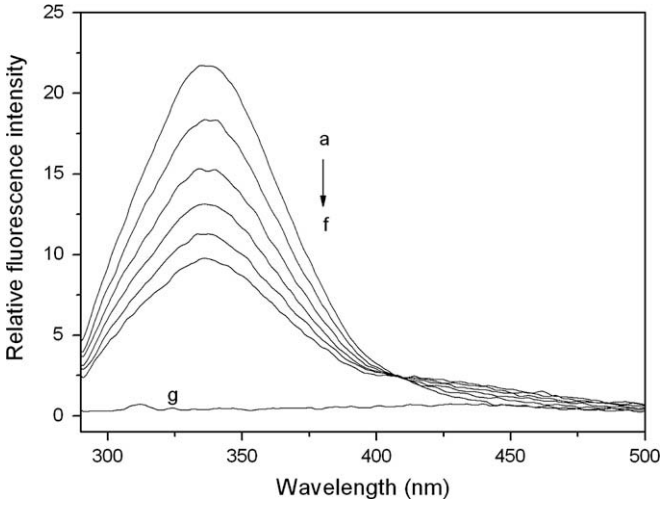

Fig. 2. Fluorescence spectra of C.I. Acid Green 1–HSA system ($T = 298$ K, pH 7.40). (a) 3.0×10^{-6} mol/L HSA; (b)–(f) 3.0×10^{-6} mol/L HSA in the presence of 3.32, 6.62, 9.90, 13.16, 16.39 $\times 10^{-6}$ mol/L C.I. Acid Green 1; (g) 3.32×10^{-6} mol/L C.I. Acid Green 1.