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Combined molecular docking and multi-spectroscopic investigation on the interaction between Eosin B and human serum albumin

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ABSTRACT

The binding of Eosin B to human serum albumin (HSA) was studied using molecular docking, fluorescence, UV–vis, circular dichroism (CD) and Fourier transform infrared (FT-IR) spectroscopy. The mechanism of interaction between Eosin B and HSA in terms of the binding parameters, the thermodynamic functions and the effect of Eosin B on the conformation of HSA were investigated. Protein-ligand docking study indicated that Eosin B bound to residues located in the subdomain IIA of HSA and Eosin B–HSA complex was stabilized by hydrophobic force and hydrogen bonding. In addition, fluorescence data revealed that Eosin B strongly quenched the intrinsic fluorescence of HSA through a static quenching procedure. Furthermore, alteration of the secondary structure of HSA in the presence of the dye was conformed by UV–vis, FT-IR and CD spectroscopy.

1. Introduction

Human serum albumin (HSA) is the most abundant of the protein in blood plasma, accounting for approximately 60% of the total protein corresponding to a concentration of 42 g/L in the blood [1]. The unique feature of albumin is its ability to bind different categories of endogenous and exogenous small molecules [2,3]. The three-dimensional structure of HSA has been determined through X-ray crystallographic measurements. This globular protein, a single polypeptide chain of 585 amino acid residues, consists of three structurally similar domains (I–III), each containing two subdomains (A and B) and stabilized by 17 disulfide bridges [4–6].

Nowadays, many studies on the binding of drugs to HSA have been carried out [7–12], but only few results have been published on the interaction between dyes and HSA [13–15]. There is an evidence of conformational changes of albumin induced by its interaction with dyes and other ligands [11–15]. The widespread commercial use of dyes today also makes it necessary to study structural dynamics of dye–protein complexes for understanding the biological effects and functions of dyes in body. Thus, the study of dyes–protein interaction is of great interest in the field of chemistry, life sciences and clinical medicine.

Eosin B (4′,5′-dibromo-2′,7′-dinitrofluorescein, structure shown in Fig. 1) has been widely used as a dye and biological stain. It is widely used in dyeing textiles, ink manufacturing, coloring cosmetic and coloring gasoline. Thus, it is very possible that the dye is diffused into human bodies. However, its effects on human serum albumin have not been studied in detail.

There are many methods which can be used to study the interaction between small molecules and albumin, such as equilibrium dialysis [16], nuclear magnetic resonance (NMR) [17], electrochemistry [18] and optical techniques. Among them, optical techniques are powerful methods because they are sensitive, rapid and relatively easy to use. In this work, the binding site of Eosin B to HSA was discussed using automated molecular docking approach. Furthermore, the interaction of Eosin B with HSA was investigated by biophysical methods mainly including fluorescence, UV–vis, CD and FT-IR studies, serving as aids to understand the mechanism of the dye binding to HSA better. The results have been discussed on the binding parameters, the identification of binding sites, the effect of Eosin B on the conformation of HSA, and the nature of forces involved in the interaction.

2. Materials and methods

2.1. Materials

Human serum albumin (HSA, 95–99% purity) was purchased from Sigma Chemical Company, and its molecular weight was

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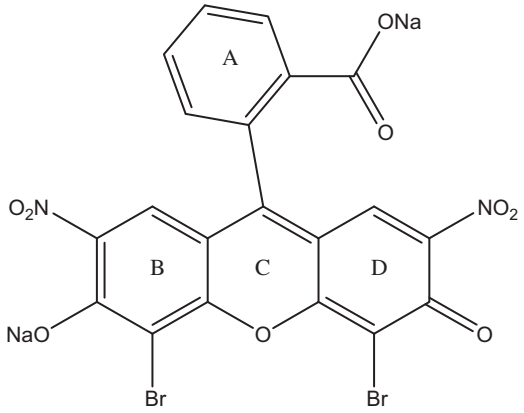

Fig. 1. The chemical structure of Eosin B.

assumed to be 66,500. Commercially available Eosin B was obtained from Shanghai Maikun Chemical Co., LTD, and used as received. 1.0 mol/L NaCl solution was used to keep the ion strength at 0.1. Tris (0.2 mol/L)–HCl (0.1 mol/L) buffer solution containing 0.1 mol/L NaCl was selected to keep the pH of the solution at 7.40. HSA stock solution (3.0×10^{-6} mol/L) was prepared in the Tris-HCl buffer solution and kept in the dark at 4 °C. 1.0×10^{-3} mol/L stock solution of Eosin B was prepared in double-distilled water. All other reagents were of analytical reagent grade and double-distilled water was used throughout the experiments.

2.2. Apparatus and methods

2.2.1. Molecular docking

The crystal structure of HSA in complex with R-warfarin was taken from the Brookhaven Protein Data Bank (entry codes 1h9z) [19]. The potential of the 3-D structure of HSA was assigned according to the Amber 4.0 force field with the 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. Then, R-warfarin was replaced by the optimized structure of Eosin B. The FlexX program was used to build the possible interaction modes between Eosin B and HSA. Some partial binding parameters of the Eosin B–HSA system were calculated by SGI FULE workstation.

2.2.2. Spectroscopic measurements

Fluorescence emission spectra were obtained with a RF-5301PC spectrofluorophotometer (Shimadzu, Japan) equipped with a xenon lamp source and 1.0 cm quartz cells. The excitation wavelength was 280 nm and the slit widths for both excitation and emission bandwidths were 5 nm. The emission spectra were recorded in the range of wavelength from 290 to 475 nm.

Fluorometric titration experiments: 3.0 mL solution containing appropriate concentration of HSA was titrated manually by successive additions of stock solution of Eosin B (to give a final concentration of $1.67 \times 10^{-6} - 1.83 \times 10^{-5}$ mol/L) with trace syringes. The fluorescence intensities were recorded at excitation and emission wavelengths of 280 and 335 nm, respectively. Fluorescence experiments were performed at four different temperatures (291, 298, 305, and 311 K). An electronic thermo regulating water-bath (NTT-2100, EYELA, Japan) was used to control the temperature of the samples.

UV–vis absorption spectra were scanned on a TU-1810 UV–visible spectrophotometer (Beijing Purkinje General Instrument Co., China) using quartz cells with 1 cm path length at room temperature.

The CD spectra of HSA in the absence and presence of Eosin B were obtained by Olis DSM1000 (USA) automatic recording spectrophotometer, using a 1 mm cell at room temperature. The induced ellipticity was gained by the ellipticity of the dye–HSA mixture subtracting the ellipticity of dye at the same wavelength and was expressed in degrees. The final results were taken as molar ellipticity ($[\theta]$) in deg cm^2 dmol^{-1}. The α-helical content of HSA was calculated from $[\theta]$ value at 208 nm according to the following Eq. [21]:

$$\alpha\text{-helix}(\%) = \{(-[\theta]_{208} - 4000)/(33000 - 4000)\} \times 100 \quad (1)$$

FT-IR measurements were performed at room temperature on a Nicolet Nexus 670 FT-IR Spectrometer (America) equipped with a germanium attenuated total reflection (ATR) accessory, a deuterated triglycine sulphate (DTGS) 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. Corresponding absorbance contributions of buffer and free Eosin B solutions were recorded and digitally subtracted in the same conditions. The subtraction criterion was that the original spectrum of protein solution between 2200 and 1800 cm^{-1} was featureless [22].

3. Results and discussion

3.1. Molecular docking study

Molecular docking study was performed to identify the primary binding site of Eosin B to HSA. Crystal structure analysis has revealed that HSA consists of three homologous domains (I, II and III): I (residues 1–195), II (196–383) and III (384–585), each of which is divided into two subdomains (A and B). The only tryptophan residue (Trp-214) is in subdomain II A [23]. Sudlow et al. [24] have suggested two main distinct binding sites on HSA, sites I and II, which locate in the hydrophobic cavities of subdomains II A and III A, respectively. The best energy ranked results of the binding mode between Eosin B and HSA are shown in Fig. 2.

It is important to note that the only tryptophan residue (Trp-214) of HSA is in close proximity to the dye molecule, which indicated that there may be an efficient fluorescence quenching of

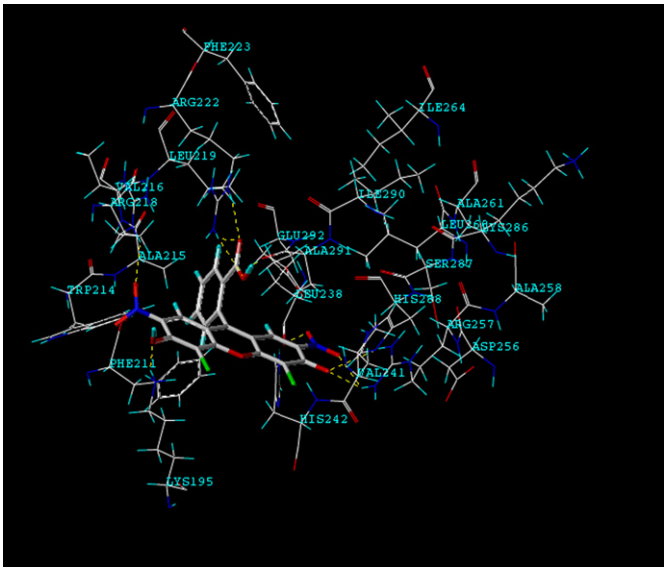

Fig. 2. The interaction model between Eosin B and HSA. The residues of HSA are represented using gray ball and stick model and the Eosin B structure is represented by a silvery white one. The hydrogen bond between Eosin B and HSA is represented by yellow dashed line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)