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Spectroscopic study on the interaction between mononaphthalimide spermidine (MINS) and bovine serum albumin (BSA)

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ABSTRACT

The interaction mononaphthalimide spermidine (MINS, **1**) and bovine serum albumin (BSA) was studied by UV/vis absorption, fluorescence and circular dichroism spectra (CD) under physiological conditions (pH = 7.4). The observed spectral quenching of BSA by compound **1** indicated compound **1** could bind to BSA. Further fluorescent tests revealed that the quenching mechanism of BSA by compound **1** was overall static. Meanwhile, the obtained binding constant and thermodynamic parameters on compound-BSA interaction showed that the type of interaction force of compound **1** and BSA was mainly hydrophobic. The analysis of synchronous, three-dimensional fluorescence and CD showed that compound **1** had weak influence on the conformational changes in BSA. Molecular docking simulation was performed and docking model in *silico* suggested that the configuration of compound **1** was localized in enzymatic drug site II in BSA. Furthermore, naphthalimide moiety of compound 1 greatly contributed to the hydrophobic interaction between compound 1 and BSA protein, as confirmed by experimental data.

1. Introduction

It is well known that protein are fundamental elements of life, and the increase or decrease of protein contents in serum can exhibit the conditions of human health [1,2]. SA, including bovine serum albumin (BSA) and human serum albumin (HSA), are the major soluble protein constituents of the circulatory system; it plays an important and efficient role in drug delivery due to their ability to reversibly bind a large variety of exogenous compounds, including fatty acids, amino acids, drugs and pharmaceuticals [3–5]. BSA is a natural globular protein that is similar to HSA by 76% in space structure and chemistry composition [6–8]. Because of its low cost and ready availability, BSA is selected as our model protein.

The 1,8-naphthalimides derivatives are proven to intercalate DNA base pairs and bind to SA [2,9,10]. Polyamines can bind to SA [11–14]. We previously reported that 2-{3-[4-(3-aminopropylamino)butyl-amino]propy}1H-benz-[*de*]isoquinoline-1,3(2*H*)-dione trihydrochloride (MINS **1**, Fig. 1) could induce apoptosis *via* caspase-dependent intrinsic and AIF-mediated caspase-independent pathways [15]. We also proved that naphthalimides-polyamine conjugates were DNA intercalators [16–18]. However, detailed interaction between compound **1** and SA is still unknown to date. In this study, the interactive pattern between MINS (**1**) and BSA was studied by UV, fluorescence, circular dichroism spectroscopy and molecular modeling method. The binding constants and main types of binding force were also investigated.

2. Materials and methods

2.1. Apparatus

UV–vis absorption spectra were measured on a Unicam UV 500 spectrophotometer using a 1.0 cm cell. Fluorescence measurements were performed with a Cary Eclipse spectrofluorimeter. Circular dichroism spectrum measurements were performed on a Modle 420 SF (USA) automatic recording spectrophotometer in a 1 mm quartz cell.

2.2. Materials

Naphthalimide-polyamine conjugate **1** was prepared previously [19]. Its solution (2.00×10^{-5} mol L^{-1}) was prepared with the

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Tris–HCl buffer solution (UV, and Fluorescence) or (2.00×10^{-4} mol L^{-1}) with the phosphate buffer saline (PBS, pH = 7.4) buffer solution (CD) and stored at 4 °C. BSA (Sino-American Biotechnology Company, Beijing, China) was used without further purification, and its stock solution, either measured at (2.60×10^{-5} mol L^{-1}), was prepared either by dissolving an appropriate amount of BSA in double Tris–HCl (pH = 7.4) buffer solution (UV, and Fluorescence) or at (2.00×10^{-4} mol L^{-1}), was mixed with PBS (pH = 7.4) buffer solution (CD), stored at 4 °C.

2.3. Procedures

2.3.1. 2.3.1.UV–Vis measurement

0.2 mL solution of BSA (2.60×10^{-5} mol L^{-1} in Tris–HCl (pH = 7.4) was mixed with 0.0, 0.10, 0.20, 0.30, 0.60, 0.90 1.20, 1.50, 1.80, 2.10, 2.40, 2.70 and 3.00 mL of compound **1** (2.0×10^{-5} mol L^{-1}) respectively. The mixture was also diluted to 5 mL with Tris–HCl (pH = 7.4). Thus, samples were prepared in concentrations of 0.0, 0.4, 0.8, 1.2., 2.4, 3.6, 4.8, 6.0, 7.2, 8.4, 9.6, 10.8 and 12.0×10^{-6} mol L^{-1}. One contained only BSA (1.04×10^{-6} mol L^{-1}) as control, while others contained different concentrations of compound **1** but had the same concentration of BSA. All of above solution was shaken for 30 min. at room temperature.

2.3.2. Fluorescence measurement

2.3.2.1. Interaction of compound 1 with BSA. Preparation of sample is the same as that of UV–Vis samples. Fluorescence wavelengths and intensity areas of samples were measured at 298, 303 and 310 K in the wavelength range of 300–550 nm with exciting wavelength at 280 nm. λ_{em} = 350 nm.

2.3.2.2. Synchronous and three-dimensional fluorescence spectroscopy of BSA. Synchronous fluorescence spectroscopy was obtained with the wavelength from 230–320 nm ($\Delta\lambda$ = 15 nm) and 480–550 nm and ($\Delta\lambda$ = 60 nm) with the emission slit width of 10 and 2.5 nm, respectively. Three-dimensional fluorescence spectroscopy was conducted with setting excitation and emission wavelength from 230 to 330 nm with an increment of 10 nm.

2.3.3. CD measurement

2 mL solution of BSA (2.00×10^{-4} mol L^{-1}) in PBS (pH = 7.4) was mixed with 0 and 3.00 mL of compound **1** (2.00×10^{-4} mol L^{-1}) respectively. The mixture was diluted to 5 mL with PBS (pH = 7.4). Thus, samples were prepared in the concentrations of 0.0 and 120.0×10^{-6} mol L^{-1}. One sample contained only BSA (40×10^{-6} mol L^{-1}) as control, while others contained different concentrations of compound **1** but had the same concentration of BSA. All of the above solution was shaken for 30 min. at room temperature.

Temperature scans were carried out between 20 °C and 95 °C, with a scan rate of 1 °C/s, using a 1 mm path length cuvette. The wavelength is 222 nm.

2.3.4. Molecular modeling study

The coordinates of three dimensional structure of BSA protein were retrieved from the Protein Data Bank (PDB code: 4JK4), and subsequently converted to DS LibDock-compatible system by adding hydrogen atoms, modifying unusual amino acids, making charge adjustments and performing additional cleanup steps on Discovery Studio 2.1 platform. Molecular docking experiments of compound **1** binding to BSA protein were performed using LibDock protocol on the Discovery Studio 2.1 platform. The whole complex was defined as a receptor and the docking site sphere was localized on the location of the ligand binding site in the BSA complex with 3,5-diodosalicylic acid. The docking result was presented by using the PyMOL software [20,21].

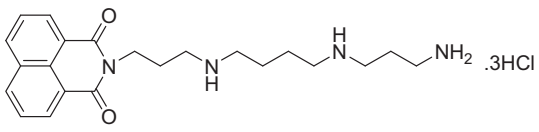

Fig. 1. The chemical structure of MINS (**1**).

3. Results and discussion

3.1. UV spectroscopic characteristics

As shown in Fig. 2, the UV spectrum of BSA in the presence of different concentrations of compound **1** was measured by Ultraviolet visible range spectrophotometer. As illustrated in Fig. 2, free BSA has no absorption at 278 nm that was inconsistent to some references which reported that free BSA has absorption at 278 nm when its concentration is at 10^{-6} M [22,23] and even if HSA is so [23,24]. As is also shown in Fig. 2, there is a continuous increase, as compound **1** increases, deducing that at least one MINS–BSA complex might be formed. Moreover, red shift of maximum peak of BSA at 210 nm was also observed, probably because of the complex formation between the MINS and BSA. In addition, the absorption of MINS at 345 nm was shown. The spectra result indicated that compound **1** penetrated the hydrophobic sites in casein sub-domain and bounded with the chromophores of tyrosine and tryptophan residues, and that the un-bound residues were buried in hydrophobic cave [25].

Utilizing absorption spectrum obtained by UV, we could also calculate the compound **1** apparent binding constant according to the following formula [26,27]:

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} \frac{1}{K_{[Comp.\mathbf{1}]}} \quad (1)$$

in which A_0 and A denote the absorbance in the absence and presence of compound **1**, respectively, and ε_G and ε_{H-G} denote the molar absorption coefficient of compound and its formed complex with BSA, respectively. The value of apparent binding constant could be measured from the intercept and slope by plotting $A_0/(A - A_0)$

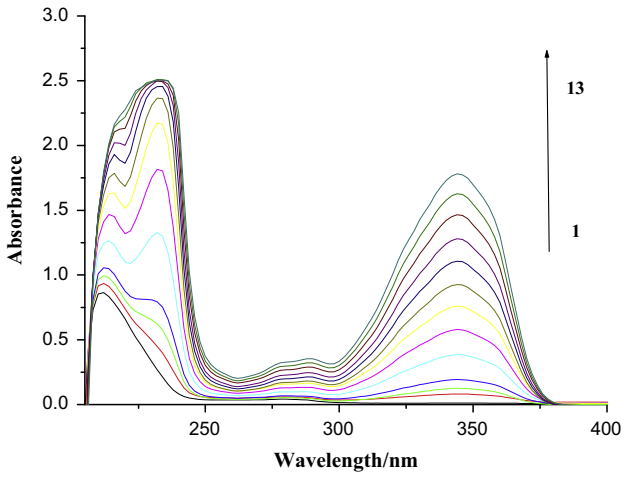

Fig. 2. UV absorption spectra of MINS with BSA. Numbers 1–13 indicates concentrations of MINS: 0.0, 0.4×10^{-6}, 0.8×10^{-6}, 1.2×10^{-6}, 2.4×10^{-6}, 3.6×10^{-6}, 4.8×10^{-6}, 6.0×10^{-6}, 7.2×10^{-6}, 8.4×10^{-6}, 9.6×10^{-6}, 10.8×10^{-6} and 12×10^{-6} mol L^{-1}, respectively. BSA concentration applied is 1.04×10^{-6}.