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Study of interaction between human serum albumin and three phenanthridine derivatives: Fluorescence spectroscopy and computational approach

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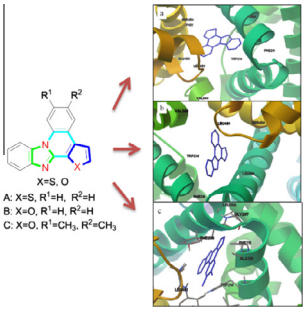
HIGHLIGHTS

- The binding of phenanthridine derivatives to HSA was investigated by different methods.
- The binding intensity was BTQ > BFQ > DFQ.
- The effects of ions on the binding affinity of phenanthridine derivatives to HSA were examined.

GRAPHICAL ABSTRACT

Molecular docking analysis of HSA with phenanthridine derivatives.



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ABSTRACT

Over the past decades, phenanthridine derivatives have captured the imagination of many chemists due to their wide applications. In the present work, the interaction between phenanthridine derivatives benzo[4,5]imidazo[1,2-a]thieno[2,3-c]quinoline (BTQ), benzo[4,5]imidazo[1,2-a]furo[2,3-c]quinoline (BFQ), 5,6-dimethylbenzo[4,5]imidazo[1,2-a]furo[2,3-c]quinoline (DFQ) and human serum albumin (HSA) were investigated by molecular modeling techniques and spectroscopic methods. The results of molecular modeling simulations revealed that the phenanthridine derivatives could bind on both site I in HSA. Fluorescence data revealed that the fluorescence quenching of HSA by phenanthridine derivatives were the result of the formation of phenanthridine derivatives–HSA complex, and the binding intensity between three phenanthridine derivatives and HSA was BTQ > BFQ > DFQ. Thermodynamics confirmed that the interaction were entropy driven with predominantly hydrophobic forces. The effects of some biological metal ions and toxic ions on the binding affinity between phenanthridine derivatives and HSA were further examined.

Introduction

Phenanthridine derivatives exhibit interesting and important biological activities, pharmacological properties and optoelectronic applications. Meanwhile, it is well known that phenanthridine derivatives have been widely used due to their versatile biological activities such as antibacterial, antitumoral, cytotoxic, and DNA intercalator [1–4]. Because of their generally broad biological importance, considerable efforts have been directed at their synthesis, characterization and functionalization. Three phenanthridine derivatives (benzo[4,5]imidazo[1,2-a]thieno

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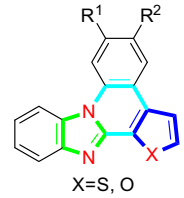

A: X=S, R^1=H, R^2=H
B: X=O, R^1=H, R^2=H
C: X=O, R^1=CH_3, R^2=CH_3

Scheme 1. Molecular structures of BTQ (A), BFQ (B), and DFQ (C).

[2,3-c]quinoline (BTQ), benzo[4,5]imidazo[1,2-a]furo[2,3-c]quinoline (BFQ), 5,6-dimethylbenzo[4,5]imidazo[1,2-a]furo[2,3-c]quinoline (DFQ)) have been obtained in our laboratory by a novel approach [5]. Despite the value of phenanthridine derivatives, few studies focus on their spectroscopic properties and biophysical studies of their interactions with protein or information on the metal ions binding to phenanthridine derivatives–HSA complex.

HSA is known to play an important role in the transport and disposition of endogenous and exogenous ligands present in blood, with a concentration of 0.6 mM in blood plasma [6,7]. Many drugs and other bioactive small molecules bind reversibly to serum albumin, which implicates HSA's role as a carrier. The crystallographic studies identified HSA has a heart shape structure composed of three major domains (I, II, III). Among them, two sites (sites I and II) located in the subdomains IIA and IIIA, have been reported as its primary ligand binding sites [8–11]. The knowledge of the mechanism of HSA binding is of great importance for understanding the process of drug transportation, and the prediction of serum concentrations of the free drug.

In summary, we attempted to investigate the most possible binding mode and the interaction mechanism between the three novel phenanthridine derivatives, (shown in Scheme 1) and HSA under simulated physiological conditions (pH = 7.40). In order to attain these objectives, multiple spectroscopic methods and molecular modeling techniques were employed. The effects of some metal ions and toxic ions on the binding affinity between the phenanthridine derivatives and HSA were also discussed. The study is helpful for understanding information of phenanthridine derivatives effects on protein conformation and information on metal ions binding to phenanthridine derivatives–HSA complex. Understanding the interactions between protein and phenanthridine derivatives may enable drug delivery systems for improved medical treatments.

Materials and methods

Reagents

HSA (fatty acid free <0.05%) was purchased from Sigma–Aldrich (St. Louis, MO, USA) and used without further purification. Final concentrations of the solutes were verified spectrophotometrically using the molar absorption coefficients determined in this work: ε_{280} = 35,500 L mol cm^{-1} for HSA [12]. The analytical reagents of phenybutazon (PB) and flufenamic acid (FA) were obtained from J&K Scientific Ltd. (Beijing, China). Other reagents were all of analytical grade. Stock solutions of HSA (3.0×10^{-5} mol L^{-1}) was prepared in Tris–HCl buffer (0.1 mol L^{-1}) of pH 7.4. The BTQ (1.0×10^{-3} mol L^{-1}), BFQ (1.0×10^{-3} mol L^{-1}), DFQ (1.0×10^{-3} mol L^{-1}), PB (1.0×10^{-3} mol L^{-1}) and FA (1.0×10^{-3} mol L^{-1}) solution were prepared in anhydrous methanol. During experiment, purified water was obtained through a Milli-Q water purification system (Millipore). All pH measurements were made with a pHS-3 digital pH-meter (Shanghai, China).

Apparatus and methods

All fluorescence spectra of HSA were recorded on FP-6500 spectrofluorometer (JASCO, Japan) equipped with 1.0 cm quartz cells and a thermostat bath. Fluorescence emission spectra were recorded in the range of 300–550 nm upon excitation at 280 nm at four temperatures (289, 296, 303 and 310 K). The slit widths of emission and excitation were set 5.0 nm. The fluorescence spectra of the buffers were subtracted and the inner-filter effect was eliminated following a reported method [13]:

$$F_{cor} = F_{obs} \times e^{(A_{ex}+A_{em})/2} \quad (1)$$

F_{cor} and F_{obs} are the corrected and observed fluorescence intensities, respectively, A_{ex} and A_{em} are the absorbance values of the systems at excitation and emission wavelengths, respectively. In this study, all fluorescence intensities were corrected prior to data analysis.

Three-dimensional fluorescence spectra were obtained under the following conditions: the emission wavelength was recorded between 220 and 600 nm, the initial excitation wavelength was set to 220 nm with increment of 5 nm, and the other scanning parameters were identical to those used for steady state fluorescence as mentioned above.

UV–vis absorption spectra were recorded with a UV-1700 spectrophotometer (Shimadzu, Japan) equipped with 1.0 cm quartz cells at 298 K.

Time-resolved fluorescence lifetime was measured with a FL 980 spectrofluorimeter (Edinburgh Instruments), using a time correlated single photon counting module with a nanoLED. Data analysis was performed with the non-linear least-square deconvolution software provided with the instrument. The instrumental response function (IRF) was recorded with Ludox showing full-width half-maximum (FWHM) around 80 ps. Average fluorescence lifetime (τ) for triexponential iterative fittings was calculated from the decay times and the normalized pre-exponential factors using the following equation:

$$(\tau) = \frac{\sum \alpha_i \tau^2}{\sum \alpha_i \tau} \quad (2)$$

where α_i are the pre-exponential factors and τ are the respective decay times.

An Avatar 360 E.S.P. FT-IR spectrometer (Perkin Elmer), equipped with a germanium attenuated total reflection (ATR) accessory, was employed to collect FT-IR spectra. All spectra were taken via the ATR method with a resolution of 4 cm^{-1} using 60 scans. Spectra processing procedures involved spectra of buffer being collected under the same conditions. The FT-IR spectra of HSA in presence and absence of sample were recorded (n = 3 replicates). First, the absorbance of buffer and free BTQ/BFQ/DFQ solutions were recorded and digitally subtracted. Then the absorbance of BTQ/BFQ/DFQ was subtracted from the spectra of HSA–BTQ/BFQ/DFQ to get the FT-IR difference spectra of HSA. For the subtraction criterion, the original spectrum of protein solution between 2200 and 1800 cm^{-1} is a smooth straight [14,15].

Binding location studies between HSA and phenanthridine derivatives in the presence of two typical site markers were executed as follows: PB (3.0 μmol/L) or FA (3.0 μmol/L) were incubated with HSA (3.0 μmol/L), pH 7.4, at 298 K for 30 min. Then a 3.0 mL sample was added to a 1 cm quartz cuvette, followed by titration of sample. An excitation wavelength of 280 nm was chosen, and the fluorescence emission wavelength was acquired from 290 to 500 nm.

The crystal structure of HSA in complex with *R*-warfarin was taken from the Brookhaven Protein Data Bank (entry codes 1h9z). The structures of the phenanthridine derivatives were