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Spectroscopic investigation on the intermolecular interaction between N-confused porphyrins-(3-methylisoxazole) diad and bovine serum albumin

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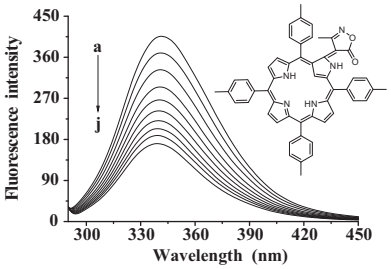
HIGHLIGHTS

- The interaction of BSA and NCP-(3-methylisoxazole) was researched.
- The fluorescence quenching mechanism is combined quenching.
- Hydrophobic interaction force plays a major role in stabilizing the complex.
- The binding constant, ΔG, ΔH, and ΔS of the interaction were calculated.
- The conformation of BSA is changed in the presence of NCP-(3-methylisoxazole).

GRAPHICAL ABSTRACT

The interaction between N-confused porphyrins-(3-methylisoxazole) diad (NCP-(3-methylisoxazole)) and bovine serum albumin (BSA) was studied by fluorescence and UV–vis spectroscopy. The quenching mechanism, binding constants, thermodynamic parameters, and binding distance were obtained.



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ABSTRACT

The intermolecular interaction between N-confused porphyrins-(3-methylisoxazole) diad (NCP-(3-methylisoxazole)) and bovine serum albumin (BSA) has been investigated through fluorescence and ultraviolet spectroscopy at different temperatures under imitated physiological conditions. The results showed that the fluorescence of BSA was quenched by NCP-(3-methylisoxazole) through a combined quenching procedure. The characteristics of NCP-(3-methylisoxazole)···BSA interaction (including interaction nature, interaction conformation, binding constants, binding sites, binding distance, thermodynamic parameters, etc.) and the effect of metal ions (Cu^{2+}, Mg^{2+}, Ca^{2+}, and Ni^{2+}) upon the NCP-(3-methylisoxazole)···BSA interaction have been detailed studied.

Introduction

Serum albumin is one of very important types of protein in God's image. Its main function is to transport various metabolites and drugs such as anesthetics, anticoagulants and sedatives in the circulatory system. Therefore, it is apparent that deeply understand the interactions between albumin and drugs may have very significant implications for design novel high-performance drugs, and indeed, such investigations have become a topic research in both current chemistry and life sciences. Bovine serum albumin (BSA) is an ideal albumin model to study the albumin···drugs interactions because BSA and human serum albumin (HSA) display approximately 76% in sequence homology, and most importantly, BSA has an inherent fluorescing property attributed to the presence of aromatic amino acids [1–4].

N-confused porphyrin (NCP) is a representative isomer of porphyrin, which exhibits general characteristics of porphyrin and

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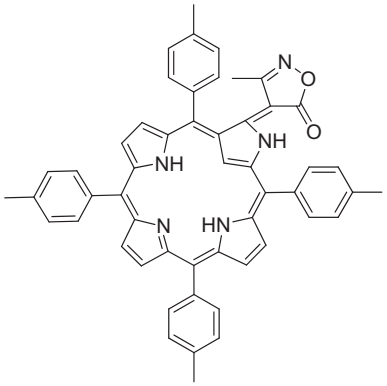

Scheme 1. Molecular structure of NCP-(3-methylisoxazolele).

some novel properties resulted form the inverse pyrrole ring in the NCP structure. Similar to porphyrin, NCP holds 18 π-electron systems and demonstrates strong aromatic. Furthermore, it may be better absorbed in the range of UV and visible light waves [5–8]. On the other hand, isoxazole is widely used in the synthesis of antiviral or anticancer drugs. Currently, as one composite of two drugs, the NCP-(3-methylisoxazole) and its derivates have been attracted great attentions owing to the potential pharmacology and important scientific significance in organic chemistry [9,10].

In the present work, we report a spectroscopic investigation on the intermolecular interaction between NCP-(3-methylisoxazole) (Scheme 1) and BSA. The purpose of this effort is to clarify the following aspects: (1) deeply understand the intermolecular interaction in the title system, including fluorescence quenching mechanism of BSA by NCP-(3-methylisoxazole), interaction nature, effect of NCP-(3-methylisoxazole) on the conformation of BSA, binding constants, binding sites, binding distance, thermodynamic parameters, and so on; (2) probe into the effect of some metal ions (Cu^{2+}, Mg^{2+}, Ca^{2+}, Ni^{2+}) on the interaction of NCP-(3-methylisoxazole)···BSA. We believe that this work will help to enrich the knowledge on these intermolecular interactions, spur further study in this area, and assist to provide useful information of the structural features that determine the therapeutic effectiveness of drugs and design of dosage forms

Experimental section

Apparatus

Fluorescence spectra were recorded on a Shimadzu RF-5301 fluorescence spectrophotometer (Tokyo, Japan) with a SB-11 water bath (Eyela) and 1.0 cm quartz cells. The emission and excitation slits were 5 and 10 nm, respectively. Fluorescence quenching spectra were measured in the range of 280–500 nm with the excitation wavelength of 280 nm at three temperatures (298, 304, and 310 K). The synchronous fluorescence spectra were obtained by setting the excitation and emission wavelength interval ($\Delta\lambda$) at 15 and 60 nm. The absorption spectra were obtained from a Shimadzu UV-2501 spectrophotometer (Tokyo, Japan). The pH measurement was made with a Leici pHS-2 digital pH-meter (Shanghai, China) with a combinational glass calomel electrode.

Reagents

BSA (⩾99%) was obtained from Huamei Bioengineering Co. (Shanghai, China) and was dissolved in a Tris–HCl (0.05 mol L^{-1}, pH = 7.4) buffer to form the BSA solution with a concentration of 1.0×10^{-5} mol L^{-1}. A Tris–HCl buffer (0.05 mol L^{-1}, pH = 7.4) containing 0.1 mol L^{-1} NaCl was selected to keep the pH value constant and to maintain the ionic strength of the solution. NCP-(3-methylisoxazole) was prepared according to the reported literature [11] and its stock solution (5.0×10^{-4} mol L^{-1}) was prepared in DMF. All other reagents were of analytical reagent grade and double–distilled water was used during the experiments.

Procedures

A 2.5 mL solution containing 1.0×10^{-5} mol L^{-1} BSA was titrated by successive additions of 5.0×10^{-4} mol L^{-1} NCP-(3-methylisoxazole) solution and the concentration of NCP-(3-methylisoxazole) varied from 0 to 1.8×10^{-5} mol L^{-1}. Titrations were done manually by using micro-injector. Fluorescence spectra were measured in the range of 280–500 nm at the excitation wavelength of 280 nm. The fluorescence spectra were performed at three temperatures (298, 304, and 310 K).

Under the same conditions, a 2.5 mL solution containing 1.0×10^{-5} mol L^{-1} BSA was titrated by DMF, and the volume of DMF varied from 0 to 90 uL.

The UV–vis absorption spectra of NCP-(3-methylisoxazole) solution with the concentration of 1.0×10^{-5} mol L^{-1} was measured in the range of 200–500 nm at 310 K.

The fluorescence spectra of BSA were also recorded in the presence of some metal ions, which contain Cu^{2+}, Mg^{2+}, Ca^{2+}, Ni^{2+} at 310 K in the range of 280–500 nm at excitation wavelength of 280 nm. In the system, the overall concentration of BSA was fixed at 1.00×10^{-5} mol L^{-1}, and the common metal ion was maintained at 4.00×10^{-5} mol L^{-1}.

Results and discussion

Fluorescence quenching of BSA by NCP-(3-methylisoxazole)

BSA fluoresces naturally at 340 nm after excitation at 280 nm, and this fluorescence is due to a tryptophan residue. When small molecules interact with BSA, this fluorescence can be quenched [12]. The fluorescence spectra of BSA in the presence of different concentrations of NCP-(3-methylisoxazole) and BSA with alone DMF were respectively shown in Fig. 1A and 1B. As depicted in Fig. 1, the influence of DMF to BSA fluorescence effect is tiny, but along with the gradual addition of NCP-(3-methylisoxazole), the fluorescence intensity of BSA remarkably decrease and a slight blue shift (from 342 to 338 nm) of the maximum emission wavelength is observed, suggesting strong intermolecular interaction between BSA and NCP-(3-methylisoxazole).

The fluorescence quenching in the NCP-(3-methylisoxazole)···BSA system can be described by the Stern–Volmer equation:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + K_q\tau_0 \quad (1)$$

where F_0 and F denotes the fluorescence intensities of BSA in the absence and in the presence of quencher, respectively; K_{SV} is Stern–Volmer quenching constant; [Q] represents the concentration of the quencher; K_q is the bimolecular quenching rate constant; τ_0 is the average lifetime of the molecule without the quencher and its value is 10^{-8} s. From Eq. (1), K_{SV} can be determine by linear regression of a plot of F_0/F against [Q] [13]. A linear Stern–Volmer plot is generally indicative of a single class of fluorophores in a protein, all equally accessible to the quencher; this also means that only one mechanism (dynamic or static) of quenching occurs. Actually, there have been several studies reporting BSA quenching due to specific interactions [14,15]. In these cases, the fluorophore can be quenched by both mechanisms with the same quencher, while the Stern–Volmer plot exhibits an upward curvature for the distinct situation, concave toward the y axis at high [Q]. Accordingly, the