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Characterization of the interaction between Fe(III)-2,9,16, 23-tetracarboxyphthalocyanine and blood proteins

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Abstract

The features of FeTCPc (Fe(III)-2,9,16,23-tetracarboxyphthalocyanine) binding to bovine serum albumin and bovine hemoglobin were investigated by fluorescence and UV/vis absorption spectroscopy. FeTCPc has the ability to quench the intrinsic fluorescence of both bovine serum albumin and bovine hemoglobin through mainly static quenching. The binding site number n, apparent binding constant K_A and the corresponding thermodynamic parameters ΔG^0, ΔH^0, ΔS^0 at different temperatures were calculated; both electrostatic and hydrophobic interactions play a major role in stabilizing the complex. The distance r between the donor (bovine serum albumin or bovine hemoglobin) and acceptor (FeTCPc) was obtained according to fluorescence resonance energy transfer. The effect of FeTCPc on the conformation of the two donors was analyzed using synchronous fluorescence spectroscopy.

Keywords: Fe(III)-2,9,16,23-tetracarboxyphthalocyanine; Bovine serum albumin; Bovine hemoglobin; Fluorescence quenching; Thermodynamic parameter; Spectroscopy

1. Introduction

Phthalocyanines have been one of the most extensively studied classes of organic functional materials because of their aromatic 18-π electron system [1]. They are important blue and green dyes, recently they have been used as photoconducting agents in photocopying machines, as sensing elements in chemical sensors [2,3], electrochromic display devices, and photodynamic reagents for cancer therapy and for other medical applications. Microbicides, which serve to prevent the initial entry of the virus into the cell, offer the possibility of protection against transmission of the virus [4,5]. There have been a number of previous studies on phthalocyanines and metallophthalocyanines as potential microbicides to kill the human immunodeficiency virus [6], the vesicular stomatitis virus [7], bovine viral diarrhea virus [8], pseudorabies virus [8], and herpes simplex virus [9]. All of these studies involve photoactivation of the phthalocyanine to produce species (singlet oxygen or free radical) that kill the virus. Conjugation of sensitizer with biological targeting agents, e.g., monoclonal antibodies or lipoproteins, increases the concentration of sensitizer on targeted tissue over normal tissue, providing additional level of selectivity and possibility to reduce effective sensitizer concentration, and thus phototoxicity to normal tissue [10,11]. To have a full understanding of the modes of drug actions, their interaction with all possible biological targets, including nucleic acids, enzymes, and other proteins, is required.

Serum albumins are the major soluble protein constituents of the circulatory system and have many physiological functions [12]. The most important property of this group of

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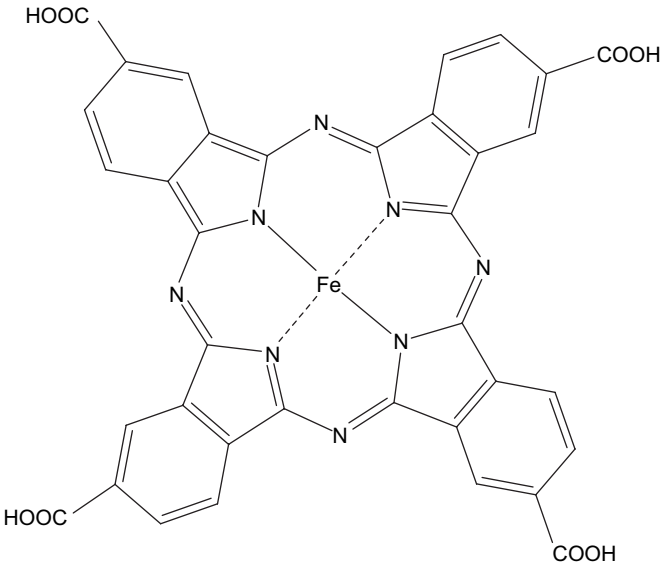
proteins is that they serve as a depot protein and as a transport protein for many drugs and other small bioactive molecules. The molecular interactions of flavonoids [13], isoquinoline alkaloids [14], insecticide [15] and dye molecules [16,17] with albumin have been investigated successfully. Another abundant blood protein, hemoglobin, accounts for 97% of dry erythrocyte weight. Hemoglobin is well known for its function in the vascular system of animals, transporting oxygen from the lungs or gills to peripheral tissues. It also aids, both directly and indirectly, the transport of carbon dioxide and regulates the pH of blood [18]. Several reports were published on the interactions of Pt drugs [19], artemisinins [20], and heteropolyacid compounds [21] with hemoglobin. In the present work, we demonstrated the affinity of Fe(III)-2,9,16,23-tetracarboxyphthalocyanine (FeTCPc) (Scheme 1) to bovine serum albumin (BSA) and bovine hemoglobin (BHb). In order to attain these objectives, we planned to carry out detailed investigation of FeTCPc–BSA and FeTCPc–BHb associations using fluorescence spectroscopy and UV/vis absorption spectroscopy. Through fluorescence resonance energy transfer and synchronous fluorescence spectroscopy, we planned to further investigate the effect of the energy transfer and the effect of FeTCPc on the conformation of BSA and BHb.

2. Materials and methods

2.1. Materials

BSA and BHb were purchased from Sigma (St. Louis, MO, USA) and used without further purification. Dr. L. Wu donated FeTCPc which was synthesized and purified according to the procedures described previously [22]. The buffer Tris was purchased from Acros (Geel, Belgium), and NaCl, HCl, etc. were all of analytical purity. BSA and BHb solutions (3.0×10^{-6} mol L^{-1}) were prepared in pH 7.40 Tris–HCl buffer solution (0.05 mol L^{-1} Tris, 0.1 mol L^{-1} NaCl). The FeTCPc solution (3.75×10^{-4} mol L^{-1}) was prepared in pH 7.40 Tris–HCl buffer containing 50% DMSO (v/v) because of its low solubility.



Scheme 1. Molecular structure of Fe(III)-2,9,16,23-tetracarboxyphthalocyanine (FeTCPc).

2.2. Equipments and spectral measurements

The UV/vis spectrum was recorded at 291 K on a GBC UV/Vis916 spectrophotometer (Australia) equipped with 1.0 cm quartz cells. All fluorescence spectra were recorded on LS-50B Spectrofluorimeter (Perkin–Elmer, USA) equipped with 1.0 cm quartz cells and a thermostat bath.

The widths of both the excitation slit and the emission slit were set to 10.0 nm/2.5 nm for BSA and 10.0 nm/5.0 nm for BHb.

2.3. Procedures

A 2.5 mL solution, containing appropriate concentration of BSA or BHb, was titrated by successive additions of a 3.75×10^{-4} mol L^{-1} stock solution of FeTCPc (to give a final concentration of 1.20×10^{-5} mol L^{-1}). Titrations were done manually by using trace syringes. The fluorescence spectra were then measured (excitation at 280 nm and emission wavelengths of 290–500 nm) at two temperatures (291 K, 305 K). The UV/vis absorbance spectra of FeTCPc with concentration of 3.0×10^{-6} mol L^{-1} were recorded at 291 K.

3. Results and discussion

3.1. UV/vis absorption studies

UV/vis absorption measurement is a very simple method and applicable to explore the structural change and to know the complex formation [23]. As shown in Fig. 1, FeTCPc gives a characteristic Q band absorption of non-aggregated

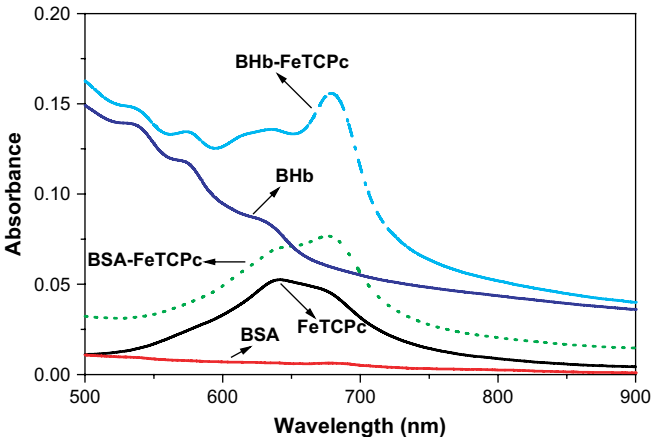


Fig. 1. Absorption spectra of BSA, BHb, FeTCPc, BSA–FeTCPc, and BHb–FeTCPc systems ($T = 291$ K, pH = 7.4). BSA or BHb concentration was 3.0×10^{-6} mol L^{-1}. FeTCPc concentration for BSA–FeTCPc or BHb–FeTCPc system was 3.0×10^{-6} mol L^{-1}. A concentration of 3.0×10^{-6} mol L^{-1} was used for FeTCPc only.