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Comparison and investigation of bovine hemoglobin binding to dihydroartemisinin and 9-hydroxy-dihydroartemisinin: Spectroscopic characterization

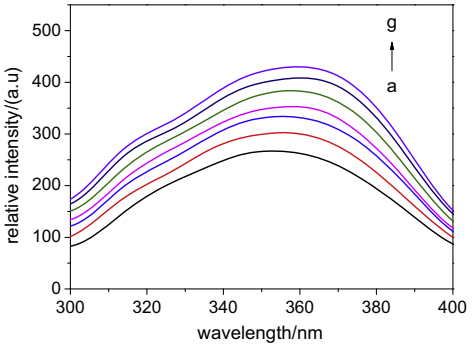
Mengsi Xiao [a,b], Lina Han [a,b], Lin Zhou [a,b], Yanhuai Zhou [c], Xiaoqin Huang [c], Xuefeng Ge [a,b], Shaohua Wei [a,b], Jiahong Zhou [a,b,*], Heming Wu [d,*], Jian Shen [a,b]

[a] Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, College of Chemistry and Materials Science, Analysis and Testing Center, Nanjing Normal University, Nanjing 210023, China
[b] Jiangsu Key Laboratory of Biomedical Materials, College of Chemistry and Materials Science, Analysis and Testing Center, Key Laboratory of Applied Photochemistry, Nanjing Normal University, Nanjing 210023, China
[c] Department of Physical Science and Technology, Nanjing Normal University, Nanjing 210023, China
[d] Institute of Stomatology, Department of Oral and Maxillofacial Surgery, Nanjing Medical University, No. 136, Hanzhong Avenue, Nanjing, China

HIGHLIGHTS

- DHA and 9-OH DHA both induce the heme group of BHb.
- DHA and 9-OH DHA both can change the polarity of BHb.
- DHA and 9-OH DHA both make BHb unfolding.
- The interaction between BHb and DHA is stronger than 9-OH DHA.

GRAPHICAL ABSTRACT



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ABSTRACT

The UV–vis absorption, steady state/time resolved fluorescence spectroscopy and synchronous fluorescence, circular dichroism (CD) spectroscopy are used to investigate the interaction mechanisms of dihydroartemisinin (DHA) and 9-hydroxy-dihydroartemisinin (9-OH DHA), respectively. The UV–vis studies present that DHA and 9-OH DHA can disturb the structure of bovine hemoglobin (BHb). Steady state/time resolved and synchronous fluorescence spectroscopy reveal that the binding constant of DHA with BHb is bigger than 9-OH DHA. CD spectra indicate DHA and 9-OH DHA can change the conformation of BHb. The comparison results suggest that the binding of BHb with DHA is more stable and stronger than 9-OH DHA.

Introduction

Artemisinin, the active component extracted from *Artemisia annua* L., is a remarkable and novel anti-malarial drug with little toxicity [1]. It contains an endoperoxide bridge that reacts with

* Corresponding authors. Address: Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, College of Chemistry and Materials Science, Analysis and Testing Center, Nanjing Normal University, Nanjing 210023, China (J. Zhou). Tel./fax: +86 025 85895170.
E-mail addresses: zhoujiahong@njnu.edu.cn (J. Zhou), whmz2002@yahoo.cn (H. Wu).

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ferrous iron to generate free radicals, such as reactive oxygen species (ROSs), which leads to macromolecular damage and cell death. Dihydroartemisinin (DHA, Fig. 1a), a more water-soluble active metabolite of artemisinin, is the most remarkable anti-malarial drug and has little toxicity to humans [2]. 9-OH DHA (Fig. 1b), a derivative of DHA, was synthesized by China Medicine University.

BHb is well known for its function in the vascular system of animals, it is a carrier of oxygen. It also helps the transport of carbon dioxide and regulates the pH of blood directly or indirectly [3]. It removes hydrogen ions in the capillaries and then to the lungs. In addition, it is involved in many clinical diseases such as leukemia, anemia, heart disease and excessive loss of blood [4].

So far, too much works have been done to learn the properties of DHA, as a new synthetic DHA derivative, the reports on the interaction of 9-OH DHA with BHb are limited.

In this study, in order to know the pharmacology of 9-OH DHA and its further use in clinic, multiple spectroscopy methods have been used to compare 9-OH DHA and DHA, including UV–vis, steady state/time resolved fluorescence spectroscopy and synchronous fluorescence, CD spectroscopy.

Experiments

Chemicals

DHA and 9-OH DHA were obtained from China Medicine University, bovine hemoglobin (from bovine blood, *Mr* = 64,500) was purchased from Sigma, and used without further purification. Other chemicals were all analytical grade.

The solution of BHb was prepared in phosphate buffers (1 μM, pH 7.0). DHA and 9-OH DHA were dissolved in deionized water ($c_{DHA} = 10^{-2}$ mol L^{-1}, $c_{9\text{-OH DHA}} = 10^{-2}$ mol L^{-1}), and stored at 0–4 °C.

Apparatus and methods

UV–vis absorption measurements

UV–vis absorption spectra were recorded on the Varian Cary 5000 spectrophotometer at 298 ± 1 K in a 1.0 cm quartz cuvette. The scanning spectrograms were in the range from 200 to 600 nm. DHA and 9-OH DHA solution of 0.33 μM was respectively added into BHb with 10.0 μL each time, the concentration of DHA and 9-OH DHA range from 0.33 to 2.0 μM.

Fluorescence spectroscopy measurements

The fluorescence spectra measurements have used Perkin–Elmer LS-50B spectrofluorimeter. The measurements were recorded in the wavelength range of 295–550 nm. As for synchronous fluorescence, the measurement wavelength ranges from 250 to 400 nm. The time resolved studies are recorded on the Fluoromax 4.

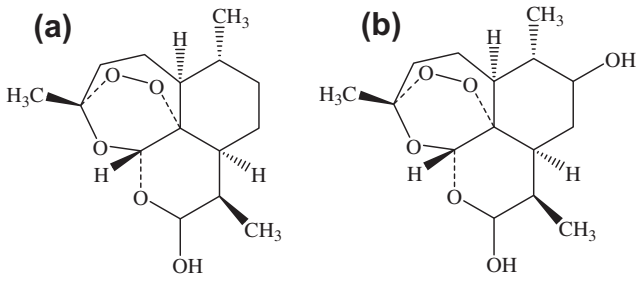

Fig. 1. (a) Molecular structure of DHA and (b) molecular structure of 9-OH DHA.

Circular dichroism (CD) spectroscopy measurements

CD measurements were taken with an Applied Photophysics Chriascan circular dichroism spectrometer using a 1 cm quartz cell. The wavelength interval is 200–260 nm.

Results and discussion

UV–vis absorption studies

UV–vis absorption spectroscopy of protein could indicate the important structure information on protein; it is a simple and convenient way to study the interaction mechanism of the medicinal molecule with biomacromolecule [5]. BHb has three absorption signals, they are 274 nm (the phenyl group of tryptophan and tyrosine residues), 307 nm (ε-band) and 404 nm (heme or Soret band) [5]. Fig. 2 shows the UV–vis absorption spectra of BHb by adding DHA and 9-OH DHA to the solution. The absorbance peaks of BHb (at 274 nm and 306 nm) both increased when DHA and 9-OH DHA were added gradually, while the peak at 404 nm decreased, suggesting that DHA and 9-OH DHA both reacted with tryptophan, tyrosine residues and heme group, which changed the chemical environment surrounding BHb.

The effects of DHA and 9-OH DHA on BHb are compared by analyzed the absorbance peak at 404 nm in Fig. 3. The results suggesting that the interaction of BHb with DHA is slightly more acute than 9-OH DHA.

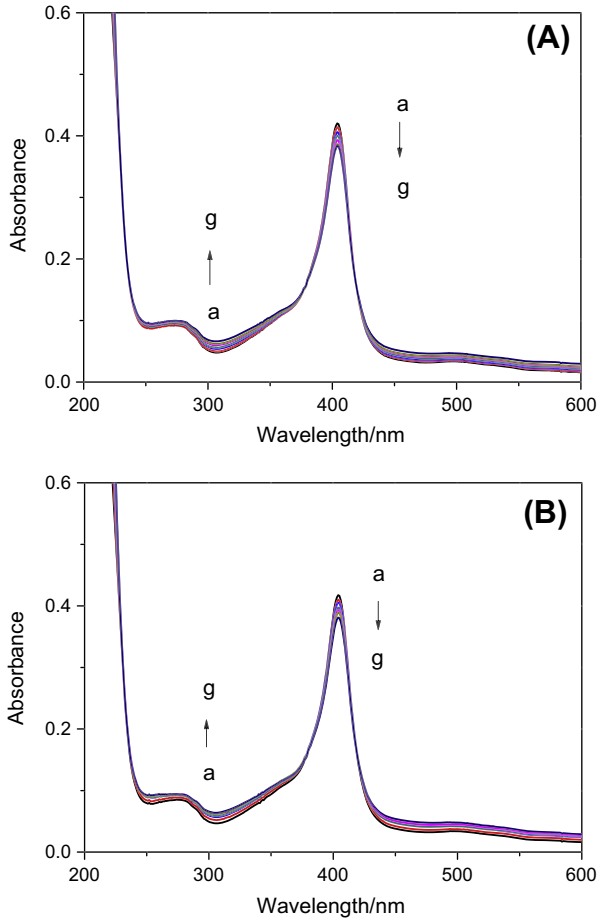

Fig. 2. UV–vis absorption spectra of pure BHb with DHA (A) and UV–vis absorption spectra of pure BHb with 9-OH DHA complex (B); [BHb] = 1.0 μM; [DHA]/(a–g) = 0, 1/3, 2/3, 1, 4/3, 5/3, 2 μM; [9-OH DHA]/(a–g) = 0, 1/3, 2/3, 1, 4/3, 5/3, 2 μM; pH = 7.0, *T* = 298 K.