Journal of Luminescence 160 (2015) 188–194

Contents lists available at ScienceDirect

Journal of Luminescence

journal homepage: www.elsevier.com/locate/jlumin

Comparison of 9-hydroxy-artemisinin with artemisinin: interaction with bovine hemoglobin

Mengsi Xiao [a], Xiuxue Yuan [a], Wenli Xie [a], Xuefeng Ge [a], Yanhuai Zhou [b], Lin Zhou [a,**], Jiahong Zhou [a,*], Jian Shen [a]

[a] Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, 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
[b] Department of Physical Science and Technology, Nanjing Normal University, Nanjing 210023, China

ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form
29 November 2014
Accepted 2 December 2014
Available online 11 December 2014

Keywords:
Artemisinin
9-hydroxy-artemisinin
Bovine hemoglobin
UV–vis
Fluorescence spectroscopy
CD spectroscopy

ABSTRACT

In this article, the UV–vis absorption, steady state/time resolved fluorescence spectroscopy and synchronous fluorescence, circular dichrosim (CD) spectroscopy are used to investigate the interaction of artemisinin (QHS) and 9-hydroxy-artemisinin (9-OH QHS) with BHb, respectively. The UV–vis studies present that QHS and 9-OH QHS can disturb the structure of bovine hemoglobin (BHb). Fluorescence data presents that the binding constant of QHS and 9-OH QHS with BHb complex at 298 K is 4.32×10^5 and 5.98×10^5 M^{-1}. CD spectra indicate QHS and 9-OH QHS can change the conformation of BHb. The comparison results suggest that the binding of BHb with 9-OH QHS is more stable and stronger than QHS, which means the structure modification of 9-OH QHS is meaningful.

1. Introduction

Artemisinin (Fig. 1 A) is an active component extracted from the antimalarial herb Qinghao, it was isolated in 1972 by Chinese researchers for the first time. Because it is of high therapeutic values in treating malaria, chloroquine-resistant Plasmodium falciparum and the cerebral infections, tremendous efforts have been made toward structure modification and analogue synthesis. So far, most of the structural modifications took place at the lactone moiety of artemisinin, modifications and structure-activity relationship on the two saturated rings remain unexplored due mainly to the difficulty of introducing functionalities on the ring systems by conventional chemical methods [1]. Therefore, microbial transformation comes to play an important role to overcome the inaccessibility to chemical reactions and to serve as a valuable tool to introduce hydroxyl group on the saturated rings [1]. Then a derivative of artemisinin, 9-OH QHS (Fig. 1 B), was produced by the biotransformation of cultured Streptomyces griseus ATCC 13273 [1] Fig 2.

Hemoglobin is one of some ordinary proteins, consisting of two identical α-chains of 141 amino acids each, and two identical β-chains of 146 amino acids each [2]. Bovine hemoglobin (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, 4].

Meunier et al. [5–7] have recently proposed that reductive homolysis of the endoperoxide function, promoted by iron (II) heme, and subsequent formation of C4 centered alkylating radicals, are crucial events for the antimalarial activity of these drugs. Moreover, the same research group demonstrated that heme may be alkylated by artemisinin in meso position; an extensive characterization of the resulting heme-artemisinin adducts was carried out [5–7]. On the ground of their results, Meunier et al. [8] suggested that heme is both the trigger and the target for artemisinin antimalarials.

Thus far, much attention has been paid to the interactions between artemisinin with BHb [9–12], as a new synthetic artemisinin derivative, the reports on the interaction of 9-OH QHS with BHb have been rather limited. Therefore, the comparison of 9-OH QHS with QHS is helpful to know the pesticide effect of 9-OH QHS.

In this study, multiple spectroscopy methods have been used, including UV–vis, steady state/time resolved fluorescence spectroscopy and synchronous fluorescence, circular dichrosim

* Corresponding author. Tel./fax: +86 025 85895170.
** Corresponding author. Tel./fax: +86 025 83591761.
E-mail addresses: zhoulin@njnu.edu.cn (L. Zhou), zhoujiahong@njnu.edu.cn (J. Zhou).

http://dx.doi.org/10.1016/j.jlumin.2014.12.002
0022-2313/

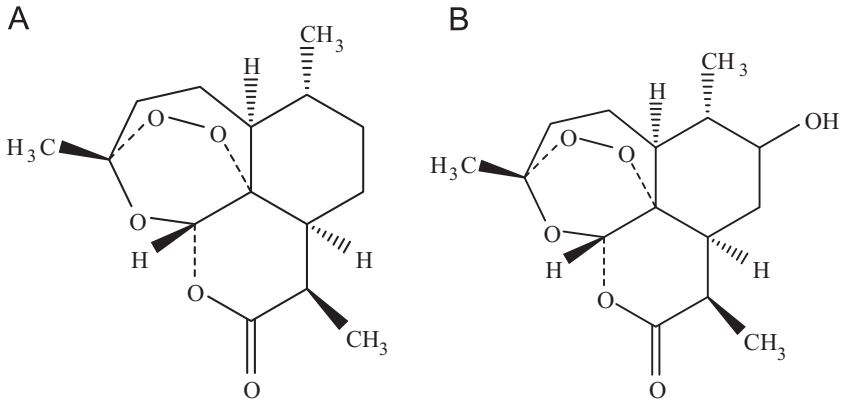

Fig. 1. (a) Molecular structure of QHS; (b) Molecular structure of 9-OH QHS.

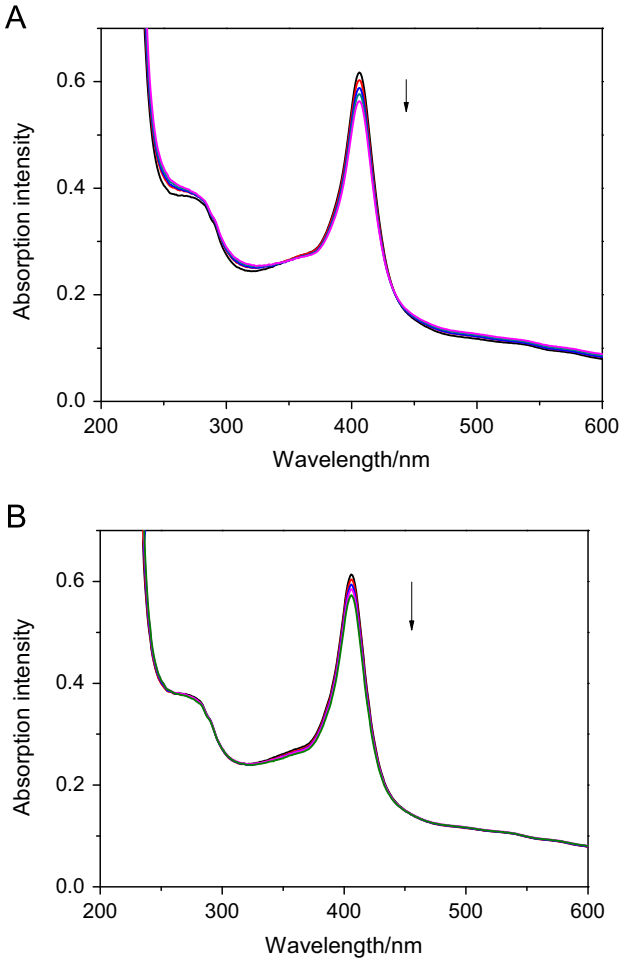

Fig. 2. (a) UV - vis absorption spectra of pure BHb and QHS complex; (b) UV - vis absorption spectra of pure BHb and 9-OH QHS complex. [BHb]= 1.5 μM, [QHS] / (a-g)=0, 1/4, 1/2, 4/3, 1 μM, [9-OH QHS]/ (a-g)=0, 1/4, 1/2, 4/3, 1 μM, pH =7.0, T=298 K.

(CD) spectroscopy. The conclusions could be of use in confirming the difference between 9-OH QHS and QHS.

2. Experiments

2.1. Chemicals

9-OH QHS was acquired from China Medicine University [1], bovine hemoglobin (BHb, Mr=64500) was purchased from Sigma, and used without further purification. Other chemicals were all analytical grade. The solution of BHb was prepared in phosphate buffers (1.5 μM, pH 7.0). 9- OH QHS was dissolved in deionized water ($c_{9\text{-OH QHS}} = 10^{-2}$ mol.L^{-1}), all chemicals were stored at 0–4 °C.

2.2. Instruments and methods

2.2.1. 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. 9-OH QHS was added into BHb successively with 10.0 μM each time, the concentration of 9-OH QHS ranges from 0.33 to 2.0 μM and its total accumulated volume was less than 100 μM.

2.2.2. Fluorescence spectroscopy measurements

The fluorescence spectra measurements have used Perkin–Elmer LS-50B spectrofluorimeter. In the steady-state fluorescence spectroscopy measurements, the band-widths of excitation and emission slits are all set at 13.5 nm. The measurements were recorded in the wavelength range of 295–550 nm at the scan speed of 600 nm / min, and the excitation wavelength was 283 nm. As for synchronous fluorescence, the measurement wavelength ranges from 250 to 400 nm, the band-widths of excitation and emission slits are also set at 13.5 nm, the Delta Lambda (Δλ) are set at 20 nm and 80 nm, respectively.

The time resolved studies are recorded on the Fluoromax 4. The Peak Rate is 5000 counts, S Detector HV is 950 V, and the Repetition Rate is 1 MHz.

2.2.3. CD spectroscopy measurements

CD measurements were taken with an Applied Photophysics Chriascan circular dichroism spectrometer using a 1 cm quartz cell.

3. Results and analysis

3.1. UV–vis absorption studies

UV–vis absorption spectroscopy is a simple and convenient way to study the interaction mechanism of the medicinal molecule with biomacromolecule, along with the red shift or blue shift, namely, hyperchromic effect or hypochromic effect, in the characteristic absorption band of the complex. Both drugs alone do not show any absorption. BHb has absorption signal at three different wavelengths, they are 274 nm (because of the phenyl group of tryptophan and tyrosine residues), 306 nm (ε-band) and 404 nm (heme or Soret band) dissolved in phosphate buffers [12]. The absorbance of BHb at 404 nm decreased, presenting the occurrence of interaction