Journal of Luminescence 142 (2013) 155–162

Contents lists available at SciVerse ScienceDirect

Journal of Luminescence

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

Interaction of Cefpiramide sodium with bovine hemoglobin and effect of the coexistent metal ion on the protein-drug association

Xiaona Yan, Baosheng Liu*, Baohong Chong, Shina Cao

Key Laboratory of Medical Chemistry and Molecular Diagnosis, Ministry of Education, College of Chemistry & Environmental Science, Hebei University, Baoding 071002, PR China

ARTICLE INFO

Article history:
Received 17 October 2012
Received in revised form
27 March 2013
Accepted 9 April 2013
Available online 19 April 2013

Keywords:
Bovine hemoglobin
Cefpiramide sodium
Metal ions
Interaction

ABSTRACT

The interaction between bovine hemoglobin (BHb) and cefpiramide sodium (CPMS) was investigated at different temperatures by fluorescence, UV absorption, and CD spectroscopy, as well as the effect of common metal ions (Mg^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Fe^{3+}, Ni^{2+}) on the BHb–CPMS system. Results showed that CPMS could quench the intrinsic fluorescence of BHb strongly, and the quenching mechanism was a static quenching process. The electrostatic force played an important role on the conjugation reaction between BHb and CPMS. The order of magnitude of binding constants (K_a) was 10^4, the number of binding site (n) in the binary system was approximately equal to 1 and the binding distance (r) was about 3.08 nm. Besides, the values of Hill's coefficients were approximately equal to 1, which indicated there was almost no cooperativity in CPMS's binding with BHb. Synchronous spectra and CD spectra revealed that the microenvironment and the conformation of BHb were changed during the binding reaction. Studies on the interaction between BHb and drug will facilitate interpretation of the drug's metabolism and transporting process in the blood, and will help to explain the relationship between structures and functions of BHb.

1. Introduction

Hemoglobin (Hb), the major protein component in erythrocytes, exists as a tetramer of globins chains that is composed of two α and two β subunits; Hb is well known for its function in the vascular system of animals, being a carrier of oxygen. It also aids, both directly and indirectly, the transport of carbon dioxide and regulates the pH of blood [1]. Bovine hemoglobin (BHb), which shares 90% amino acid sequence homology with human hemoglobin, has a few advantages over its human counterpart. BHb is a better oxygen carrier than human hemoglobin [2]. It is a tetrameric protein with 572 amino acid residues, consisting of two identical α-chains of 141 amino acids each and two identical β-chains of 145 amino acids each (as seen in Fig. 1). The crystal structure of BHb was taken from the Protein Data Bank (entry PDB code 1G09). In addition, the α1β2 subunit interface is considered to play a pivotal role in the quaternary structure transition [3]. Studying the binding mechanism is much important for life science, chemical, pharmaceutical and clinical medicine. Cefpiramide sodium (CPMS) is a semisynthetic third-generation cephalosporin whose structure, antibacterial activity, and stability to β-lactamases have been described and researched extensively (the structure shown in Fig. 2). A number of studies have documented its broad spectrum of antibacterial activity against aerobic, anaerobic gram-positive and gram-negative bacteria effectively. Compared with other cephalosporins, it is particularly active against *Pseudomonas aeruginosa* [4]. At present, the molecular interactions between protein and many ligands have been investigated successfully in biochemistry domain [5–8]. However, the interaction between CPMS and BHb has not been investigated, especially the effect of coexistent metal ion on the binding of drugs to BHb. In this report, we took BHb as the representative protein and provided investigations on the interaction of CPMS with BHb by using fluorescence quenching, UV absorption spectrometry, synchronous fluorescence and circular dichroism under physiological pH 7.4 as well as the effect of the coexistent metal ion. This study is expected to provide important insight into the essence, potential toxicity between drugs and protein in real terms, and can also provide a useful clinical reference for future combination therapy.

2. Experimental

2.1. Apparatus

All fluorescence spectra were recorded with a Hitachi F-4600 spectrofluorophotometer. Absorption were measured with an UV–vis recording spectrophotometer (UV-265 Shimadzu, Japan). CD spectra were recorded on a MOS-450/SFM300 circular dichroism spectrometer (Bio-Logic, France). All pH measurements were

* Corresponding author. Tel.: +86 312 5079385; fax: +86 312 5079525.
E-mail address: lbs@hbu.edu.cn (B. Liu).

0022-2313/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jlumin.2013.04.009

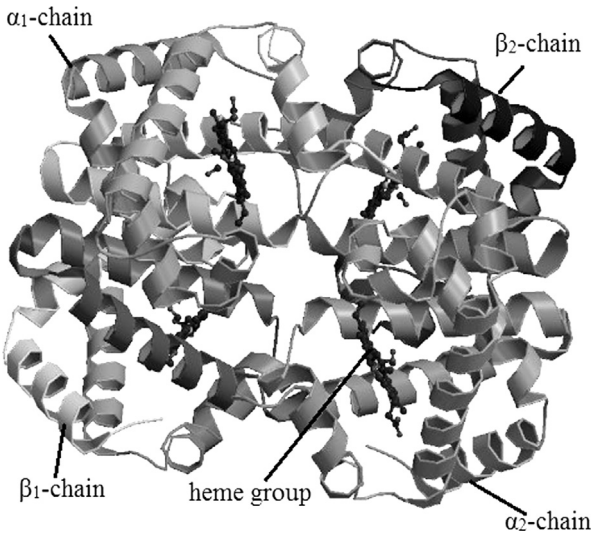

Fig. 1. Schematic drawing of the BHb molecule Globin chains are marked with α_1, α_2, β_1, and β_2 chain.

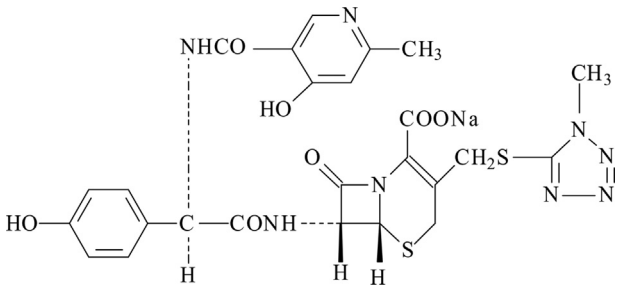

Fig. 2. Chemical structure of Cefpiramide Sodium.

made with a pHS-3C precision acidity meter (Leici, Shanghai). All temperatures were controlled by a CS501 super-heated water bath (Nantong Science Instrument Factory).

2.2. Materials

Cefpiramide Sodium (CAS#, 74849-93-7) was obtained from Monitor of Chinese Veterinary Medicine (the purity grade inferior 99%). Bovine Hemoglobin (CAS#, 9008-02-0) was purchased from Sigma Company (the purity grade inferior 99%). Stock solutions of BHb (2.0×10^{-4} M), CPMS (1.0×10^{-3} M) and metal ions (1.0×10^{-3} M) were prepared. All the stock solutions were further diluted as working solutions prior to use. Tris–HCl (0.05 M Tris) buffer solution [9] containing NaCl (0.15 M) was used to keep the pH of the solution at 7.4, NaCl solution was used to maintain the ionic strength of the solution. All other reagents were of analytical grade and all aqueous solutions were prepared with newly double-distilled water and stored at 277 K.

2.3. Procedures

2.3.1. Fluorescence measurements

In a typical fluorescence measurement [10], 1.0 mL Tris–HCl (pH=7.4), 1.0 mL BHb solution (4.0×10^{-5} M) and different volume of CPMS were added into 10 mL colorimetric tube successively. The samples were diluted to scaled volume with water, mixed thoroughly by shaking, and kept static for 25 min at different temperatures (293, 303 and 313 K). Excitation wavelength for BHb was 280 nm (or 295 nm), with a 10 mm pathlength cell. The excitation and the emission slits were set at 5 nm each. The solution was subsequently scanned on the fluorophotometer and determined the fluorescent intensity at 336 nm. Otherwise, we recorded the fluorescence spectra of BHb–CPMS system when the $\Delta\lambda$ value between the excitation and emission wavelengths was constant at 15 and 60 nm [11], respectively. The synchronous fluorescence spectra were obtained at 293 K; when $\Delta\lambda$ was set at 15 or 60 nm, the excitation wavelength was 265 nm [12] or 240 nm [13].

2.3.2. UV–vis absorption measurements

1.0 mL Tris–HCl (pH=7.4), 1.0 mL BHb solution (4.0×10^{-5} M) and different volume of CPMS were added into 10 mL colorimetric tube successively. The reference was different concentrations of CPMS (from 0 to 8.0×10^{-5} M) solution. The samples were diluted to scaled volume with water, mixed thoroughly by shaking, and kept static for 25 min at 293 K. The UV–vis absorption spectra of BHb in the presence and absence of CPMS were recorded with 10 mm quartz cells in the range from 190 nm to 500 nm.

2.3.3. Circular dichroism measurements

On the one hand, 1.0 mL Tris–HCl (pH=7.4), 0.5 mL BHb solution (4.0×10^{-5} M) and different volume of CPMS were added into 10 mL colorimetric tube successively. On the other hand, 1.0 mL Tris–HCl (pH=7.4), 0.5 mL BHb solution (4.0×10^{-5} M), 0.1 mL metal ions solution (1.0×10^{-4} M) and different volume of CPMS were added into 10 mL colorimetric tube successively. The samples were diluted to scaled volume with water, mixed thoroughly by shaking, and kept static for 25 min at 293 K. Circular dichroism (CD) measurements were carried out with a 1.0 mm path length quartz cuvette. Each spectrum was recorded at wavelengths between 200 and 300 nm and a scan speed of 1 nm sec^{-1}. The α-helix content were obtained by software DicroProt-2000.

3. Results and discussion

3.1. UV–vis absorption spectra studies

UV–vis absorption measurement is a very simple and applicable method to explore the structural and micro-environmental changes of proteins [14]. The UV–vis absorption spectra of BHb in the absence and presence of CPMS are shown in Fig. 3. It can be seen that BHb has three absorption peaks. The strong absorption peak at 215 nm, the weak absorption peak at 275 nm and the peak at 405 nm corresponds to the porphyrin-Soret band of BHb [15]. With gradual addition of CPMS to BHb solution, the intensity of

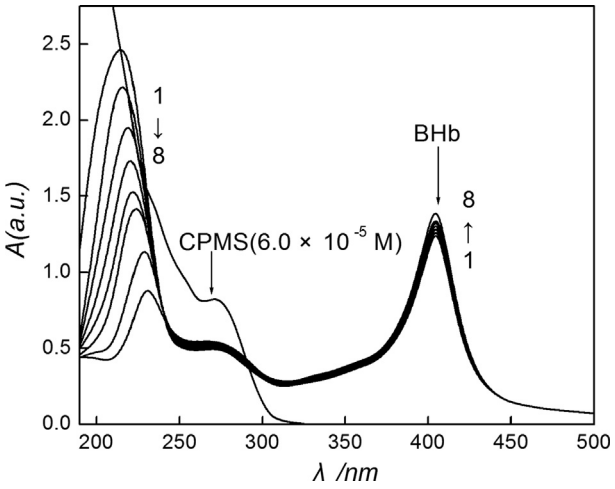

Fig. 3. UV absorption spectra of BHb-CPMS system (T=293 K). $C_{BHb}=4.0 \times 10^{-6}$ M, 1–8 $C_{CPMS}=(0, 0.8, 1.6, 2.4, 3.2, 4.0, 6.0, 8.0) \times 10^{-5}$ M.