Journal of Luminescence 140 (2013) 87–94

Contents lists available at SciVerse ScienceDirect

Journal of Luminescence

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

Studies on the interactions of chloroquine diphosphate and phenelzine sulfate drugs with human serum albumin and human hemoglobin proteins by spectroscopic techniques

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ARTICLE INFO

Article history:
Received 5 December 2012
Received in revised form
7 February 2013
Accepted 5 March 2013
Available online 16 March 2013

Keywords:
Human serum albumin
Hemoglobin
Interaction
Chloroquine diphosphate
Phenelzine sulfate
Fluorescence spectroscopy

ABSTRACT

The interactions of chloroquine diphosphate (CQP) and phenelzine sulfate (PS) drugs with human serum albumin (HSA) and human hemoglobin (HMG) proteins were investigated by various spectroscopic methods. It was found that CQP caused the fluorescence quenching of protein molecules through a static quenching mechanism, but PS did not. The values of Stern–Volmer quenching constant, bimolecular quenching constant, binding constant and number of binding site on the protein molecules were calculated for HSA–CQP and HMG–CQP systems at pH 7.4 and different temperatures. For CQP, there was only one binding site on HSA and HMG proteins and the binding affinity of HSA was higher than that of HMG. The binding constants decreased with increasing temperature. The values of negative enthalpy change and positive entropy change indicated that electrostatic interactions play an important role in the binding processes. In addition, the binding processes were spontaneous and carried out by exothermic reactions. According to Förster resonance energy transfer theory, the average binding distance between proteins and CQP was calculated as 3.72 nm for HSA–CQP system and 3.45 nm for HMG–CQP system. Circular dichroism analysis displayed that the addition of CQP led to a decrease in the α-helix amount of HSA and HMG proteins.

1. Introduction

The interactions between proteins and drugs affect the pharmacological behaviors, side effects, distributions and eliminations of drugs [1,2]. In addition, the binding of various drugs to a protein may cause some conformational changes in the secondary structure of protein. When a conformational change occurs in the protein structure, some alterations may be observed in the normal functions of protein. The therapeutic effect of a drug is directly related with the free drug concentration in blood [3]. Only unbound drug can diffuse from blood to tissue or organ where the pharmacological activity and/or the side effect occur [1]. Strong binding between protein and drug molecules decreases the concentration of free drug in blood and also causes a decrease in the pharmocodynamic effect of drug. On the other hand, weak binding leads to a short lifetime or poor distribution [4,5]. Therefore, the knowledge of the nature and magnitude of protein–drug binding can help us in understanding the pharmacokinetics and pharmacodynamics of a drug [3].

Several methods including the use of ion selective electrodes [6,7], Fourier transform infrared (FTIR) spectroscopy [8], nuclear magnetic resonance (NMR) spectroscopy [9], UV–visible spectroscopy, fluorescence spectroscopy [10], circular dichroism (CD) spectroscopy [11,12], affinity chromatography [4], high performance liquid chromatography (HPLC) [1] and equilibrium dialysis [13] have been used for studying the binding of various small ions or molecules to proteins. Among them, fluorescence and CD spectroscopic techniques are used widely to clarify the protein–drug interactions owing to their sensitivity, selectivity, convenience and short analysis time [14,15].

Generally, human serum albumin (HSA) and human hemoglobin (HMG) proteins are of high affinity for drug molecules. A lot of drugs are bound to these proteins reversibly. HSA is a single-chain consisting of 585 amino acids with one tryptophan [16]. It is the most abundant protein constituent of blood plasma and synthesized by the liver [17]. This protein is composed of three domains (I, II and III) and each of them consists of two subdomains (A and B) [18]. HSA has many physiological functions. It contributes 80% to colloid osmotic blood pressure and is responsible for the maintenance of blood pH. This protein is the principal carrier of many compounds. Additionally, HSA performs other functions such as sequestering oxygen free radicals and inactivating various toxic lipophilic metabolites. The crystal structure of albumin reveals a heart-shaped molecule that contains an equilateral triangle with sides of ~8 nm and a depth of 3 nm [19].

HMG, a major component in erythrocytes, consists of two identical α-chains and two identical β-chains. Each of α-chain

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0022-2313/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jlumin.2013.03.015

has 141 amino acids and each of β-chain is of 146 amino acids [20]. HMG is roughly spherical molecule with $6.4 \times 5.5 \times 5.0$ nm [21]. It carries oxygen from lung to peripheral tissues, provides electron transfer in all organs and parts of the body and adjusts the pH of blood. Moreover, HMG plays an important role in the transport of H^+ and CO_2 from tissues to lung [22,23].

Chloroquine diphosphate (CQP) and phenelzine sulfate (PS) are two important drugs. CQP is used commonly in the treatment of various rheumatic diseases including rheumatoid arthritis [24] and the treatment and prevention of malaria [25–27] due to its low cost and effectiveness [28]. This drug is also used against acute chikungunya infections and some viral infections such as influenza. It has been proposed as a HIV-1 therapeutic agent [29]. On the other hand, CQP has various side effects. One of the important side effects of CQP is the retinopathy [24]. As an antidepressant drug, PS is used extensively in the treatment of some psychiatric disorders such as panic disorder, social anxiety disorder and depression [30–32]. In addition, it has a number of important neurochemical actions [33]. Although the various pharmacological properties of CQP and PS drugs have been determined, the interactions of these drugs with HSA and HMG proteins have not been investigated comprehensively.

The aim of the present study is to determine the binding mechanisms, binding constants, the numbers of binding sites per protein molecule, thermodynamic parameters and binding distances for the interactions of CQP and PS drugs with HSA and HMG proteins at 288.15, 298.15, 310.15 and 318.15 K and to investigate the effect of drug molecules on the structural changes of proteins.

2. Materials and methods

2.1. Materials

Human serum albumin (HSA) and human hemoglobin (HMG) proteins, chloroquine diphosphate (CQP) and phenelzine sulfate (PS) drugs, tris(hydroxymethyl)aminomethane buffer used to maintain the pH value of solutions at physiological pH and NaCl used as an ionic strength adjuster were purchased from Sigma. The chemical structures of drugs are shown in Fig. 1. The average molecular weight values of 66500 and 64500 g/mol were used in the preparation of HSA and HMG solutions, respectively. Prior to each experiment, all solutions were prepared freshly in tris(hydroxymethyl)aminomethane buffer solution (0.05 M, pH 7.4) containing 0.1 M NaCl. Deionized water was used in the preparation of buffer solution.

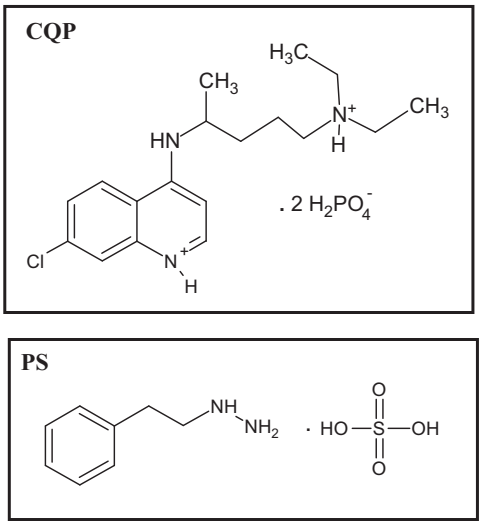

Fig. 1. Chemical structures of chloroquine diphosphate (CQP) and phenelzine sulfate (PS).

2.2. Methods

Firstly, the stock solutions of HSA and HMG proteins (5.0×10^{-5} M) and CQP and PS drugs (1.0×10^{-4} M) were prepared with buffer solution and kept in a refrigerator at 4–6 °C. Then, a series of protein–drug mixture solution containing various drug concentrations was prepared from the stock solutions of proteins and drugs to investigate the interactions between protein and drug molecules. In protein–drug interaction experiments, the concentrations of HSA and HMG proteins were fixed at 5.0×10^{-6} M and 2.5×10^{-6} M, respectively, and the concentrations of drugs were varied in the range of $0.0–30.0 \times 10^{-6}$ M for HSA–CQP system, $0.0–70.0 \times 10^{-6}$ M for HSA–PS system and $0.0–15.0 \times 10^{-6}$ M for HMG–CQP and HMG–PS systems.

The fluorescence spectra of protein–drug mixture solutions were recorded by a Varian-Carry Eclipse model fluorescence spectrophotometer using a quartz cell with 1.0 cm path length in a thermostatically controlled cell holder at 288.15, 298.15, 310.15 and 318.15 ± 0.10 K after an equilibration time of 15 min at each temperature. An excitation wavelength of 280 nm was used for HSA and HMG protein solutions [18,22]. The widths of excitation and emission slits were set to 5 nm in fluorescence measurements. Then, the observed fluorescence intensities were corrected to eliminate the inner filter effect of proteins and drugs. Therefore, the absorption measurements of solutions were carried out by a Varian-Cary 5000 model UV–vis–NIR spectrophotometer using a matched pair of quartz cells (path length: 1 cm) in a peltier thermostatted cell holder at 288.15, 298.15, 310.15 and 318.15 ± 0.10 K. Then, for each system, the absorbance values were determined at the excitation and emission wavelengths of the fluorescence measurements. The corrected fluorescence intensity (F_{cor}) values were obtained by the following equation [34]:

$$F_{cor} = F_{obs} 10^{(A_{exc} + A_{em})/2} \quad (1)$$

where F_{obs} is the observed fluorescence intensity and A_{exc} and A_{em} are the absorbances of the system at the excitation and emission wavelengths, respectively. In this study, the corrected fluorescence intensity values were used.

To investigate the effect of drugs on the secondary structure of proteins, a series protein–drug solution was prepared and the CD spectra of 5.0×10^{-6} M HSA and 2.5×10^{-6} M HMG solutions containing various drug concentrations were obtained on a Jasco-J-815 model spectropolarimeter using a quartz cell with 0.05 cm path length at 298.15 K. CD measurements were carried out in the range of 200–250 nm at 1 nm intervals and CD spectra were collected with the scan speed of 20 nm/min. Moreover, each CD spectrum was the average of 3 scans.

It should be indicated that the spectra of appropriate blanks corresponding to the buffer solution for pure protein and pure drug solutions and the drug-buffer solutions for protein–drug mixture solutions were subtracted from the sample spectra in all fluorescence, absorption and CD measurements.

In addition, the pH values of solutions were checked with a Jenway 3040 ion analyzer using combined glass electrode, which was calibrated with standard buffer solutions before use.

3. Results and discussion

3.1. Fluorescence spectra and quenching mechanism

The fluorescence spectra of HSA and HMG proteins in the absence and presence of CQP at different concentrations are displayed in Fig. 2A and B. These figures indicate that HSA and HMG exhibit a fluorescence emission peak at 335 nm and 331 nm, respectively, when they were excited at 280 nm. These results are