Journal of Molecular Liquids 211 (2015) 584–590

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

Studies on the interaction between neutral red and bovine hemoglobin by fluorescence spectroscopy and molecular modeling

Ruiqiang Wang [a,*], Zhigang Li [b], Lingling Yang [b], Ting Ren [b], Lijiao Zhang [b], Ruiyong Wang [b,*]

[a] First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China
[b] Department of Chemistry, Zhengzhou University, Zhengzhou 450001, China

ARTICLE INFO

Article history:
Received 22 April 2015
Received in revised form 19 July 2015
Accepted 25 July 2015
Available online xxxx

Keywords:
Fluorescence spectroscopy
Bovine hemoglobin
Neutral red
Interactions

ABSTRACT

The binding interaction of neutral red (NR) with bovine hemoglobin (BHb) was studied by fluorescence spectroscopy in combination with molecular modeling. NR quenched the intrinsic fluorescence of BHb via a static mechanism. According to relevant data, the binding constants were calculated at two different temperatures. The thermodynamic parameters obtained from the fluorescence data showed that the hydrophobic and electrostatic interactions played a major role in stabilizing the complex. Synchronous and three-dimensional fluorescence spectra of BHb were investigated in the presence of NR. The results showed that the environment of tryptophan and tyrosine residues was altered by the dye. The fluorescence experimental results were in agreement with the results obtained by molecular modeling study.

1. Introduction

Hemoglobin (Hb, Mr = 64,500 Da), an important functional protein for reversible oxygen carrying and storage in the vascular system of animals, consists of two identical α-chains of 141 amino acids each, and two identical β-chains of 146 amino acids each [1]. Hb emits intrinsic fluorescence mainly due to Trp and Tyr residues [2]. The fluorescence intensity and the location of fluorescence peak reflect the microenvironment of the chromophore group of protein [3]. Except for albumin, as a kind of intracellular protein, Hb can also function as binders of drugs [4]. What's more, the concentration of Hb is 330 mg/mL [5], bigger than the serum albumin 40 mg/mL [6]. Hb can reversibly bind with many kinds of small bioactive molecules, such as alkaloids [7], analogs of biphenyldicarboxylate [8], Troxerutin [9], tannic acid [10], FNC [11], artemisinin [12], dihydromyricetin [13], herbicide [14] and surfactant [15], and so on.

It is known that organic dyes are widely used as a working medium in dye lasers [16], in analytical chemistry to determine the various trace elements [17]. Moreover, dyes are applied to textile, cosmetic, printing and food processing industries extensively [18]. If the wastewater enters the environment, the dye-containing wastewaters will pose a great threat to the environment, that affects the health of living beings and fertility of the soil. Therefore, investigation of the dye–protein complexes is of vital importance. There have been a number of previous studies on the interaction between dyes and proteins, such as phenosafranin [19], tricarbocyanine dyes [20]. The interactions of hemoglobin with some dyes have been reported, such as C.I. acid red 27 [21], the food additive amaranth [22], Toluidine blue [23], tartrazine [24], Sudan dyes [25] and gentian violet [26]. Neutral red (NR, Fig. 1) is a kind of mixed anthracycline-based cationic dye, which not only used for cytosol dyeing and cell identification, but also can be used as pH indicator, adsorption indicator, redox indicator and biological fluorescent probe. The interaction of neutral red with bovine serum albumin has been reported [27,28]. However, the interaction mechanism between neutral red and Hb has not been reported. Furthermore, NR is an interacting mode spectroscopic probe [29]. NR gives an emission maximum at 650 nm (λ_{ex} = 540 nm), while NR had no intrinsic fluorescence from 310 nm to 500 nm at the excitation wavelength of 290 nm.

The present paper deals with the mechanism of binding of NR with bovine hemoglobin (BHb) at simulated physiological conditions by fluorescence, UV/vis absorption and molecular modeling. The energy transfer between NR and BHb and the characteristics of resonance light-scattering spectra (RLS) are also reported. Moreover, the conformational changes of BHb occurring in the presence of NR have been analyzed by using synchronous and three-dimensional fluorescence techniques. This paper helps understand the hemoglobin's binding mechanisms to dyes and provides clues to the biological effects and functions of dyes in body.

2. Materials and methods

2.1. Materials

BHb (Sigma) was dissolved in ultra pure water to form 1.0 × 10^{-4} mol · L^{-1} solution, then preserved at 4 °C and diluted as required.

* Corresponding authors.
E-mail addresses: wangruiqiang@zzu.edu.cn (R. Wang), wangry@zzu.edu.cn (R. Wang).

http://dx.doi.org/10.1016/j.molliq.2015.07.066
0167-7322/

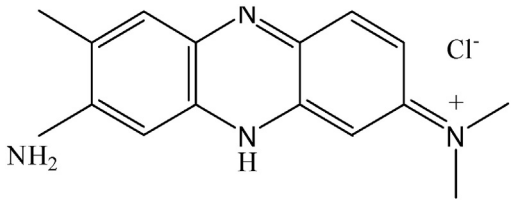

Fig. 1. The molecule structure of NR.

NR (Three Elsevier, Shanghai, China) was dissolved in ultra pure water to form 1.0×10^{-3} mol · L^{-1} solution, then diluted as required. The buffer tris (Shanpu, Shanghai, China), NaCl and HCl were all of analytical purity.

2.2. Equipment and spectral measurements

The UV/vis spectra were recorded on an UV-1800 spectrophotometer (Mapada, Shanghai, China) equipped with 1.0 cm quartz cells. Fluorescence quenching and synchronous fluorescence spectra were recorded on a 970-CRT spectrofluorimeter (San Ke, Shanghai, China) equipped with 1.0 cm quartz cells. The widths of excitation and emission slits were set to 5.0 nm/10.0 nm respectively. RLS and three-dimensional fluorescence measurements were performed on an F-4500 fluorescence spectrophotometer (Hitachi, Japan) equipped with 1.0 cm quartz cell, using 10.0 nm/20.0 nm slit widths.

2.3. Procedures

The fluorescence measurements were carried out as follows: to each of a series of 10 mL test-tube, 2.0 mL Tris–HCl buffer (pH 7.4), 2.0 mL of 0.5 mol · L^{-1} NaCl and 1.0 mL of 2.0×10^{-5} mol · L^{-1} BHb were added, followed by 1.0 mL of different concentrations NR. The fluorescence spectra were then measured (excitation at 290 nm and emission wavelengths range 310–500 nm) at two temperatures (307 K, 322 K). The synchronous fluorescence spectra were obtained through simultaneous scanning of the excitation and emission monochromators while maintaining a constant wavelength interval between them (Δλ, 15 nm and 60 nm). The three-dimensional fluorescence spectrum was performed under the following conditions: the emission wavelengths at 280–470 nm, the excitation at 200 nm with an increment of 10 nm, excitation and emission slit widths were 10 nm/20 nm respectively, and the scan speed was 1200 nm · min^{-1}, PMT (Photo Multiplier Tube) voltage was 700 V. The UV/vis absorbance spectra of BHb with different concentrations NR were recorded at room temperature. RLS were obtained by synchronous scanning with the wavelength range of 200–800 nm on the spectrofluorophotometer at room temperature.

2.4. Molecular modeling study

The AutoDock4.2 [30] program was used to calculate the interaction modes between NR and BHb. Lamarckian genetic algorithm (LGA) implemented in AutoDock was applied to calculate the possible conformation of NR that binds to the protein. The structure of NR was downloaded on website (http://zinc.docking.org/). The crystal structure of BHb was taken from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) (PDB ID: 1G09). Water molecules were removed, and hydrogen atoms were added. A grid map of $126 \times 106 \times 126$ grid points in size with a grid-points pacing of 0.553 Å was created for the protein. The scoring functions of the empirical free energies for the docked configurations have been tested for all docking models. According to the binding energy and the geometry matching after 250 runs, the most favorable docking model was selected for further analysis.

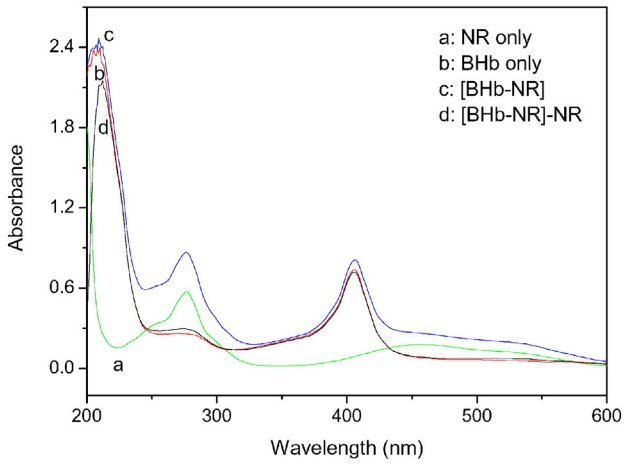

Fig. 2. Absorption spectra of NR bound to BHb at pH 7.4. (a) Absorption spectra of NR only, c(NR) = 16.0 μM; (b) absorption spectra of BHb only, c(BHb) = 2.0 μM; (c) absorption spectra of BHb–NR; (d) absorption spectra of [BHb–NR]–NR.

3. Results and discussion

3.1. UV/vis absorption spectra studies

UV/vis absorption measurement is a simple but efficacious method to explore the structural changes of protein and investigate protein–ligand complex formation [31]. Hence, absorption spectra of NR and NR-BHb system were recorded. Fig. 2 showed the absorption spectral changes of BHb in the presence of NR in the wavelength 200–600 nm. It can be seen that BHb has three absorption peaks. The strong absorption peak at 210 nm not only reflects the framework conformation of protein but also corresponds to the peptide bond [32]. The weak absorption peak at 278 nm appears due to the aromatic amino acids (Trp, Tyr and Phe) [33]. The peak at 405 nm corresponds to the porphyrin Soret band of BHb [34]. Spectra b and d should be identical in Fig. 2 if no interaction occurred between BHb and NR. The UV/vis absorption spectrum of BHb shows a strong band in the near-UV region with a maximum at 210 nm, which appears due to peptide bond absorption of tryptophan. By comparing the spectra b with d, it can be found that the absorbance at 210 nm of BHb decreases. This result indicates that the binding interactions occur between BHb and NR, which may cause the slight change of the conformation of protein [35].

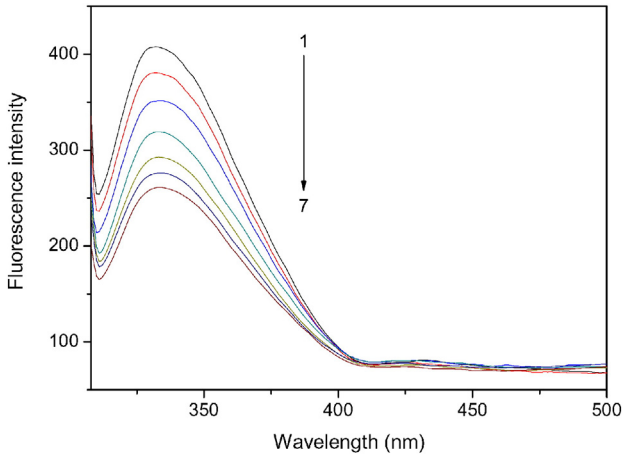

Fig. 3. Effect of NR on fluorescence spectra of BHb (T = 307 K, pH = 7.40 and λ_{ex} = 290 nm). Curves (1–7): c(BHb) = 2.0 μM, c(NR): 0, 4.0, 8.0, 12.0, 16.0, 20.0, 24.0 μM, respectively.