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Study of the interaction between bovine hemoglobin and analogs of biphenyldicarboxylate by spectrofluorimetry

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ABSTRACT

The interaction between bovine hemoglobin and analogs of Biphenyldicarboxylate was investigated by fluorescence, synchronous fluorescence, ultraviolet–vis absorbance, resonance light-scattering spectra and three-dimensional fluorescence spectra at pH 7.40. The quenching mechanism and binding constants were determined by the quenching of bovine hemoglobin fluorescence in presence of analogs. Results showed that the nature of the quenching was of static type. Both the van der Waals and hydrogen bonding played a major role in stabilizing the complex. The distance between donor and acceptors was obtained to be 2.11–2.25 nm according to Förster's theory. The influence of analogs on the conformation of bovine hemoglobin was investigated.

1. Introduction

DDB (dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dicarboxybiphenyl-2,2′-dicarboxylate, Biphenyldicarboxylate) which was discovered in the process of studying the anti-hepatotoxic components from Sehizandra chinensis, has been well known as an effective agent for liver protect. It can decrease hepatic damage and hepatocytes necrosis induced by some chemieal substance such as carbon tetrachloride, D-galaetosamin or thioacetamide, lower the level of syrum glutamic-pyrumic transaminase and glutamic-oxalacetic transaminase. In addition, it can relieve the symptom of intokicant heptitis caused by chemotherapic agents [1]

Since the overall distribution, metabolism and efficacy of many drugs are correlated with their affinities towards hemoglobin [2,3], the investigation of drugs with respect to BHb-drug binding is imperative and of fundamental importance. Hemoglobin, the major protein component in erythrocytes, exists as a tetramer of globins chains that is composed of two α and two β subunits. Hemoglobin 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 [4]. 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. BHb has a less exothermic oxygen binding and delivers oxygen even at low temperatures [5]. In addition, it is involved in many clinical diseases such as leukemia, anemia, heart disease, excessive loss of blood, etc. [6]. Reports were published on the interactions of BHb with resveratrol [7], caffeine [8], semiconductor zinc oxide nanoparticles [9], C.I.Solvent Red 24 [10], didodecyldimethylammonium bromide [11], and colloidal TiO_2 [12]. Since BHb is an important functional protein for reversible oxygen carrying and storage, as well as a mode protein with high a-helical content, the potential changes of conformation and function for BHb after binding of small molecules have been a focus of study.

In this paper, for the first time the interaction between BHb and DDB or analogs were investigated by fluorescence, UV–vis absorption, resonance light scattering spectra, synchronous fluorescence and three-dimensional fluorescence spectra techniques under physiological pH 7.40. Based on Stern–Volmer equation, the quenching constants were calculated. According to thermodynamic equations, ΔH, ΔG and ΔS and the acting forces between analogs of DDB and BHb were discussed. According to Förster's theory, the distance r between donor and acceptors were obtained.

2. Materials and methods

2.1. Materials

BHb from bovine blood (Sigma) was directly dissolved in 0.1 mol L^{-1} Tris–HCl buffer solution (pH 7.40) with 0.08 mol/L NaCl. The stock solution (9.5×10^{-5} mol L^{-1} BHb) was kept in the dark at 0–4 °C. 1.0×10^{-3} mol L^{-1} DDB and analogs of DDB (as shown in

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Fig. 1) solutions were obtained by dissolving them in absolute ethyl alcohol, which were synthetized in our lab [13]. Double distilled water was used throughout and all other chemicals were of analytical grade.

2.2. Apparatus

The fluorescence was tested on 970 CRT Spectrofluorophotometer (Shanghai). The UV–vis absorption spectra were obtained using a UV/V-16/18 vis spectrophotomete (Shanghai). The temperature was regulated by using D2KW–4 thermostat water-bath (Beijing), and the pH value was checked through a pH-3 digital pH meter (Shanghai). The 3D fluorescence spectra and resonance light-scattering spectra were obtained by F-4500 Spectrofluorophotometer (Japan). Unless otherwise mentioned, all chemicals were of analytical reagent grade and were used without further purification. All the experiments were carried out at room temperature.

2.3. Procedures

Under the optimum conditions, Tris–HCl buffer solution, NaCl solution, appropriate amounts of BHb and DDB or analogs were added to 10.0 mL colorimetric tube and diluted to 10.0 mL with double distilled water. The fluorescence spectra were measured (excitation at 288 nm and emission wavelengths of 308–400 nm) at three temperatures (295 K, 305 K, 315 K), respectively. Synchronous fluorescence spectra of BHb in the presence of different concentration of DDB or analogs were recorded with the D-value ($\Delta\lambda$) between excitation wavelength and emission wavelength stabilized at 20 nm or 60 nm. The three-dimensional fluorescence spectrum was performed under the following conditions: the emission wavelengths at 290–470 nm, the excitation at 200–300 nm, with other parameters just the same as those of the fluorescence quenching spectra. The UV/vis absorbance spectra of DDB or analogs and BHb were obtained at room temperature. Resonance light-scattering spectra were measured by synchronous scanning with the wavelength range of 200–800 nm on the spectrofluorophotometer.

3. Results and discussion

3.1. Fluorescence quenching of BHb by DDB or analogs

BHb is a multi-tryptophan protein, which contains two α-14 Trp, two β-15 Trp, and two β-37 Trp. The β-37 Trp residues are the most dominant fluorophores and play an important role in the quaternary state change upon ligand binding [14]. These tryptophans can be used as intrinsic fluorophores to study the binding of

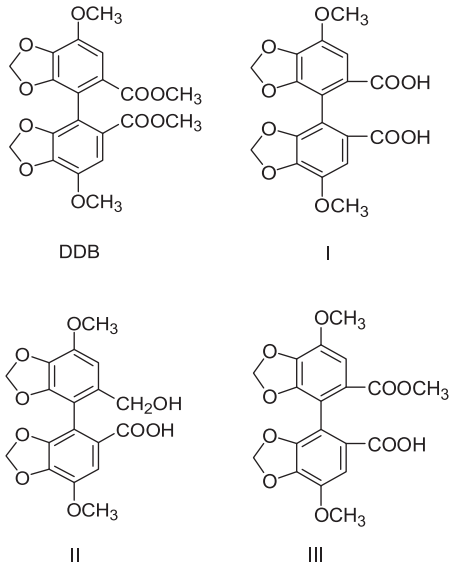

Fig. 1. The molecular structure of DDB and analogs of DDB (I, II and III).

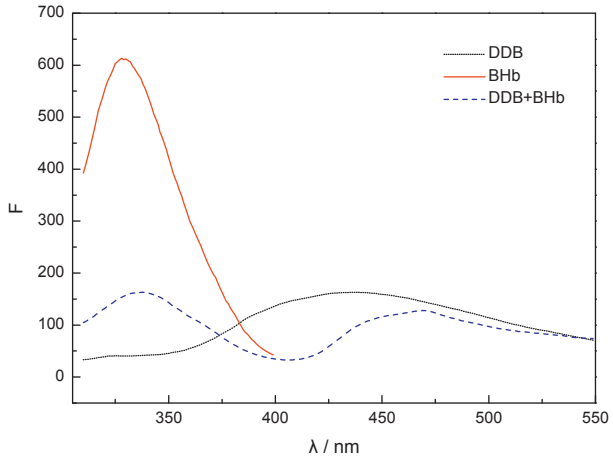

Fig. 2. The fluorescence emission spectra of DDB, DDB–BHb and BHb. Tris–HCl 2.50 ml pH=7.40, NaCl 0.08 mol L^{-1}, BHb 9.50×10^{-6} mol L^{-1} and DDB 1.0×10^{-4} mol L^{-1}.

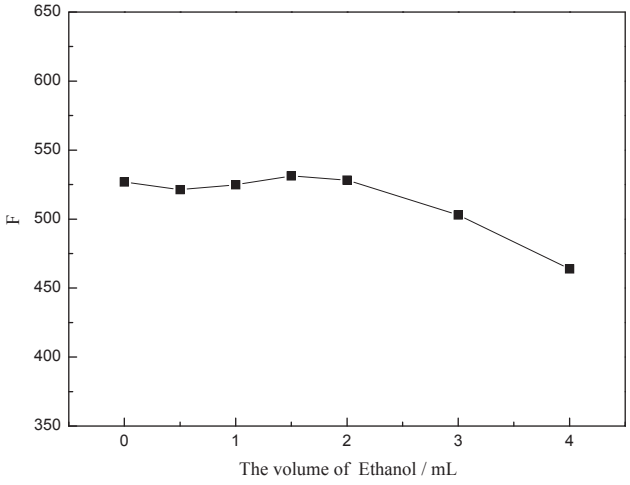

Fig. 3. The effect of ethyl alcohol amount on the fluorescence of BHb. Tris–HCl 2.50 mL pH=7.40; NaCl 0.08 mol L^{-1} and BHb 9.50×10^{-6} mol L^{-1}.

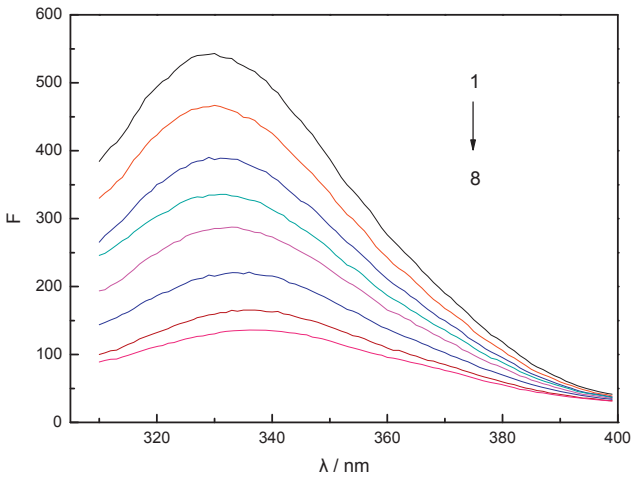

Fig. 4. The fluorescence spectra of BHb with different concentration of DDB. Tris–HCl 2.50 ml pH=7.40; NaCl 0.08 mol L^{-1}; BHb 9.50×10^{-6} mol L^{-1}; DDB 0, 1.0×10^{-5}, 2.0×10^{-5}, 3.0×10^{-5}, 4.0×10^{-5}, 6.0×10^{-5}, 8.0×10^{-5}, 1.0×10^{-4} mol L^{-1} (From top to bottom).