Journal of Luminescence 137 (2013) 186–190

Contents lists available at SciVerse ScienceDirect

Journal of Luminescence

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

Spectroscopic study on interaction between bovine hemoglobin and salmon DNA and the analytical applications

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ARTICLE INFO

Article history:
Received 29 September 2012
Received in revised form
11 December 2012
Accepted 27 December 2012
Available online 7 January 2013

Keywords:
Bovine hemoglobin
Salmon DNA
Absorption spectrum
Fluorescence spectrum
Circular dichroism spectrum
Resonance Rayleigh scattering

ABSTRACT

In weak acidic medium, bovine hemoglobin would bind with salmon DNA (sDNA) to form a complex. This resulted in changes of absorption and circular dichroism spectra, fluorescence quenching of hemoglobin and remarkable enhancement of resonance Rayleigh scattering (RRS), as well as the appearance of a new RRS spectrum. The spectral characteristics of these spectra were investigated. The type of fluorescence quenching was discussed via the fluorescence lifetime of hemoglobin before and after the reaction as well as effects of temperature on fluorescence intensity. The conformational change of bovine hemoglobin was explored through circular dichroism spectra. The reasons of RRS enhancement were also discussed. In addition, it was found that the fluorescence quenching and RRS methods using bovine hemoglobin as a probe could be used to the determination of DNA. The detection limits (3σ) for sDNA were 5.5 ng mL^{-1} (RRS method) and 202.3 ng mL^{-1} (fluorescence quenching method). The optimum reaction conditions of the two methods were tested. The selectivity of RRS method was examined owing to its higher sensitivity. The RRS method was used for the determination of DNA in synthetic samples with satisfactory results.

1. Introduction

Bovine hemoglobin (BHb), a water soluble protein, has a molecular weight of approximately 67,000 and contains two α and two β subunits, each of which has one redox iron heme as its prosthetic group, the heme is located in crevices at the exterior of the subunit [1]. It is the oxygen carrier in red blood cells. It also makes contribution to the transport of carbon dioxide and the maintenance of blood pH [2]. Interactions between BHb and small molecules, including medicines (e.g., 4-aminoantipyrine [3], oxytetracycline [4], lomefloxacin hydrochloride [5] and paraquat [6]), metal ions (e.g., Ni^{2+} [7], Ti (IV) [8], Ag(I) [9]), inorganic substances (e.g., NO_2^- [10], NO [11], O_2 [12] and CO [13]) and organic substances (e.g., dietary polyphenols [14], surfactants [15], glycerin [16] and dextran sulfate [17]), have been researched, providing much useful information on the chemical and biological properties of BHb. Studies of the interaction between BHb and biomacromolecules are of significance for elucidating the biological function of BHb. At present, its interactions with peptides [18] and other proteins [19,20] have been reported, while its interaction with DNA has seldom researched.

The reported methods for studying the interactions between proteins and biomacromolecules mainly include mass spectrometry (MS) [21], nuclear magnetic resonance (NMR) spectroscopy [22] and X-ray diffraction methods [23]. They have made remarkable contributions to elucidating the nature of protein–nucleic acid interactions. However, these methods require expensive instruments that are complicated to operate. For example, it is very difficult to prepare suitable crystals for X-ray diffraction. Therefore, when developing these large, sophisticated, instrumental analytical methods, it is also very significant to develop low-cost, simple and rapid spectroscopic methods to provide new information that enriches the research area of biological macromolecular interactions. In 1993, Pasternack firstly used resonance Rayleigh scattering (RRS) technique to research the aggregation of chromophore on nucleic acids, showing the high selectivity and sensitivity of resonance light scattering method [24]. After that, the RRS method has been widely applied to researching interactions between DNA and dyes [25,26], drugs [27–29], nanoparticles [30,31], and some biomacromolecules [32,33]. Similarly, the UV/vis absorption, fluorescence quenching, time resolved fluorescence and circular dichroism spectra are important methods to study the interaction of proteins with other substances because they are sensitive and relatively easy to use. They can provide some important structural and dynamic information.

In this work, we observed that interaction between BHb and sDNA resulted in great change of absorption and circular

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http://dx.doi.org/10.1016/j.jlumin.2012.12.041

dichroism (CD) spectra, fluorescence quenching of BHb and obvious enhancement of resonance Rayleigh scattering (RRS). Their spectroscopic characteristics were researched. The quenching effect of sDNA on BHb fluorescence is a dynamic quenching event based on the increased quenching constant (k_{SV}) values for increased temperatures and especially the change of fluorescence lifetime observed before and after the BHb–sDNA interaction. All Trp residues were influenced by the interaction because the fluorescence could be totally quenched by excessive sDNA. The secondary structure composition of BHb was calculated through CD spectra. The RRS enhancement attributed to an increased molecular volume, an enhanced hydrophobicity, a partial liquid–solid interface, a resonance energy transfer effect and a conformational change. The RRS and fluorescence quenching (FQ) method for the quantification of sDNA were highly sensitive, and the detection limits (3σ) were 5.5 ng mL^{-1} (RRS method) and 202.3 ng mL^{-1} (FQ method).

2. Experimental

2.1. Apparatus and reagents

An RF-5301PC spectrofluorophotometer (Shimadzu, Japan) was used to acquire the fluorescence and RRS spectra, and a FL-TCSPC spectrofluorophotometer (Horiba Jobin Yvon Inc., France) was employed to measure the fluorescence lifetime of insulin. A J-810 circular dichroism (CD) spectrometer (JASCO, Japan) was used to acquire CD spectra, and the secondary structure composition of BHb was calculated using Jasco Secondary Structure Estimation software. A UV-2450 spectrophotometer (Shimadzu, Japan) was employed to acquire absorption spectra. A PHSJ-3 F pH meter (Jingke, Shanghai) was used to adjust the pH.

Salmon DNA was obtained from Sigma. A solution of stDNA was prepared by weighing a certain amount of sDNA in a 100-mL beaker and by adding approximately 50 mL of a 0.5% NaCl solution. At least 24 h (with occasional, gentle shaking) was required for the dissolution of sDNA at 4 °C. The concentration of the sDNA was calculated based on its absorbance at 260 nm after establishing that the absorbance ratio A_{260}/A_{280} was 1.80–1.90. Molarities were calculated (when necessary) using the following expression: $\varepsilon_{260\ nm}$= 6600 L mol^{-1} cm^{-1}. A typical DNA concentration of 20.0 μg mL^{-1} was used for experiments. Bovine hemoglobin (Sigma) solution was prepared by dissolving its commercial products in doubly distilled water. Its working solution was 100 μg mL^{-1} and be kept at 4 °C.

Britton–Robinson buffer is a pH buffer used for the range pH 2–pH 12. It consists of a mixture of 0.04 mol L^{-1} H_3BO_3, 0.04 mol L^{-1} H_3PO_4 and 0.04 mol L^{-1} CH_3COOH that has been titrated to the desired pH with 0.2 mol L^{-1} NaOH. Its pH values were judged using pH meter.

All other reagents were of analytical reagent grade and used without further purification. Doubly distilled water was used throughout.

2.2. General procedure

Appropriate amounts of BHb, 1 mL of BR buffer solution and suitable amounts of sDNA were added to a 10-mL volumetric flask, diluted with water to the mark and then mixed thoroughly by gentle shaking. After incubation for 5 min, absorption, CD, fluorescence and RRS spectra of the solution were acquired. The enhanced RRS intensity (ΔI_{RRS}) of the system is represented by $\Delta I_{RRS}=I_{RRS}-I^0_{RRS}$, where I_{RRS} and I^0_{RRS} are the RRS intensities of the complex and the reagent blank, respectively. The quenched fluorescence intensity (ΔF) is represented by $\Delta F=F-F_0$.

3. Results and discussion

3.1. Absorption spectrum

Fig. 1 shows the absorption spectra of BHb, sDNA and BHb–sDNA complex. It shows that sDNA has a characteristic peak at 260 nm. BHb has two absorption peaks at 266 nm and 395 nm. The former mainly contributes to $\pi\rightarrow\pi*$ transition of indole ring of Trp residues. The latter was just the characteristic absorption of the porphyrin–soret band [34]. After the interaction, the shape of absorption spectrum of BHb changes and the absorbance between 238 nm and 500 nm increased. In protein molecules, the change of microenvironment affected by protein conformation will cause the change of absorption. Thus, after the interaction with sDNA, the BHb conformational change results in the change of microenvironment leading to the increase of absorbance.

3.2. Fluorescence quenching of BHb by sDNA

BHb contains three Trp residues in each αβ dimmer, for a total of six in the tetramer: two α-14 Trp, two β-15 Trp, and β-37 [35]. The intrinsic fluorescence of BHb primarily originates from β-37 Trp that plays a key role in the quaternary state change upon ligand binding [36]. In pH 2–4 media, the maximum excitation (λ_{ex}) peak is at 274 nm, and there is a weaker λ_{ex} at 230 nm. The maximum emission peak (λ_{em}) is at 346 nm. The sDNA is not fluorescent, but it can quench the fluorescence of BHb (Fig. 2) without changing peak shape. The quenched fluorescence intensity (ΔF) is proportional to the concentration of sDNA which providing a way for quantification of DNA.

The influence of the temperature on the fluorescence was investigated. Based on the Stern–Volmer graph of F_0/F versus [sDNA], the quenching constant (k_{SV}) increases with increasing temperatures (Fig. 3). In addition, the fluorescence lifetime of BHb before and after the interaction was measured (Fig. 4). BHb has three fluorescence lifetimes of 0.8158, 3.497 and 7.752 ns, and these three lifetimes altered when BHb binds to sDNA (Table 1). The total fluorescence lifetime of BHb before and after the interaction was 3.787 and 1.518 ns. The above two results showed that the quenching effect of sDNA on BHb fluorescence is a single static quenching event. All Trp residues were influenced by the interaction because the fluorescence could be totally quenched by excessive sDNA.

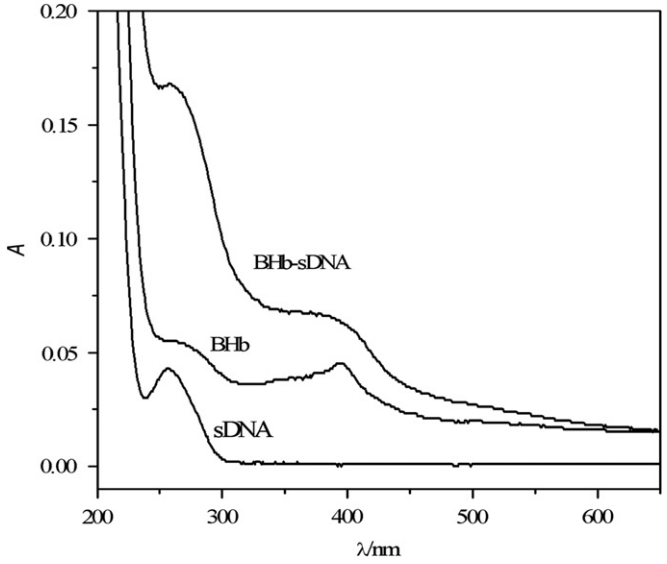

Fig. 1. Absorption spectra BHb: 20.0 μg/mL; sDNA :2.0 μg/mL; pH 3.5.