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Studies on the binding behavior of prodigiosin with bovine hemoglobin by multi-spectroscopic techniques

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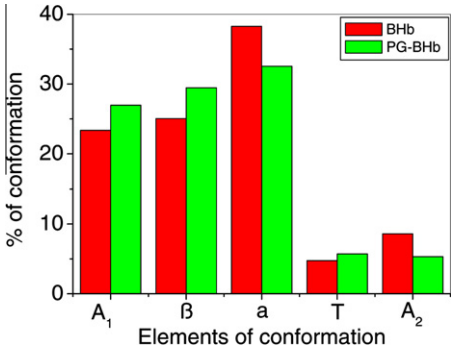
HIGHLIGHTS

- PG can bind to BHb to form a stable complex with one binding site.
- The interaction follows the static quenching mechanism.
- The hydrophobic interactions play a major role in the binding reaction.
- The microenvironment and conformation for BHb is changed in the presence of PG.
- Some α-helix structures have been converted into the β-sheets after conjugation.

GRAPHICAL ABSTRACT

Bar diagram of the different conformations of BHb before and after conjugation with PG.



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ABSTRACT

In this article, the interaction mechanism of prodigiosin (PG) with bovine hemoglobin (BHb) is studied in detail using various spectroscopic technologies. UV–vis absorption and fluorescence spectra demonstrate the interaction process. The Stern–Volmer plot and the time-resolved fluorescence study suggest the quenching mechanism of fluorescence of BHb by PG is a static quenching procedure, and the hydrophobic interactions play a major role in binding of PG to BHb. Furthermore, synchronous fluorescence studies, Fourier transform infrared (FTIR) and circular dichroism (CD) spectra reveal that the conformation of BHb is changed after conjugation with PG.

Introduction

Prodigiosin (PG, Fig. 1) is classical secondary metabolites appearing in later growth stages of *Serratia* [1], *Vibrio* and *Streptomyces* [2,3]. It is the parent member of a family of red pigments with methoxyrrole ring, and possesses promising antimicrobial [4], immunosuppressive [5], and anticancer activities [6]. Such as, National Cancer Institute (NCI) has also determined PG can strongly resist cancer cell [7]. However, PG has not obvious toxicity to nonmalignant cell-lines [8]. The therapeutic potential of PG has stimulated research into their mechanism of action, and the understanding of interaction between PG and proteins is the key issue for the biomedical use of PG.

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Proteins in the blood and plasma are the major biomolecules in lives and the major target of many kinds of medicines [9], viruses [10] and artificial drugs [11]. In general, hemoglobin (Hb) can be considered from all points of view an ideal model for investigating the properties of proteins. It is an important respiratory protein in red cells, being indispensable for oxygen transport, H_2O_2 dispersion and electron transfer to all organs and parts of the body [12]. Hb exists as a tetramer of globin chains, composed of two α and two β-subunits [13], each of which has one redox iron heme group. In addition, Hb contains four oxygen-binding sites per monomer and can bind with many kinds of endogenous and exogenous agents [14]. Hb has long been used as the paradigm for understanding the structure–function relationships of proteins [15–17], and many drugs used for therapeutic purposes may cross the membrane barriers and interact with it [18]. So, study on binding behavior of PG with BHb is of critical importance for understanding transportation, distribution and bioavailability of PG *in vivo*. This can directly help in the advancement of molecular diagnostics, therapeutics, molecular biology, and so on [19–22].

Recently, various methods have been used to characterize the conformational changes of proteins [23–26]. In this work, the interaction between PG and BHb is investigated by UV–vis absorption spectra, steady-state and time-resolved fluorescence spectra, CD spectra and FTIR spectra. The experimental results indicate that the fluorescence decay of BHb induced by PG follows the static quenching mechanism, and the hydrophobic interactions play a major role in binding of PG to BHb. In addition, the secondary structure of BHb is changed in the presence of PG.

Experimental

Materials

Prodigiosin (PG) was produced by *Serratia marcescens* and purified according to the procedures described previously [27]. Bovine hemoglobin (BHb) was purchased from Sigma, and used without further purification. Other chemicals were all analytical grade.

The solution of BHb was prepared in phosphate buffers (0.1 M, pH 7.0) just before each experiment. Concentrated methanolic PG solutions were added to 3 mL solutions of various amounts of BHb, and the final concentrations of methanol were kept <1% (by volume) in all samples, then incubated at 4 °C for at least 24 h.

Apparatus and methods

UV–vis absorption spectra were obtained on a Varian Cary 5000 spectrophotometer. The steady-state fluorescence spectra were performed using a Perkin-Elmer LS-50B fluorescence spectrophotometer.

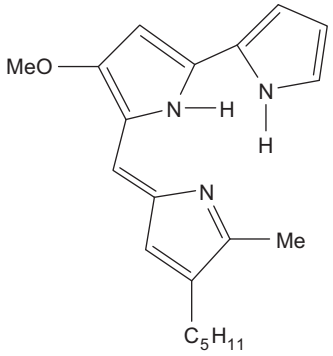

Fig. 1. Molecular structure of prodigiosin (PG).

The fluorescence lifetime measurements were done in a Horiba Jobin Yvon Fluoro Max-4 time correlation single photon counting (TCSPC) system, using a 283 nm diode excitation source (IBH, NanoLED, pulse Fwhm ~3 μs). The time resolution was estimated at 1.5 ns and repetition rate up to 1 MHz. The time ranges are 0.219 ns/channel, in 4096 effective channels. Data were globally fitted to the appropriate exponential model after deconvolution of the instrument response function by an iterative deconvolution technique, using the IBH DAS6 fluorescence decay analysis software.

FTIR spectra were made on a Nicolet Nexus 670 FT-IR infrared spectrometer. All spectra were taken via the Attenuated Total Reflection (ATR) method with resolution of 4 cm^{-1} and 512 scans. The samples were prepared by casting the BHb or PG–BHb solution (100 μL) on the surface of horizontal places ZnSe crystal to form a liquid thin film.

CD measurements were taken with an Applied Photophysics Chriascan circular dichroism spectrometer in a 1 cm quartz cell at room temperature. Spectra were scanned with 1 nm spectral bandwidth and 0.5 nm step resolution. Measurements were recorded in the range of 200–260 nm.

Results and discussion

UV–vis absorption spectra

Fig. 2 shows the UV–vis absorption spectra of BHb and PG–BHb system with increasing concentration of PG over a range of 0–6.0 μM. As can be seen from Fig. 2, BHb has three absorption bands located at 278 nm (due to the phenyl group of tryptophan and tyrosine residues), 348 nm (ε band), and 405 nm (heme or Soret band) [28–30] from 225 to 425 nm in phosphate buffers. With addition of PG to BHb, the intensity of the bands centered at 278 and 348 nm both increase, while the peak at 405 nm decrease, which suggest that PG can access both the heme group and the aromatic acid residues, and disturb the chemical environment surrounding BHb.

Steady-state fluorescence spectra

Fluorescence measurements of proteins can provide considerable information about the accessibility of ligands to the fluorophores. Generally, the fluorescence of protein arises from three intrinsic fluorophores (tryptophan, tyrosine, and phenylalanine residues). The intrinsic fluorescence of BHb is mainly contributed by the tryptophan residue alone, because the fluorescence quan-

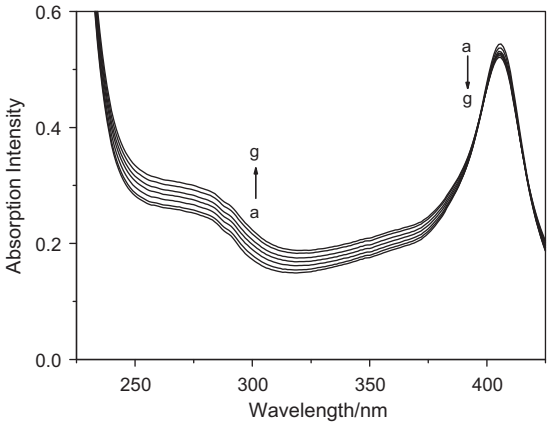

Fig. 2. UV–vis absorption spectra of pure BHb and PG–BHb conjugate; [BHb] = 1.0 μM; [PG]/(a–g) = 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 μM; T = 298 K.