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Spectroscopic studies on the interaction of hypocrellin A and hemoglobin

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ABSTRACT

The spectrophotometric and spectrofluorimetric studies revealed that hemoglobin (Hb) could interact with hypocrellin A, a photosensitizing drug used in photodynamic therapy. It was found that this kind of interaction can induce the conformational changes in Hb. In addition, based on fluorescence quenching titration and electron paramagnetic resonance spectroscopy results, the binding parameters, thermodynamic parameters are obtained. The quenching mechanism is also proposed.

1. Introduction

Hypocrellin A (4,9-dihydroxyperylene-3-10-quinone, HA), one natural pigment extracted from *Hypocrella baHbuase*, a parasitic fungus of Siramudinaria, has been used as a phototherapeutic agent to cure various skin diseases. It has also been used as an orally taken folk medicine for several centuries in China [1,2]. Recently, it was found that this natural perylenequinonoid compound possesses the antitumoral and antiviral activities against several types of viruses, including the human immunodeficiency virus [3]. Comparing with the presently used hematoporphyrin derivatives (HPD), it possesses several advantages, such as easy preparation and purification, small aggregation tendency, strong red light absorptivity and high quantum yields of singlet oxygen [4,5]. According to the photodynamic therapeutic theory, in the procedure of the medical treatment, HA or its analogues could be injected intravenously into the patients. An incubation period for some hours is required before further treatment with the light. This interval allows HA to bind with the serum proteins, such as albumins, globulins and lipoproteins. The protein-bound HA would gradually release in either vascular stroma or intercellular loci, such as lysosomes, golgi, endoplasmic reticulum and cellular membranes [6]. In order to obtain the details of the binding characteristic of HA with these proteins in the photodynamic therapeutic procedure at molecular level, the interaction of HA with the protein should be further studied.

Hb is a protein in the blood and its function is mainly to transfer oxygen. In this paper, Hb was used to systematically investigate its interactive mechanism with HA under the physiological conditions.

2. Materials and methods

2.1. Reagents

Hb (Aldrich Chemical Co. Inc.) was dissolved in 0.04 mol/L phosphate buffer solution (pH 7.4) with 0.10 mol/L HA NaCl. The concentration of Hb was 3.3×10^{-5} mol/L. HA was obtained according to the procedure described in the literature [7], and its purity was >97% measured with HPLC technique. Due to the low solubility of HA in water, 1.0 mmol/L HA solution was prepared with adding small amounts of the concentrated DMSO solution to double distilled water [8]. The solutions were protected from exposure to the light.

2.2. Apparatus

The UV–vis absorption spectra in the range of 220–440 nm were obtained using a Lambda 17 UV–vis spectrophotometer (PerkinElmer, USA). All the fluorescence measurements were car-

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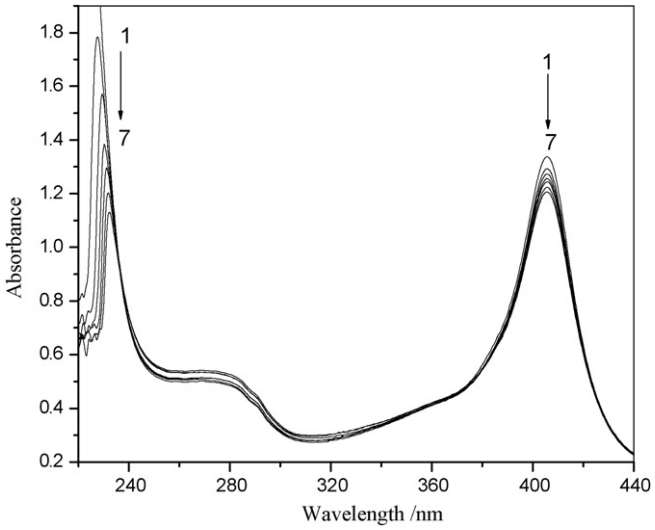

Fig. 1. UV–vis absorption spectra of 33 μmol/L Hb solution with (1) 0 μmol/L, (2) 5 μmol/L, (3) 10 μmol/L, (4) 15 μmol/L, (5) 20 μmol/L, (6) 25 μmol/L and (7) 30 μmol/L HA at 25 °C.

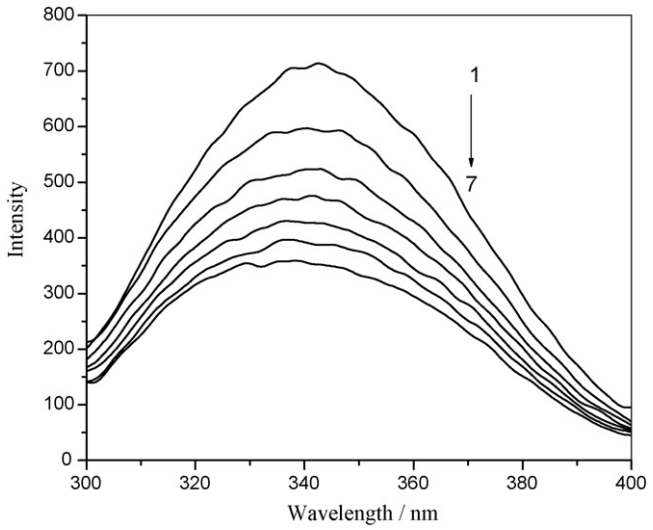

Fig. 2. Fluorescence emission spectra of 26 μmol/L Hb solution with (1) 0 μmol/L, (2) 5 μmol/L, (3) 10 μmol/L, (4) 15 μmol/L, (5) 20 μmol/L, (6) 25 μmol/L and (7) 30 μmol/L HA at 25 °C. λ_{ex}: 280 nm.

ried out using a LS 50 B spectrofluorimeter (PerkinElmer, USA). The excitation wavelength was 280 nm. Usually, all the experiments were carried out at 25 ± 2 °C.

EPR spectra were obtained using a Bruker ESP-300E spectrometer. The operating conditions were as follows—Microwave bridge: X-band with 100 Hz field modulation; sweep width: 100 G; modulation amplitude: 1.0 G; modulation frequency: 100 kHz; receiver gain: 1×10^5; microwave power: 5 mW; operation temperature: room temperature. The samples were injected into the specially made quartz capillaries for EPR analysis. Then, nitrogen, air or oxygen was purged for 30 min in the dark according to the experimental requirements. Finally, the samples were illuminated directly in the cavity of the ESR spectrometer with a Nd:YAG laser (355 nm, 5–6 ns of pulse width, repetition frequency: 10 Hz, 10 mJ/pulse energy).

3. Results and discussions

3.1. UV–vis absorption spectroscopy

Fig. 1 shows the UV–vis absorption spectra of 33 μmol/L Hb solution with the different concentration of HA. It can be observed from Fig. 1 that two characteristic absorption peaks of Hb are located at 220 and 406 nm (Fig. 1, Curve 1). The addition of HA into a solution of Hb, red-shifted the 220 nm absorption peak of Hb and reduced its absorbance. The position and absorbance of the peak at 406 nm does not change significantly. For example, when the concentration of HA in the solution is 30 μmol/L, the peak at 220 nm is shifted to 232 nm and the absorbance is decreased 37% (Fig. 1, Curve 7) compared to a solution of Hb in absence of HA (Fig. 1, Curve 1). The absorption peak at 220 nm is due to the absorption of the amidic acid residues in the peptide of Hb and the absorption peak at 406 nm is due to the absorption of the heme group of Hb [9]. Above results illustrate that HA can interact with the amidic acid residues of Hb, and the interaction does not affect the structure of the heme group. To check the effect of DMSO, controlled experiments were carried out using 30 μmol/L HA and 0.003 mg/mL DMSO. The controlled experiments suggested that DMSO does not affect the results of above experiments under our experimental conditions, and this is consistent with the published literature [10].

3.2. Fluorescence spectroscopy

The fluorescence emission spectra of 26 μmol/L Hb solution with different concentrations of HA are shown in Fig. 2. It can be found that the fluorescence peak of Hb is at 340 nm in the absence of HA. The peak intensity will decrease and the peak position is slightly blue-shifted with increasing the concentration of HA, indicating that there is an interaction between Hb and HA.

There are two kinds of fluorescence quenching, i.e. static quenching and dynamic quenching. For dynamic quenching, the interaction would increase the effective collision number, enhance the energy transfer and increase the quenching constant of the fluorescence substance with increasing the temperature. If it is static quenching, the stability of the compound formed and the quenching constant would decrease with increasing the temperature.

In order to reveal the quenching mechanism, the Stern–Volmer graph at various temperatures was plotted (Fig. 3). It was found from Fig. 3 that the ratio of the initial fluorescence intensity (F_0) and the fluorescence intensity at certain condition (F) should be

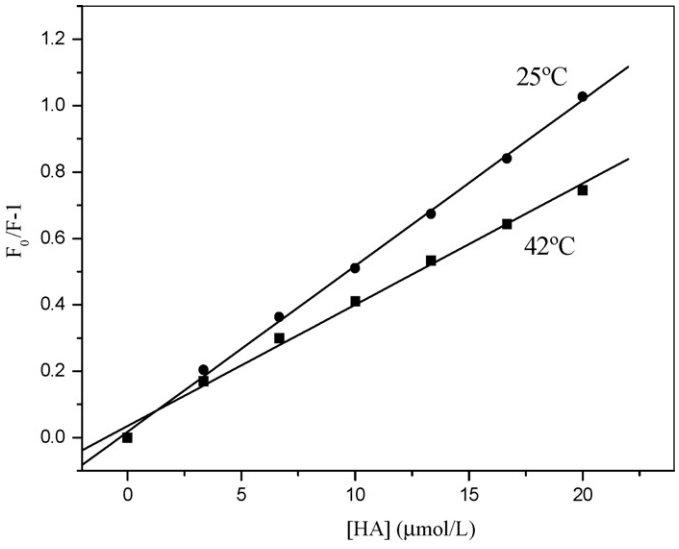

Fig. 3. Plots of F_0/F vs the concentration of HA at 25 and 42 °C.