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The interaction between hemoglobin and two surfactants with different charges

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Abstract

The interactions of hemoglobin (Hb) with sodium dodecyl sulfate (SDS) and dodecyl trimethylammonium bromide (DTAB) are investigated by several methods. We observed the formation of hemichrome below the critical micelle concentration (cmc) of surfactant and the release of heme from Hb above the cmc. When pH value of Hb/surfactant system is lower than isoelectric point (p*I*) of Hb, the interaction of SDS with Hb is both electrostatic and hydrophobic, while the interaction of DTAB with Hb is hydrophobic mainly. On the contrary, when pH > p*I*, the interaction of SDS with Hb is hydrophobic mainly, while the interaction of DTAB with Hb is both electrostatic and hydrophobic. In the case where both the electrostatic interaction and hydrophobic interaction exist, the electrostatic interaction plays a more important role. Thus, SDS tends to interact with Hb more obviously than DTAB does when pH < p*I* and the interaction between DTAB and Hb is stronger when pH > p*I*.

Keywords: Hemoglobin; DTAB; SDS; Hemichrome; Isoelectric point

1. Introduction

Hemoglobin (Hb), a kind of respiratory protein of vertebrate erythrocytes, is important in oxygen transport, H_2O_2 dispersion and electron transfer to all organs and parts of the body [1]. Now, we have understood its structure, the mechanisms of its oxygen transport function and its electron transfer on electrode surface [2–7]. And the research on its interaction with other molecules, such as bacterial endotoxin [8,9], hydroxyurea [10] and trehalose [11], indicates that some biological molecules can convert oxyhemoglobin (oxyHb) to methemoglobin (metHb) and hemichrome, while some others may slow or reverse the auto-oxidation process of Hb [8,11]. Since hemichrome accumulation in red cells is typical of some blood diseases [12,13] and aging of the erythrocyte [14], the interrelated study of the conversion between metHb and hemichrome will be worthwhile not only in theory but also in practice.

Surfactant–protein interactions are very common in the fields of medicine, chemistry, biology, and so on [15–18]. Recently, the study of the interaction between surfactants and proteins is focused on the surfactant–protein complex structure and the change of protein structure. And according to the transient kinetic study, surfactant can induce the transformation of the form R → T of Hb [19,20]. Venkatesh et al. [21] studied the heme release from reconstituted Hb in CTAB and SDS and concluded that CTAB were capable of releasing heme better than SDS, due to the stronger hydrophobicity of CTAB. Since the study of the interaction between surfactants and Hb and of the effect of Hb on the aggregation behavior of surfactants would contribute to the elucidation of the essence of the interaction between surfactants and Hb, in the present paper, we choose surfactants SDS and DTAB, with a same hydrophobic tail and a different charged head group in structure, to discuss the interaction between surfactant and Hb, which should be helpful to expatiate upon the influence of Hb on different types of surfactant aggregations, and understand the change of the structure of Hb by surfactant more thoroughly.

2. Experimental

2.1. Materials

Sodium dodecyl sulfate (SDS, Sigma, >98%, recrystallized twice in ethanol), dodecyl trimethylammonium bromide

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(DTAB, Sigma, 99%), hemoglobin (bovine, Sigma). Water used was distilled twice.

2.2. Methods

2.2.1. Measurement of conductance

The conductances of DTAB/H_2O and SDS/H_2O systems with and without Hb were measured with a conductor meter (DDS-11A, Shanghai Weiye Apparatus Co., China) at 25.0 ± 0.1 °C, which was calibrated by 0.01 mol/L KCl solution before measurement. The error limitation for conductivity values was within ±0.5%.

2.2.2. Measurement of UV–vis absorption spectra

The UV–vis spectra of Hb with DTAB and SDS in water and phosphate buffer solution were measured with a Shimadzu UV-2501 ultraviolet spectrophotometer at 25.0 ± 0.1 °C. A 1 cm × 1 cm (length × width) sample cuvette was chosen and water was used as reference solution.

2.2.3. Measurement of fluorescence spectra and synchronous fluorescence spectra

The fluorescence spectra and synchronous fluorescence spectra of Hb in different DTAB/H_2O and SDS/H_2O systems were measured with a Shimadzu model RF-5301PC fluorescence spectrophotometer at 25.0 ± 0.1 °C. The excitation wavelength was 280 nm and the excitation and emission slits were 3 nm. The wavelength interval (Δλ) for synchronous fluorescence spectra was 20 nm for tyrosine residue (Tyr) and 80 nm for tryptophan residue (Trp), respectively.

2.2.4. Measurement of the images

Micrographs were obtained with a transmission electronic microscope (TECNAI 12, Philip Apparatus Co., Netherlands) by negative staining and freeze-fractured (BAF 400 D, Balzers, Germany) technique.

2.2.4.1. Negative staining method. Before being stained, the sample solution was adsorbed onto a copper grid by laying a carbon Formvar-coated copper grid (230-mesh) on one drop of the sample solution; the excess liquid was wiped away with filter paper. Then, one drop of the uranyl acetate solution (1%) as the staining agent was placed onto the copper grid. The excess liquid was also wiped away with filter paper.

2.2.4.2. Freeze-fractured technique. For the preparation of replica, a small amount of sample was placed in a sample cell. Then the cell was swiftly plunged into liquid nitrogen, and then the frozen sample was fractured and replicated in a freeze-fractured apparatus [17].

2.2.5. Measurement of isothermal titration microcalorimetry

The interaction of hemoglobin with SDS and DTAB was detected by MicroCal VP-ITC (USA) at 25.0 ± 0.1 °C. Hemoglobin solution was loaded into the sample cell (1.44 mL), and the surfactant solutions (0.2 mol/L) were loaded into the injector (250 μL), which titrated with approximately 50 aliquots of 5 μL surfactant solution into the cell. The syringe is tailor-made such that the tip acts as a blade-type stirrer to ensure an optimum mixing efficiency at 400 rpm. The heat evolved or absorbed by each injection in the course of titration is directly measured by the ITC unit, producing the raw heat signal, also known as cell feedback (CFB). Integration of each CFB gives the differential enthalpy curve for the titration.

3. Results and discussion

3.1. Conductance and TEM study

Fig. 1 shows the UV–vis spectra of Hb in water, which is same as the characteristic spectra of metHb. By the method reported by Ajloo et al. [20], the concentration of metHb, 92%, can be calculated by Eq. (1) according to the absorbances at 540 nm (A_{540}), 560 nm (A_{560}) and 576 nm (A_{576}). Furthermore, due to the Q band around 500 nm and the ligand-to-metal charge transfer (LMCT) band, approximately at 630 nm, which are spectral fingerprints of the aquometHb species, metHb used in the paper is an aquometHb species [19].

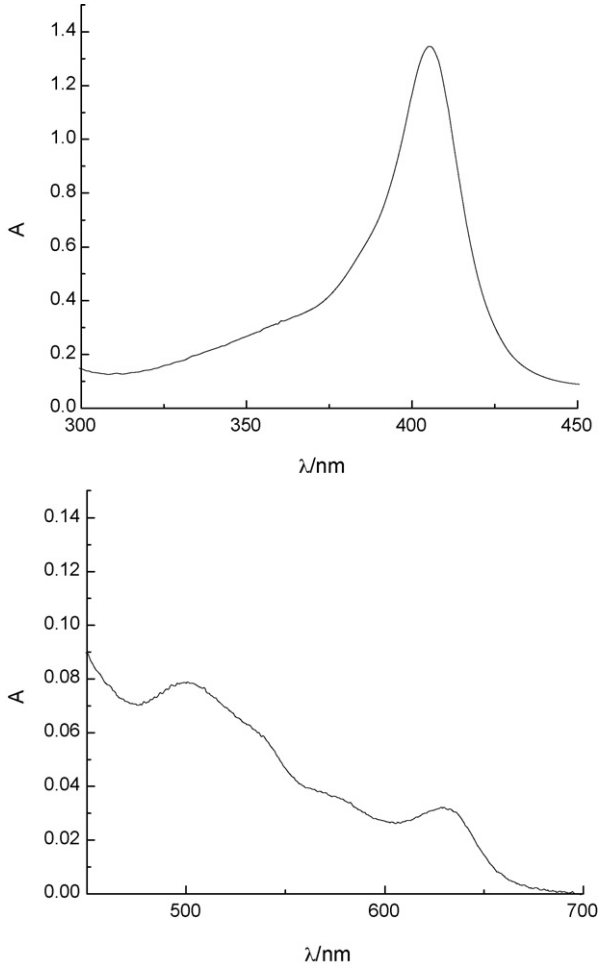

Fig. 1. UV–vis spectra of Hb (3.0×10^{-6} mol/L) in water.