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Study on the interaction between hemoglobin and didodecyldimethylammonium bromide by spectroscopic technique

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Abstract: The interaction between hemoglobin (Hb) and didodecyldimethylammonium bromide (DDAB) was investigated by UV-vis spectra, fluorescence spectra and circular dichroism spectra (CD). With the addition of DDAB into the solution of Hb, the Soret absorption decreased gradually, the fluorescence intensity of Hb increased remarkably and α-helical content of Hb increased. According to Scatchard analysis, the binding constants of DDAB to Hb were obtained at different temperature. The parameters of thermodynamics, ΔH, ΔS and ΔG, indicated that the hydrophobic force should be mainly interaction of DDAB with Hb.

Key words: hemoglobin; fluorescence spectra; CD spectra; thermodynamic parameters

牛血红蛋白与双十二烷基二甲基溴化铵相互作用的光谱研究

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摘要: 运用荧光光谱、紫外-可见吸收光谱和圆二色谱法研究了双十二烷基二甲基溴化铵(DDAB)与牛血红蛋白(Hb)的相互作用。从紫外-可见吸收光谱观察到, 随着 DDAB 的浓度增大, Hb 在 406nm 处的特征吸收峰强度下降, 且峰位蓝移, 说明 DDAB 导致血红素辅基微观环境变化。由荧光光谱研究可以得出随着 DDAB 的浓度增大, Hb 在 340nm 处的荧光强度逐渐增强, 说明导致色氨酸荧光淬灭的血红素辅基与色氨酸的距离增大。由 Scatchard 方程计算了不同温度下该反应的表观结合常数、结合位点数及结合热力学参数, 热力学参数的变化表明 DDAB 与 Hb 之间以疏水作用力为主。圆二色谱的研究进一步表明 DDAB 使 Hb 产生轻微的二级结构改变, α-螺旋含量增加.

关键词: 牛血红蛋白; 荧光光谱; 圆二色谱; 热力学参数
中图分类号: O657.3 **文献标识码:** A

Surfactants are widely employed in biochemistry and biotechnology for the purpose of protein solubilization, purification, characterization, and structure determination because microemulsions and lamellar liquid crystals can be used to simulate some biological systems [1]. As a biological environmental simulation, the surfactant was presumed or expected that there was no interaction with protein studied.

However, most surfactants can interact with proteins and then affect the properties and structures of protein [2-6]. The interaction of anionic sodium n-dodecyl sulfate(SDS) and cationic cetyltrimethylammonium bromide (CTAB) with hemoglobin and other proteins was recently performed and the associative bind-

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ing constants of the surfactants to hemoglobin were determined [7-11].

Didodecyldimethylammonium bromide (DDAB) is an ionic surfactant with double lipophilic chain. It was immobilized on suface of electrode in the electrochemical study of various heme proteins to increase the electrochemical response compared to bare electrodes [12,13]. Koper and other authors [14-16] argued that DDAB most likely induced the release of the heme group from heme protein, such as myoglobin and hemoglobin, when DDAB was modified on a pyrolytic graphite electrode.

Hemoglobin (Hb), a tetramer protein with an Mr of approximately 67 kDa, is composed of two α and two β subunits. Hb is an important functional protein for reversible oxygen carrying and storage, as well as a model protein with high α-helical content.

In order to understand the influence of DDAB on Hb, in the present work, the interaction of DDAB with Hb was investigated by spectroscopic methods including UV-vis spectra, fluorescence spectra and circular dichroism (CD). The binding parameters of the reaction were calculated on basis of Scatchard plots. The results may provide some helpful information about the interaction of hemeprotien with surfactants.

1 Experimental section

1.1 Materials

Bovine hemoglobin (Hb, Sigma Chemical Co.) was used without further purification, and was dissolved in 0.1 mol·L^{-1} phosphate buffer (KH_2PO_4 – K_2HPO_4) at different pH values. The concentration of Hb solution was determined on the basis of the molar extinction coefficient ε_{406} =41,000 L·mol^{-1}·cm^{-1} [17]. All other chemicals used were of analytical reagent grade. Water used was distilled twice. Didodecyldimethylammonium bromide (DDAB, 98%) purchased from Aldrich.

1.2 Methods

1.2.1 Effect of pH on the fluorescence of Hb/DDAB system Hb was dissolved in phosphate buffer at different pH values, and its fluorescence intensity with each addition of DDAB stock solutions was measured at the excitation wavelength of 280 nm and emission wavelength of 340 nm.

1.2.2 UV-vis spectra The UV-vis spectra were recorded on a HP-8453 spectrophotometer with 1-cm quartz cells in the range of 200-700nm at 296 K.

1.2.3 Fluorescence spectra The slits for excitation and emission widths were both 5 nm. 2.5mL of Hb solution were placed into the quartz cuvettes of 1 cm optical path and were recorded the intrinsic fluorescence spectra at 300-450 nm using an excitation wavelength of 280 nm. Emission spectra were recorded after each addition of DDAB stock solutions (10mmol·L^{-1}) into Hb solution at 296 K and 310K.

1.2.4 Far-UV CD spectra Circular dichroism measurements were carried out using a Jasco J-715 spectropolarimeter, purged with N_2 at a flow rate of 3-5 L/min. The resolution was 0.2 nm. The scan speed was 50 nm/min and the response time was 0.25 sec. Utilization of the software supplied with the instrument allowed the estimation of the secondary structure of the proteins by Yang's method [18].

2 Results and discussion

2.1 Effect of reaction time and pH on the Hb/DDABsystem

Dependence of the fluorescence intensity of Hb on reaction time was investigated. The result was showed in Fig. 1. Obviously, the fluorescence intensity of Hb with DDAB increased first, and then decreased during beginning 5 min. After 5 min it was steady, therefore, the recording time was chosen at 5 min in this paper.

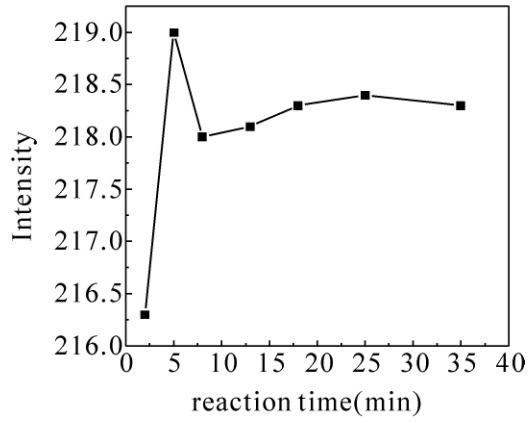

Fig. 1 Dependence of the fluorescence intensity of Hb on reaction time

C_{Hb} = 4.5 × 10^{-5} mol·L^{-1},

C_{DDAB} = 1.2 × 10^{-5} mol·L^{-1}

The effect of pH on the fluorescence of Hb/DDAB system was examined. As can be seen from Fig. 2, the fluorescence intensity of Hb in pH 5.5 phosphate buffer increased gradually with increasing

concentration of DDAB, and the intensity values was bigger than that of Hb in pH 7.0 and 9.0. The results indicated that the proton can increase the interaction of DDAB with Hb, so pH 5.5 was chosen in this study.

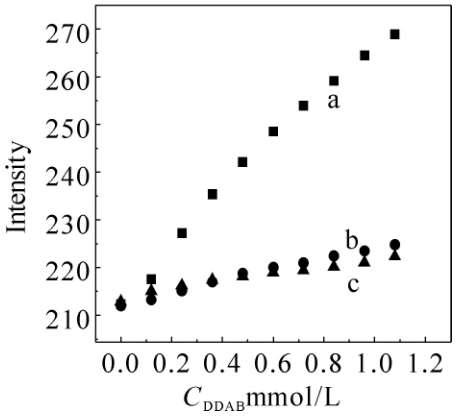

Fig. 2　Effect of pH on the fluorescence intensity of Hb/DDAB system
$C_{Hb} = 4.5 \times 10^{-5}$ mol · L^{-1},
(a) pH 5.5; (b) pH 7.0; (c) pH 9.0

2.2　UV-vis spectrum

Fig. 3 shows the Soret absorption of Hb with different concentration of DDAB. The Soret absorption decreased gradually and went with a blue shift from 406 to ~400nm with the increasing concentration of DDAB, which showed that the DDAB micelle could change the microenvironment around heme [19].

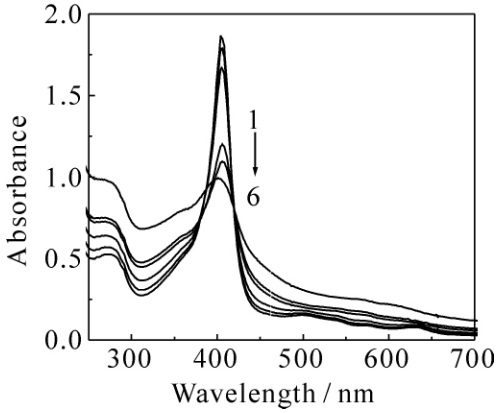

Fig. 3　Effect of the concentration of DDAB on the UV-vis spectra of Hb in pH 5.5
$C_{Hb} = 4.5 \times 10^{-5}$ mol · L^{-1};
the concentration of DDAB ($\times 10^{-4}$ mol · L^{-1}) was 0, 0.2, 0.4, 1.0, 1.2, 3.0. (from 1 to 6)

2.3　Fluorescence spectrum

The fluorescence of Hb is mainly from tryptophan (Trp), however, the efficient energy transfer from Trp to heme significantly quenches the protein fluorescence [20]. Fig. 4 showed that the fluorescence intensity of Hb increased gradually with increasing concentration of DDAB and the maximum emission wavelength of Hb shifted slightly from 340 to 345 nm. These phenomena could be deduced that the quenching reaction of Trp by heme was depressed as a result of the enlarged distance between heme and Trp [7,21]. With the further increase of DDAB, the fluorescence intensity of system increased tardily, indicating the beginning of saturation in the Hb binding site.

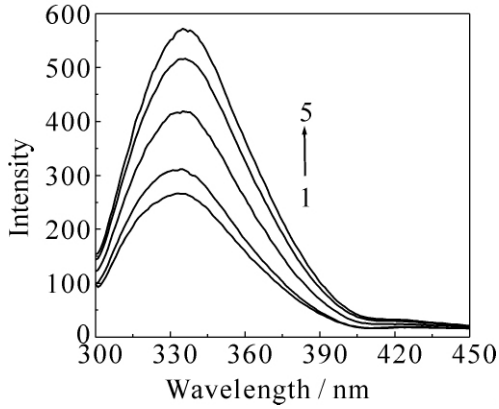

Fig. 4　Effect of the concentration of DDAB on the fluorescence of Hb in pH 5.5
$C_{Hb} = 4.5 \times 10^{-5}$ mol · L^{-1};
the concentration of DDAB ($\times 10^{-5}$ mol · L^{-1}) was 0, 1.2, 1.6, 2.0, 2.8 (From 1 to 5)

A simple binding model A + B = C may be fit to illustrate a binding of a small ligand to a macromolecule, where A is ligand, B is macromolecule, C is the complex. The binding parameters were calculated using the Scatchard's equation [22,23]:

$$\frac{r}{C_f} = nK - Kr$$

where r is the moles number of ligand bound with per mole of macromolecule, n is the number of binding sites on the macromolecule, K is the binding constant, C_f is the concentration of free ligand. The fluorescence data of Hb were collected after interacted with different concentration of DDAB. The Scatchard plot of r/C_f versus r was shown in Fig. 5. The curve represented an intersection of two straight lines, indicating two classes of binding sites in the process of binding. The K and n for each type of site were obtained in Table 1. In this study, the Scatchard plot for binding of DDAB to Hb is nonlinear, which shows that the process of DDAB binding is cooperative [3]. The cooperative binding indicates that there is large-scale binding induced by the formation of micelle-like clusters of DDAB around the Hb. Hb consists of two α and two β subunits,

each of four heme monomer is combined in the hydrophobic cavity of Hb. The binding of DDAB to Hb may occur at the hydrophobic pocket of the protein molecule, therefore, the hydrophobic force might play a driving role in the binding process.

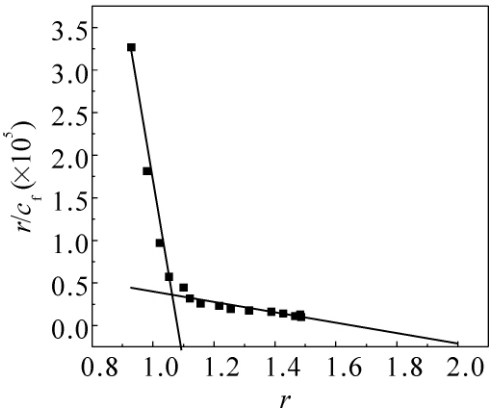

Fig. 5 The Scatchard plot for DDAB binding to Hb ([DDAB] < 1mmol? L^{-1}) at 37 °C

Table 1 The values of K and n for DDAB binding to Hb obtained from Scatchard analysis

t (°C)	K_1, K_2 (M^{-1})	n_1,	n_2
23	6.01×10^5, 7.60×10^4	1.1	1.5
37	2.20×10^6, 4.86×10^5	1.1	1.8

2.4 Determination of interaction type

In order to characterize further the binding mode of DDAB to Hb, some thermodynamic parameters of the interaction of DDAB and Hb were got according to the equation of thermodynamics expressed as follows [24, 25]:

$$\ln \frac{K_2}{K_1} = \Delta H(1/T_1 - 1/T_2)/R \quad (1)$$

$$\Delta G = -RT \ln K \quad (2)$$

$$\Delta S = (\Delta H - \Delta G)/T \quad (3)$$

where K_1, K_2 is the binding constant at the different temperature; R is the gas constant; G is Gibbs energy; H is enthalpy; and S is entropy. If $\Delta H > 0$ and $\Delta S > 0$, the main force is hydrophobic interaction; if $\Delta H < 0$ and $\Delta S > 0$, the main force is electrostatic action, however, if $\Delta H < 0$ and $\Delta S < 0$, the main force is hydrogen bond or van der waals force. It can be simply deduced which type of interaction between Hb and DDAB exists by the parameter of thermodynamics.

According to the binding constant of DDAB with Hb at different temperature in Table 1, ΔH, ΔS and ΔG can be calculated. They are 70.5 kJ · mol^{-1} (ΔH), 0.35 kJ · mol^{-1} (ΔS), and -32.75 kJ · mol^{-1} (ΔG), respectively. Based on the above principle ($\Delta H > 0$, $\Delta S > 0$) the hydrophobic force should be main interaction of DDAB with Hb on the whole binding process, suggested that the hydrophobic chain of DDAB could permeate into the hydrophobic cavity of Hb.

2.5 Far-UV CD spectra and the secondary structural changes

In addition to fluorescence and Soret absorption, CD is another useful conformational probe for the study of proteins. It provides additional evidence for the possible conformational changes of Hb. The CD spectrum of Hb exhibits an intensive positive peak about 195 nm and pronounced negative bands about 208 and 222 nm (Fig. 6), which are characteristics of a high α-helical content [26, 27]. The addition of DDAB increased the intensity of the negative bands at 208 and 222 nm in molar ellipticity. The calculation based on analysis of the curves using Yang´s method [18] shown that α-helical content increased from 46.9% to 53.6%, indicating that DDAB did not destroy the α-helical regions of protein molecules and only resulted in slight conformational changes.

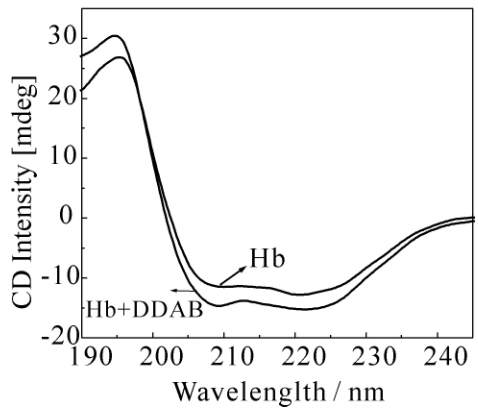

Fig. 6 Far-UV CD spectra of Hb in absence and presence of DDAB
$C_{Hb} = 2.1 \times 10^{-6}$ mol · L^{-1},
$C_{DDAB} = 5.0 \times 10^{-7}$ mol · L^{-1}.

3 Conclusion

The interaction of DDAB with Hb was investigated by different spectroscopic techniques. The data of UV-vis and fluorescence spectrum indicated the change of microenvironment of heme in Hb and an enlarged distance between heme and Trp. The analysis of CD spectrum showed that the binding of DDAB to Hb induced a slight conformation change of Hb. The parameters of thermodynamics suggested that the type of interaction between DDAB and Hb was mainly hydrophobic force.

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