Electrochemical Investigation of Bovine Hemoglobin at an Acetylene Black Paste Electrode in the Presence of Sodium Dodecyl Sulfate

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Electrochemical behaviors of bovine hemoglobin (Hb) at an acetylene black paste electrode based on the enhancement effect of sodium dodecyl sulfate (SDS) were investigated. In the optimal conditions, a very weak reduction peak was observed at an acetylene black paste electrode for hemoglobin in the absence of SDS. However, the reduction peak current increased remarkably after the addition of 4.0×10^{-4} mol L^{-1} SDS, suggesting that SDS exhibits obvious enhancement effect to the determination of hemoglobin. All the experimental parameters, such as pH value, concentration of SDS, accumulation time and accumulation potential were optimized for hemoglobin analysis. The proposed method possesses high sensitivity (detection limit is 3.0×10^{-9} mol L^{-1}), wide linearity (6.0×10^{-9} to 6.0×10^{-7} mol L^{-1}), rapid response and low cost. Finally, the method was successfully employed to determine hemoglobin in a spiked sample.

Key Words : Bovine hemoglobin, Surfactant, Acetylene black, Voltammetry

Introduction

Hemoglobin (Hb) is a physiologically important globular protein.[1-3] The physiological function of Hb is to store and transport dioxygen to the body where it is used in aerobic metabolic pathways. It is also involved in many clinical diseases such as leukemia, anemia, heart diseases, etc.[4] Therefore, the determination of Hb is of great importance for clinical diagnosis and physiological research. To date, several means had been reported to determine Hb, including spectrophotometry,[5,6] fluorimetry,[7] chemiluminescence[8] and high performance liquid chromatography.[9,10] Spectrophotometric or fluorimetric methods for the determination of Hb were mainly based on its enzymatic activity for the oxidation of chromogens with hydrogen peroxide or other oxidizers, and they have the problems with regard to selectivity, sensitivity and stability of the reagents. Whereas these can be overcome in the electroanalytical detection, consequently, many methods based on electrochemical response of Hb were constructed for its determination.[11-16]

Compared with cytochrome c, hemoglobin (Hb) usually exhibit more sluggish electron transfer on electrodes because the electroactive section (heme group) in Hb is much more buried with respect to the protein surface. Therefore, to open the electroactive section of Hb is a key step for the electrochemical investigation of Hb at an electrode surface directly. Surfactants are compounds which had a hydrophobic group and a hydrophilic group. They can combine with the protein by the hydrophobic group in solution, and possibly open its electroactive section, while the hydrophilic group might combine with water, which was beneficial to the dissolving of the macromolecules.[17]

In the present paper, we use an anion surfactant SDS to act on Hb and to investigate the electrochemical behavior of Hb at an acetylene black paste electrode. The objective of the work is to develop a convenient and sensitive procedure for the determination of Hb based on the unusual properties of acetylene black coupling with surfactant. Acetylene black, a special type of carbon black, is made by the controlled combustion of acetylene in air under pressure. Due to its excellent electric conductivity, large specific surface area and strong adsorptive ability, acetylene black has been widely used in electrochemistry and electroanalysis.[18-22] Compared with the other paste electrodes in routine use, acetylene black can significantly improve the electrochemical responses of the analytes, so as to the determining sensitivity can remarkably enhanced. Surfactants are a kind of amphiphilic ions or molecules with a hydrophilic head on one side and a long hydrophobic tail on the other side. They have been widely used in electrochemistry and electroanalysis chemistry field to change the electrical properties of the electrode solution interface and the electrochemical process through adsorption at interfaces *via* electrostatic interaction and/or hydrophobic interaction.[23,24] In a phosphate buffer (pH = 6.0), Hb can react with SDS through static interaction. The current response of Hb at the electrode was enhanced remarkably and a very low detection limit of 3.0×10^{-9} mol L^{-1} (S/N = 3) was obtained. Based on these, a simple and sensitive electrochemical method was developed for the determination of Hb. Its successful application in the determination of Hb in a spiked sample proves that it was a reliable method which was sensitive, simple and low cost.

Experimental Section

Reagents. Hemoglobin from bovine blood (Shanghai Bo'ao, China) was used without further purification. 1.0 mg mL^{-1} hemoglobin stock solution was prepared by dissolving

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hemoglobin into 1/15 mol L^{-1} phosphate buffer. Working solutions were prepared daily by suitable dilution with phosphate buffer. Sodium dodecyl sulfate (SDS) (Shanghai Reagent Corporation, China) was made into 1.0×10^{-2} mol L^{-1} aqueous solutions. Spectroscopic acetylene black powder and paraffin oil were purchased from Shanghai Reagent Corporation, China. Other chemicals were of analytical-reagent grade and used without further purification. All solutions were prepared with redistilled water. All experiments were carried out at room temperature.

Apparatus. All the electrochemical measurements were performed with a CHI 660A Electrochemical Workstation (CH Instrument, USA). A three-electrode system, including acetylene black working electrode (3.5 mm in diameter), a platinum wire counter electrode and a saturated calomel reference electrode (SCE), was employed.

Preparation of the acetylene black paste electrode. The acetylene black paste electrode was prepared by mixing 10.0 mg acetylene black powder and 15.0 μL paraffin oil in a small mortar, then, this mixture was homogenized. After that, the paste was pressed into the cavity of the electrode body, and the surface was smoothed against weighing paper. Unless otherwise stated, the paste was carefully removed and another new acetylene black electrode was remade after each measurement.

Determination of hemoglobin. Unless otherwise stated, 1/15 mol L^{-1} phosphate buffer containing 4.0×10^{-4} mol L^{-1} SDS was used as the supporting electrolyte for Hb determination. The accumulation step was carried out at 0.20 V with 80 s stirring solution, then the differential pulse voltammograms from 1.00 to -0.80 V were recorded after 5 s quiet time, and finally the peak current at -0.26 V was measured.

Results and Discussion

Electrochemical responses of hemoglobin. Figure 1(c) illustrates the cyclic voltammogram (CV) of a acetylene black electrode in the phosphate buffer containing 4.0×10^{-4} mol L^{-1} SDS. No redox peak was observed in the potential range, suggesting that SDS is an electrochemically inactive compound. As shown in Figure 1(b), however, a reduction peak which was not well-defined could be seen at about -0.56 V without the effect of the surfactant. Figure 1 (a) shows the cyclic voltammogram obtained at the acetylene black electrode for 7.0×10^{-7} mol L^{-1} hemoglobin after the addition of SDS. We observed that Hb would give a much larger current response if we added 4.0×10^{-4} mol L^{-1} SDS to the phosphate buffer solution containing Hb. The CV curve show that Hb could present a satisfying result after it was acted on by SDS. Because of the effect of the surfactant, the peak current of the protein increase greatly and the shape of the reduction peak was also improved.

Differential pulse voltammetry was further applied for the electrochemical investigation of Hb at the acetylene black electrode in the presence of SDS. From the voltammograms of Figure 2(c) and (d), in the potential range from 0.60 V to -
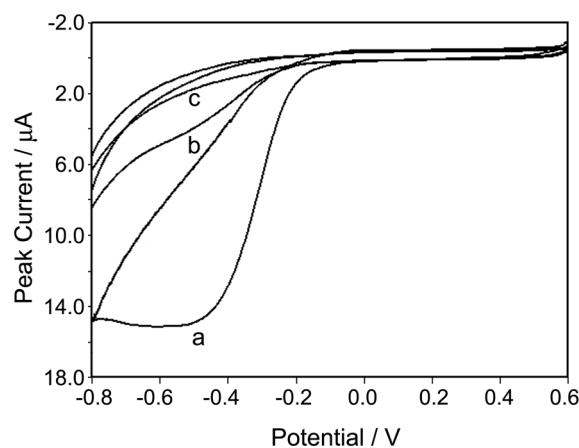

Figure 1. Cyclic voltammetric response of the acetylene black electrode in the presence of (a) 7.0×10^{-7} mol L^{-1} hemoglobin and 4.0×10^{-4} mol L^{-1} SDS; (b) 7.0×10^{-7} mol L^{-1} hemoglobin; (c) 4.0 $\times 10^{-4}$ mol L^{-1} SDS.
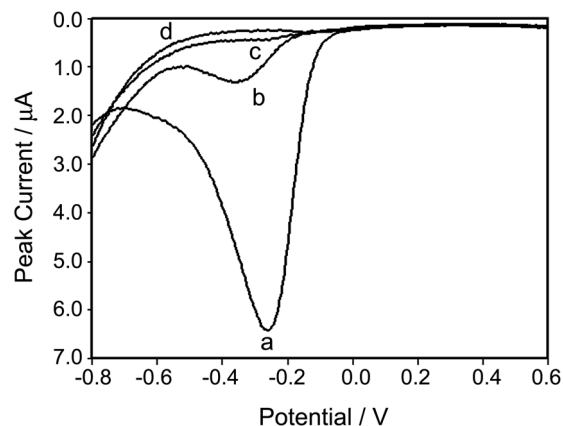
Figure 2. Differential pulse voltammograms for the acetylene black electrode in the presence (a) 3.0×10^{-7} mol L^{-1} hemoglobin and 4.0×10^{-4} mol L^{-1} SDS; (b) 3.0×10^{-7} mol L^{-1} hemoglobin; (c) 4.0×10^{-4} mol L^{-1} SDS and (d) phosphate buffer.

0.80 V, no reduction peaks were observed at the electrode in 1/15 mol L^{-1} phosphate buffer in the presence and absence of SDS. However, when 3.0×10^{-7} mol L^{-1} Hb was added, an obvious reduction peak can be observed at -0.36 V without the effect of SDS. But after the addition of SDS, perfect peak of Hb could be obtained at -0.26 V. The positive shift of the peak potential suggested that the reduction process of Hb at the acetylene black electrode was facilitated by the added anionic surfactant SDS. This not only proves once again that SDS could make Hb give a gratifying voltammetric response, but also means the possibility of microanalysis for Hb.

Effect of pH value. The electrochemical responses Hb at the acetylene black electrode showed remarkable dependence on the pH value of supporting electrolyte. Figure 3 is the voltammetric measurement results for 0.3 μmol L^{-1} Hb in 1/15 mol L^{-1} phosphate buffer solutions at different pH values. It could be observed that, as the pH value increased in the range of 4.5-5.8, the peak currents for Hb increased while the peak potentials shifted negatively. The maximum
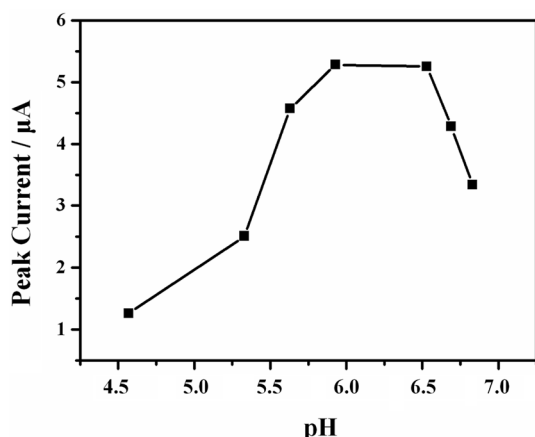
Figure 3. Effect of pH on the peak current response of 3.0×10^{-7} mol L^{-1} hemoglobin in the presence of 4.0×10^{-4} mol L^{-1} SDS.

Figure 4. Dependence of peak current of 2.0×10^{-7} mol L^{-1} hemoglobin on the concentration of SDS.

peak current response can be obtained in pH range from 5.8 to 6.5. When the pH value was higher than 6.5, the peak current decreased rapidly. Therefore, pH 6.0 was selected for the electrochemical investigation of Hb in the further experiments.

Influence of pH on the electrochemical behavior of Hb at the acetylene black past electrode coupling with SDS was due to the electrostatic interaction between Hb and SDS. The isoelectric point (pI) of bovine hemoglobin is known to be 7.1. In a strong acid solution, the amount of protonated amino acid residues will increase. However, the electrostatic force of $-SO_3^{2-}$ will be decrease, so as to a few amount of Hb-SDS aggregates can be formed and adsorbed to the electrode surface. When pH values higher than the protein pI value (pI = 7.1) for bovine hemoglobin, there is a decrease of available cationic sites and that would reduce the electrostatic contribution of SDS. According to literature reports both ionic as well as hydrophobic interactions between SDS and heme proteins at about pH 7.0 might be important for the formation of a complex equilibrium involving several species.[25] Hydrophobic interactions promoted by SDS may be an important factor for the adsorption of Hb to the electrode surface. Therefore, a maximum current response can be obtained at pH range from 5.8 to 6.5. A similar phenomenon can be observed in the absence of SDS, indicating that the electrode process is not changed after the addition of SDS.

Effect of SDS concentration. In addition, the effects of SDS concentration on the reduction of Hb were depicted in Figure 4. The reduction peak current increased with the increase of SDS concentration when SDS concentration was lower than 4.0×10^{-4} mol L^{-1}. A maximum current response was obtained in the presence of 4.0×10^{-4} mol L^{-1} SDS. However, with the further increase of SDS concentration, the reduction peak current decreased. It is due to the formation of SDS layer on the electrode surface, and the electron transfer between Hb and the electrode was blocked. To sum up, 4.0×10^{-4} mol L^{-1} SDS can more effectively improve the reduction peak current of Hb at the carbon electrode. Moreover, the effect of different surfactants on the
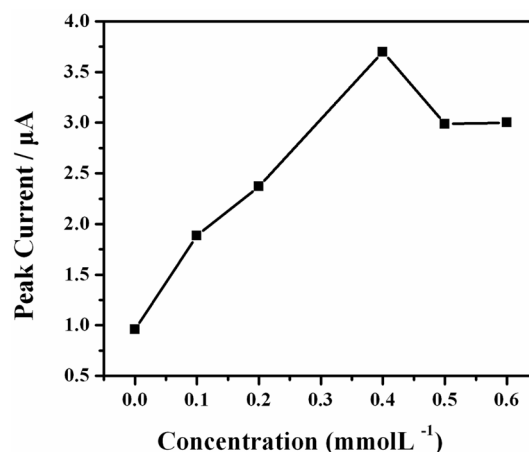
current response of Hb at the acetylene black electrode was also investigated. The results indicated that neutral surfactants (trition X-100) has almost no influence on the electrochemical response of Hb, cationic surfactants such as hexadecyl trimethylammonium bromide (CTAB) and cetyl pyridine bromide (CPB) can improve its current response on a certain degree especially in a base solution. Whereas, anionic surfactant both sodium dodecyl sulfate (SDS) and sodium dodecyl benzene sulfonate (SDBS) can remarkably enhance the current response of Hb in acid solution. The enhancement of peak current in the presence of SDS is larger than that in the presence of SDBS, thus, SDS is chosen for the further determination of Hb. Above results indicated that the enhancement of the peak current mainly come from the increase of the amount of surface adsorption via electrostatic and/or hydrophobic interaction.

Effect of accumulation conditions. Because accumulation can improve the amount of Hb on the electrode surface, then obviously improve the determining sensitivity. So, the two main parameters of accumulation step-accumulation potential and time were examined, respectively. As shown in Figure 5(A), when the accumulation potential shifts from 0.70 to 0.20 V, the peak current of Hb increases slightly and a maximum current response was obtained at about 0.20 V. With further increase of the accumulation potential from 0.20 V to 0.40 V, the peak current varied slightly. That is to say, the further increase of accumulation potential has no visible influence on the adsorption of Hb on the surface of the acetylene black electrode. Therefore, the accumulation was carried out at 0.20 V with the solution stirring. The influence of accumulation time ranged from 0 to 90 s on the reduction peak current of Hb at the acetylene black electrode was shown in Figure 5(B). The peak current increases with extending the accumulation time in the range 0-60 s. Under the same experimental conditions, the longer the accumulation time, the more Hb was adsorbed to the electrode surface. Afterward, the peak current tends to be stable with further increasing accumulation time from 60 s to 90 s. This may be attributed to the saturated adsorption of
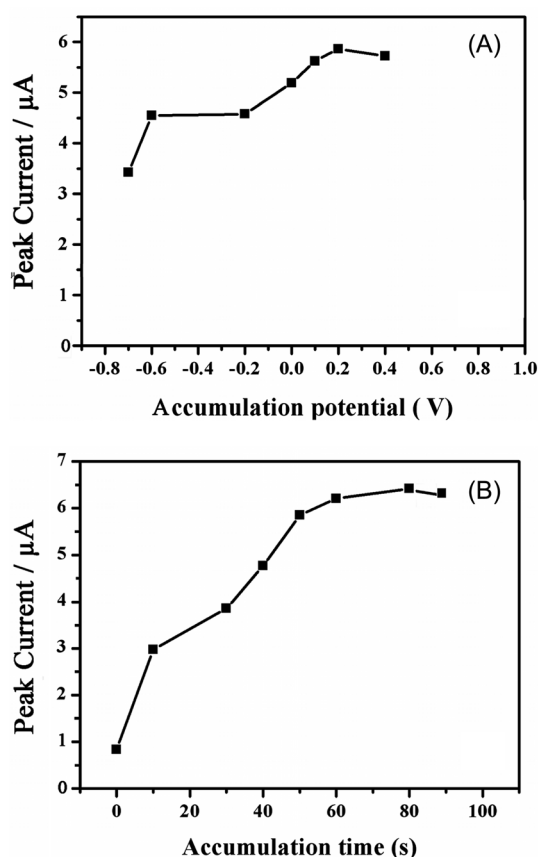
Figure 5. Dependence of peak current on the accumulation potential (A) and the accumulation time (B).

Hb at the electrode surface. Therefore, the optimal accumulation time of 80 s was employed in the further experiment.

Calibration curve. The calibration curve for the determination of Hb in the presence of SDS in 1/15 mol L^{-1} at the acetylene black electrode under optimal working conditions was characterized by DPV and presented as Figure 6. The reduction peak current was linearly related to the concentration of Hb in the range of 6.0×10^{-9} to 6.0×10^{-7} mol L^{-1}. The linear regression equation can be described as following: Ip (μA) = 14.2814 C ($\mu mol \cdot l^{-1}$) + 0.7547 (r = 0.9991). A detection limit of 3.0×10^{-9} mol L^{-1} (based on S/N = 3) was obtained with 80 s accumulation time. With the same electrode which was rinsed and then polished on a piece of weighting paper to remove the previous deposits on the surface of the electrode, the relative standard deviation (R.S.D.) is 2.46% for 7 times parallel detections of 3.0×10^{-7}
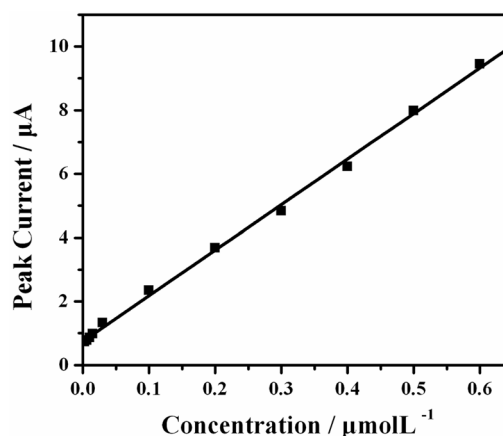
Figure 6. Calibration curve for the determination of hemoglobin.

Table 1. Interferences of some species on the current response of 3.0×10^{-7} mol L^{-1} hemoglobin

Interferences	Concentration/ ($\mu mol \cdot L^{-1}$)	Peak current change (%)
BSA	0.15	7.6
proline	46.5	1.3
arginine	15.7	3.8
L-threonine	83.9	7.4
L-histidine	6.5	−5.1
serine	19.0	0.61
tryptophan	97.9	0.26
valine	18.2	5.2
alanine	24.3	2.8
glycin	17.1	3.7
leucine	27.2	4.2
Co^{2+}	0.15	−7.1
Zn^{2+}	17.9	−1.0
Pb^{2+}	4.8	−1.0
Ni^{2+}	15.7	−1.0

mol/L Hb, suggesting satisfactory reproducibility.

Interference. Under optimal experimental conditions, the interferences of some metal ions, amino acids and bovine serum albumin (BSA) have been evaluated. The peak currents of 3.0×10^{-7} mol L^{-1} Hb in the absence and presence of foreign species were measured by DPV, respectively, and the error was consequently obtained. The results are listed in Table 1. It was found that praline, arginine, serine, tryptophan, alanine, glycin, leucine, Zn^{2+}, Pb^{2+} and Ni^{2+} almost do not interfere with the reduction current response of Hb

Table 2. Determination of hemoglobin in a spiked sample

sample	Spiked concentration (mol·L^{-1})	Co-existing species	Found (mol·L^{-1})	Recovery (%)	RSD
1	1.50×10^{-7}		1.45×10^{-7}	96.7	
2	1.50×10^{-7}	L-threonine, L-histidine,	1.48×10^{-7}	98.7	
3	1.50×10^{-7}	tryptophan, praline, arginine, BSA, Co^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}	1.49×10^{-7}	99.3	2.22
4	1.50×10^{-7}		1.53×10^{-7}	102.0	

(Co^{2+}: 1.0×10^{-5} mol/L; Cd^{2+}: 2.2×10^{-5} mol/L; Ni^{2+}: 4.2×10^{-5} mol/L; Zn^{2+}: 1.6×10^{-5} mol/L; L-Threonine: 2.1×10^{-5} mol/L; L-histidine: 1.9×10^{-6} mol/L; tryptophan: 1.0×10^{-6} mol/L; proline: 2.3×10^{-5} mol/L; arginine: 2.9×10^{-6} mol/L; BSA: 3.7×10^{-8} mol/L)

(signal change below 5.0%). However, BSA, L-threonine, L-histidine, valine and Co^{2+} have relatively obvious interference on the determination of Hb.

Determiantion of Hb in a spiked sample. In order to test the practical applicability of the proposed method, the concentration of Hb in a spiked sample was determined, and the results are shown in Table 2. The relative standard deviation for four-time parallel detections was 2.22%. In addition, the recovery on the basis of the above method was in the range of 96.7-102.0%. The result indicates that the determination of Hb at the acetylene black electrode using SDS as enhancing element is effective and sensitive.

Conclusion

In the present paper, electrochemical behaviors of Hb in the absence and presence of SDS at the acetylene black electrode were investigated. The results indicated that the electrochemical responses of Hb were facilitated by the anion surfactant SDS. The experimental parameters which would affect the reduction peak current response of Hb have been optimized. Advantages of this method are simple, sensitive and accurate, which were demonstrated by its application in the determination of Hb in a spiked sample.

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