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Voltammetric Studies of the Interaction of Tris (1, 10-phenanthroline) Cobalt (III) with Bovine Serum Albumin

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Abstract: The voltammetric methods were used to prove the interaction of metal complex $Co(phen)_3^{3+}$ with bovine serum albumin (BSA). The interaction of BSA with $Co(phen)_3^{3+}$ molecules using BSA-modified electrode is described. Information of the binding ratio and interaction mode can be obtained from their electrochemical behavior and electrochemical data. Furthermore, attenuated total reflection infrared experiment was performed to prove the interaction between complexes and BSA.

Keywords: Bovine serum albumin, nano-gold, modified electrode, cyclic voltammetry.

Recently, the studies on the interaction of DNA with small molecules have been attracting much interest[1-6]. These researches not only gave the important information about thermodynamics and kinetics, but also can prompt the combination between the electrode substances and biomacromolecule with super catalysis and sensor character. It is of great significance in understanding energy conversion and metabolism in live body, biomolecular structure, various physical and chemical characters for exploring the physiological action and action mechanism in organism, in exploiting new type biologic sensor *etc.*. As a carrier of albumin, the content of serum albumin is the largest in plasma. It can bind extensively with many endogensis and extergensis materials. There are many kinds of micro- and macro- elements in the living bodies, which participate many important life courses. The interactions of serum albumin with many remedy molecules are already studied[7-8].

In this paper, we focused our attention on the interaction of tris (1, 10-phenanthroline) cobalt (III) with BSA. A series of important data were obtained, such as binding ratio and interaction model with electrochemical and spectrochemical methods. The results showed that there is an interaction between BSA and the complexes.

Experimental

Bovine serum albumin (BSA) (Sino-American Biotechnology Company) and $HAuCl_4$ (Shanghai Chemical Reagent, China) were used without further purification. The BSA solution was prepared by dissolving it in 0.02 mol/L phosphate buffer (pH 7.0).

$Co(phen)_3(ClO_4)_3$, tris(1, 10-phenanthroline) cobalt (III) perchlorate, was synthesized as reported in the literature[9]. Colloidal gold sols with average particle diameter of 20 nm were prepared according to Frens[10]. As the supporting electrolyte, 0.1 mol/L KCl was used. Other reagents were of analytical grade. All solutions were prepared with double distilled water.

Electrochemical experiments were carried out using a three-compartment cell with BSA modified electrode or GCE as the working electrode, a platinum wire counter electrode, and an Ag/AgCl as the reference electrode. All reported potentials are against an Ag/AgCl without regard for the liquid junction. Experiments were carried out at 25°C. Cyclic voltammetric (CV) measurements were performed with a BAS-100A electrochemical analyzer (Bioanalytical systems Inc. USA) and Potentiostat/Galvanostat Model 283 (EG&G Instruments USA). Fourier transform infrared spectra were measured by a Nexus-870 (Nicolet, USA) equipped with Attenuated Total Reflection Spectra (ATR) accessory. An MCTA detector cooled with liquid nitrogen was used. The spectroscopy was manipulated with ATR corrector.

Modified electrode and electrochemical examination

Glass carbon electrodes (GCE), sealed in glass, were used throughout. The area of the GCE used is 0.07 cm^2. GCE were polished to a mirror with 0.05 μm alumina polishing suspension (Buehler) and then cleaned ultrasonically in acetone and water respectively, for 3 minutes. A freshly polished GCE was first treated by applying +1.5 V for 30 min in colloidal gold sols for immobilization of nano-gold, rinsed with doubly distilled water. Then, the electrode was immersed in the BSA solution for accumulation of BSA for 5 min at +1.5 V. Finally, the electrode was rinsed with doubly distilled water to remove unabsorbed BSA. Thus, BSA-modified electrode was obtained. It is denoted as BSA/NG/GCE.

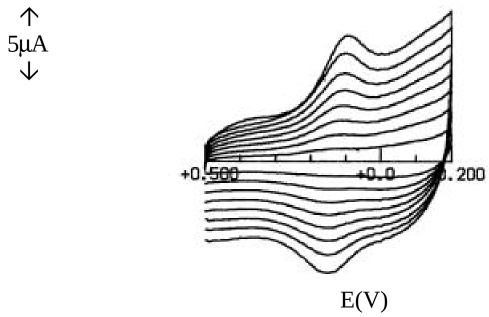
$Co(phen)_3^{3+}$ cations were accumulated onto the modified electrodes by immersing the electrodes in 1 mmol/L $Co(phen)_3^{3+}$ pH 7.0 phosphate buffer solution for 5 min. Then the electrode was rinsed with double distilled water and ready for cyclic voltammetric experiments. The CV of the solution of $Co (phen)_3^{3+}$ containing BSA was measured using the bare GCE with different concentration of the $Co (phen)_3^{3+}$.

Results and Discussion

Figure 1 shows CVs of $Co(phen)_3^{3+}$ on BSA/NG/GCE in pH 7.0 phosphate buffer solution at various scan rates. The linear dependence of the cathodic peak current on scan rate in the range of 10 to 200 mV/s indicates that $Co(phen)_3^{3+}$ is bound to the BSA/NG/GCE surface. From the slope of the $i_p \sim v$ curve, a total surface concentration of $\Gamma = 2.91 \times 10^{-9}$ mol/cm^2 $Co(phen)_3^{3+}$ on the BSA/NG/GCE surface can be calculated, according to the equation[11]: $i_p = n^2F^2\Gamma vA/4RT$. All above experiment results indicate that there is an interaction between BSA and $Co(phen)_3^{3+}$.

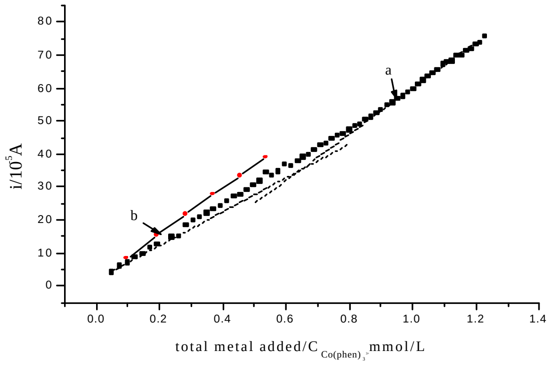
For explain the interaction of BSA with $Co(phen)_3^{3+}$ more clearly, CV experiments were carried out in which the ratio of BSA to $Co(phen)_3^{3+}$ was varied, furthermore, cyclic voltammograms of Co(phen)$_3^{3+}$ (varied concentration) in pH 7.0 phosphate buffer solution (no BSA) was carried out, too. Curve b shows a linear dependence of the cathodic peak current on the concentration of Co(phen)$_3^{3+}$ (without BSA). The results of titration of 1.14×10^{-8} mol/L BSA with Co(phen)$_3^{3+}$ was shown in **Figure 2a**. Two limiting regions are found (dash line). At large ratio of BSA to Co(phen)$_3^{3+}$, because of binding of Co(phen)$_3^{3+}$ to the large, slowly diffusing BSA, the current decreased. While at the very small ratio of BSA to Co(phen)$_3^{3+}$ the main contribution to current is free Co(phen)$_3^{3+}$ in solution[5] (the slope approximately is the same as of the curve in **Figure 2b**). One can from the intersection of the two limiting regions to set the bond ratio. It is probably 9.09×10^{-3}. The diffusion coefficient of the free Co(phen)$_3^{3+}$ was obtained from the $i_p/v^{1/2}$ data as D_f=2.65×10^{-6}cm^2/s ($i_p=269An^{3/2}D^{1/2}CV^{1/2}$).

Figure 1 Cyclic Voltammograms of Co(phen)$_3^{3+}$ on BSA/NG/GCE



in pH=7.0 phosphate buffer solution at different scan rates (v=10, 20, 30, 40, 50, 60, 70, 80 mv/s)

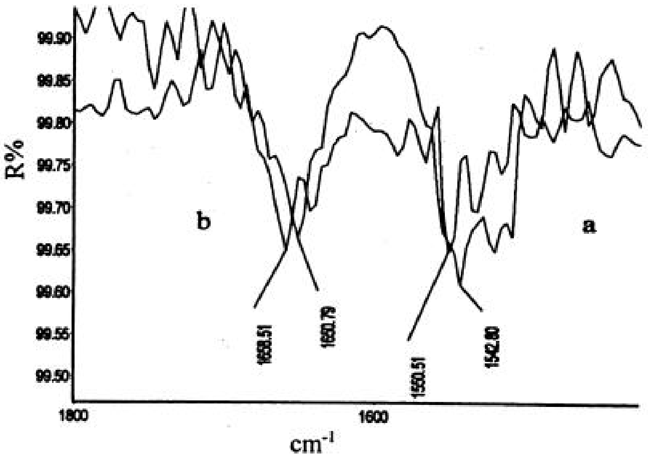
Figure 2 CV peak current for total Co(III) reduction for 5.68×10^{-8}mol BSA titrated with 5.0 mmol/L Co(phen)$_3^{3+}$.



Initial solution volume is 10 cm^3. Other conditions as in **Figure 1**.
a: square points represent the titration experiment data.
b: circle points are the data of free Co(phen)$_3^{3+}$. Dash lines are the limiting slopes.

The IR spectra of the BSA and BSA- Co(phen)$_3^{3+}$ compound in phosphate buffer (pH 7.0) are displayed in **Figure 3**. The strong absorption peak at 1680-1630 cm^{-1} is attributed to C=O group while absorption at 1570-1510 cm^{-1} was assigned to –CONH- group. Comparing the corresponding absorption peaks of the BSA and BSA-Co(phen)$_3^{3+}$, the later shifts to a longer wavelength (red shift). This indicate that the center cation (Co^{3+}) may bond to the BSA, which bring about a great variation of the spectroscopic character of coordination compounds due to the difference in micro-structure and micro-environment.

Figure 3 The infrared spectra of the BSA and BSA-Co(phen)$_3^{3+}$



a. BSA b: BSA-Co(phen)$_3^{3+}$

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