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Studies on the Erythromycin-Modified Glassy Carbon Electrode and Interaction with DNA

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Abstract: The chemically modified electrode (CME) which was constructed by covalent attaching **erythromycin** (ERM) to the glassy carbon (GC) surface was investigated in Tris-HCl buffer (pH=6.0) by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). In the potential range of -0.5~0.4V, CME yields a pair of stable redox wave. It is the carbonyl group of the ERM molecule immobilized on the GC surface that undergoes two electron redox process involving two protons. The interaction of CME with DNA was also studied by DPV. The CME shows the same interaction with DNA as that in the solution. And the result was proved by fluorescence.

Keywords: Erythromycin, chemically modified electrode, voltammetry, interaction, DNA.

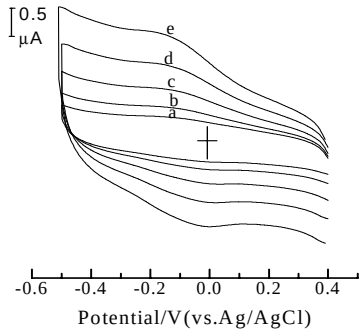
ERM belonging to tetradecabasic macrolide is one of the antibiotics obtained from the fermentation liquor of *Streptomyces erythreus*. It is mainly used for the infections of *Gram-positive bacteria* and *Gram-negative coccus*[1], and has important usage in clinical practice[2]. In the body, ERM prevents the proteins of germs from synthesis for it has stronger affinity to the bacterial nucleoprotein[1]. It was also reported[3] that ERM bound tightly with DNA. Until today, ERM is still one of the antibiotics that is most widely used in clinical practice for its low toxicity. However, little has been published[4] on the voltammetric behavior and quantitation of the saccharide-related antibiotics, novobiocin and erythromycin, despite their increasing pharmaceutical use. In this study, ERM was immobilized on the GC electrode surface and studied by voltammetry.

Preparation of the CME

Glassy carbon electrodes (No. 7 Telecommunicative Factory of Shandong in China, 4 mm diam) were polished successively with emery paper (600 mesh), diamond grinding paster (W 14) and Al_2O_3 (0.03 and 0.05 μm) to a shiny finish, and rinsed copiously with doubly distilled water between steps and at the end of the polishing. After being rinsed, the electrodes were sonicated in dilute acid, alcohol and doubly distilled water respectively, 10 min each time. The electrodes prepared as above were refluxed in the mixture (*ca.* 3 ml freshly distilled $SOCl_2$ and 2 ml freshly distilled acrylic acid in 15 ml of sodium dried benzene) for 2 hours, briefly rinsed with dry benzol, and left for 5 hours in a solution of *ca.* 1 mg of ERM in 15 ml of dry benzene with stirring at room temperature.

Thorough rinsing with dry benzene and reagent grade ethyl acetate were carried out to remove adsorbed ERM.

Figure 1. Cyclic voltammograms of CME at different scan rates (a)20 (b) 30 (c)50 (d) 80 (e)120 mV/s in **HCl-Tris** buffer(pH=6). $c_{\mathbf{NaCl}}$=50mmol/L, $c_{\mathbf{Tris}}$= 5mmol/L



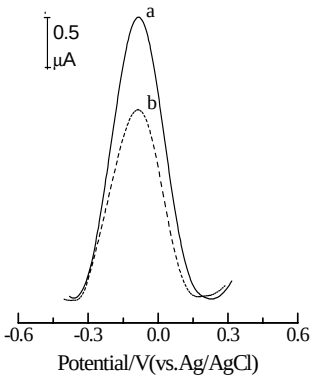
Electrochemical behavior of CME

Cyclic voltammograms of ERM attached to glassy carbon electrode prepared as above with the potential scan rate as a variable are illustrated in **Figure 1**. Unlike the $v^{1/2}$ dependence of i_p for the solution-confined ERM, CME shows a direct dependence on v (0.02V/s ~ 0.2V/s, correlation coefficient 0.9945) diagnostic of the trend expected for surface-bound species. In Tris-HCl buffer (pH 6.0), the redox reactions occur at potential similar to the $E^{0'}$(the formal potential $E^{0'}=(E_{pa}+E_{pc})/2$) values for unattached ERM (0.065V vs. Ag/AgCl), typical of modified electrodes[5, 6].

The electrochemical behaviors of unattached ERM are dependent on the concentration of hydrogen ion in aqueous solutions[7]. The influence of $[H^+]$ on the reaction of attached ERM was studied by DPV. With the pH value decreasing, the reduction peak potential shifts to more positive, and the peak current becomes larger, indicating that the hydrogen ion must be taking part in the electrode reactions.

According to the literature[8], we studied the relationship between $E_{1/2}$ and the pH value by DPV. We assume that E_p of DPV is equal to $E_{1/2}$ when ΔE is small enough. In the range of the pH value (3.45~7.44), $E_{1/2}$ is linear with the pH value (R=0.9855), and the slope is 27 mV/pH that is close to 26 mV/pH[8]. Recently, we studied[7] ERM in solutions, and reported that the carbonyl group of the ERM molecule is electroactive and experiences a two-electron process. From the results, we conclude that the redox reaction of the surface functionalities on CME is a two-electron redox process involving two protons. We believe that it is the carbonyl group that is reduced in the solution.

Figure 2. Differential pulse voltammograms of CME before (a) and after (b) addition of **DNA** (c_{DNA}=7.0×10^{-9}mol/L) in **HCl-Tris** buffer (pH=6). c_{NaCl}=50mmol/L, c_{Tris}=5mmol/L.

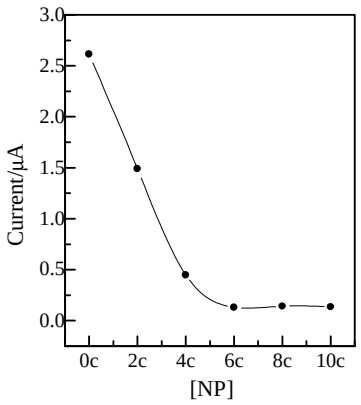


The interaction with DNA

The interaction between CME and DNA was studied by DPV for further accurate determination of the peak current and the peak potential. The differential pulse voltammograms of CME in the absence and presence of DNA is shown in **Figure 2**. After the addition of DNA in the solution, change in peak current suggests that binding has taken place between ERM attached to the electrode and DNA.

On the addition of different amounts of DNA, the peak current of ERM attached to the electrode is plotted as a function of concentration of DNA ([NP]) (**Figure 3**). The magnitude of the peak current decreases largely, and the decrease in peak current becomes slight when [NP] is 6c (c=3.546x10^{-9}mol/L), then remains independent of the concentration of DNA. This illustrates that ERM attached to the electrode interacts with DNA quantitatively, and the interactive equilibrium is reached at the [NP] value of 6c. It is similar to the interaction of unattached ERM with DNA. In solutions, the decrease in current in CV experiments may be attributed to the diffusion of ERM bound to the large, slowly diffusing DNA molecule. The change in current upon DNA addition can be explained in terms of diffusion of an equilibrium mixture of free and bound ERM to the electrode[7]. But, for interaction of ERM attached to the electrode surface with DNA, we can not explain by that, because there is no diffusion to the electrode surface. We believe that the interaction of ERM attached to the electrode surface with DNA is similar to unattached ERM— both partially intercalate in the helix of DNA *via* tetradecabasic macrocycle of the ERM molecule. However, ERM attached to the electrode surface intercalates between adjacent bases of DNA *via* the side of tetradecabasic macrocycle including carbonyl group. The decrease in current can be explained by that after ERM intercalating in the helix of DNA, the carbonyl group is surrounded by the helix of DNA, and then can not be reduced.

Figure 3. Relationship between peak currents and concentrations on addition of **DNA**. c=3.546×10^{-9}mol/L. in **HCl-Tris** buffer (pH=6) c_{NaCl}=50mmol/L, c_{Tris}=5mmol/L.



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