Trade Science Inc.

April 2006 Volume 2 Issue 2

Analytical CHEMISTRY
An Indian Journal

Full Paper

ACAIJ, 2(2), 2006 [60-64]

Studies Of The Electrochemical Behavior Of Epinephrine Immobilized On Self-Assembled Monolayers

Lu Xiaoquan
Collge of Chemistry and Chemical Engineering, Northwest Normal University, Lanzhou 730070 (P.R.CHINA)
E-mail: Luxq@nwnu.edu.cn

Zuo Guofang, Chen Jing, Zhang Yan, Liu Xiuhui, Liu Hongde, Zhang Limin
Collge of Chemistry and Chemical Engineering,
Northwest Normal University,
Lanzhou 730070, (P.R.CHINA)

Received: 12th October, 2005
Accepted: 12th December, 2005
Web Publication Date: 5th April, 2006

ABSTRACT

Pure and mixed 3-mercaptopropionic acid monolayers were prepared on gold electrode surface with the intention of studying the electrochemical behaviors of epinephrine (EP). The self-assembled monolayers (SAMs) were characterised by electrochemical reductive desorption of the thiolate from the gold surface. The cyclic voltammetry was used to investigate monolayers which reveals that the $2e^-$ transfer of EP at the electrode by one step and the electron transfer coefficient ks is calculated as 0.185 cm s^{-1}. The experiments have also shown that the proportions of the two compounds yield modified electrodes exhibiting corresponding electrochemical behaviors for the EP. With increased the proportion of mercaptopropionic acid on electrodes, the electrochemical respondes of EP corresponding increased. The reaction mechanism of EP immobilized on SAMs were also discussed.

© 2006 Trade Science Inc. - INDIA

KEYWORDS

Epinephrine(EP);
Immobility;
Self-assembled monolayers (SAMs);
Electron transfer;
Reaction mechanism.

INTRODUCTION

Epinephrine (EP) is an important catecholamine neurotransmitter in the mammalian central nervous system. The oxidative reactive of catecholamines are involved in various enzymatic and nonenzymatic processes in human body. The studies of their electrochemical behaviors can reveal their physical function. But at the conventional electrodes, the electrochemical characteristics of EP is irreversible, which results in the difficulty to research its electron transfer[1,2].

Self-assembled monolayer approach is a good way to control the surface of electrodes at the molecular level[3]. Such assemblies could provide a means to control the chemical and physical properties of interfaces for a variety of heterogeneous phenomena. Such chemically modified electrodes also have good stability and have been widely used to study the electrochemical behavior of some biomolecules.

In this paper, 3-mercaptopropionic acid was used as the assembling molecule. Through the covalenting attachment at the activation with EDAC and NHS, EP were immobilized on the surface of modified electrodes. At these electrodes, a pair of well-defined redox waves of EP were obtained in PBS solution and the oxidation of EP was proved to be a two-electron transfer quasi-reversible process.

EXPERIMENTAL

Instruments and reagents

3-Mercaptopropionic acid (MPA, Fluka), 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide (EDAC, Sigma), N-hydroxysuccinimide (NHS, Sigma), 1-propanethiol (C_3SH, Fluka), epinephrine (EP, Sigma), were used as received. All other reagents used were of analytical grade. Water was dual distilled from an all-quartz still. High purity nitrogen was used for deaeration.

Cyclic voltammetry were performed with a CHI 832 Electrochemical Workstation (CH Instrument, USA) in a conventional three-electrode cell. The working electrodes used were bare or modified gold electrode (Model CHI101, 2-mm diameter). A twisted platinum wire was used as the counter electrode and a Ag/AgCl electrode as the reference electrode.

Electrode modification

Before each experiment, the surface of the gold electrode was first polished with 0.3 and 0.05 μm α-Al_2O_3 powder successively, and washed ultrasonically in deionized water for 10 minutes. After that, the substrates were cleaned in piranha solution for a few minutes at 90°C. Rinsed with distilled water and ethanol, and dried. The gold electrode were immersed immediately in a 1mM solution of MPA or mixed solutions of MPA:C_3SH in ethanol for 2h. After assembly, the electrodes were thoroughly rinsed with ethanol and water and dipped either in a 1mg/mL EDAC-1mg/mL NHS solution for 1 h, or in a 1 mmol/L EP in PBS, pH=7.0 for about 12 h at 4°C.

RESULTS AND DISCUSSION

Characterization of the MPA and C_3SH self-assembled monolayers with electrochemical method

The redox behavior of a reversible couple can be used to probe the packing structure of the monolayer[4,5]. Figure 1 shows the cyclic voltammograms of bare and MPA self-assembled gold electrodes in 1mM $Fe(CN)_6^{3-}$+0.1MKCl solution. Comparing figure 1a with b, it can be seen that the peak current was decreased and the peak-to-peak separation (ΔE_p) was increased. The modified electrodes with pure self-assembled monolayers of MPA and C_3SH were also characterized by electrochemical reductive desorption in 0.5M KOH solution, and representative cyclic voltammograms are illustrated in figure 2. In agreement with the expected behaviour for the desorption of a thiolate monolayer the modified electrodes exhibit a main cathodic peak assigned[6] to the break of the sulphur–gold bond, a one-electron reduction process. Based on the relation $Q = nFA\Gamma$ (where Q is the total charge (C), A is the electrode surface area (cm^2), after correcting for surface roughness (roughness factor = 1.13). Γ is the surface coverage (mol/cm^2), n and F have their usual electrochemical meaning, the charge under a desorption wave was then used to provide a measure of the surface coverage of MPA.

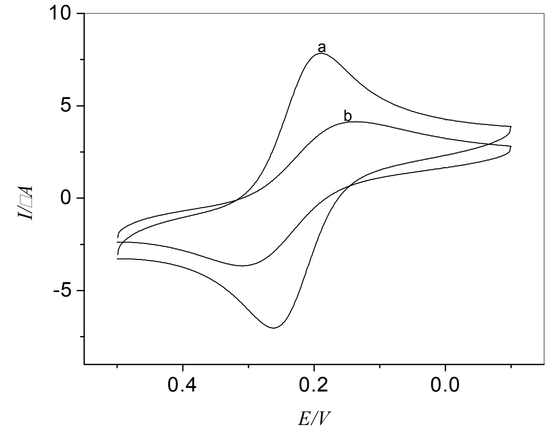
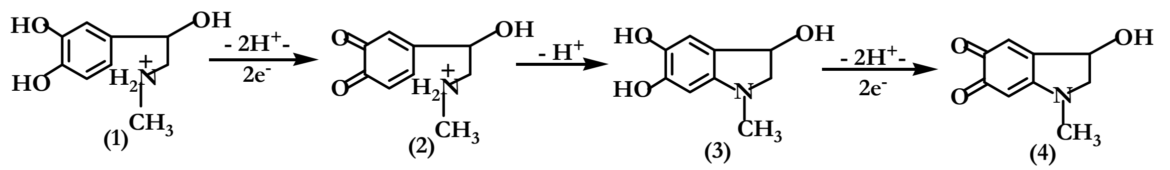


Figure 1: Cyclic voltammograms of 1mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ at a bare gold electrode (a) and MPA SAM/Au electrode (b). 0.1MKCl; scan rate: 50mV/s.



SCHEME 1: The reaction mechanism of EP in bare gold electrode.

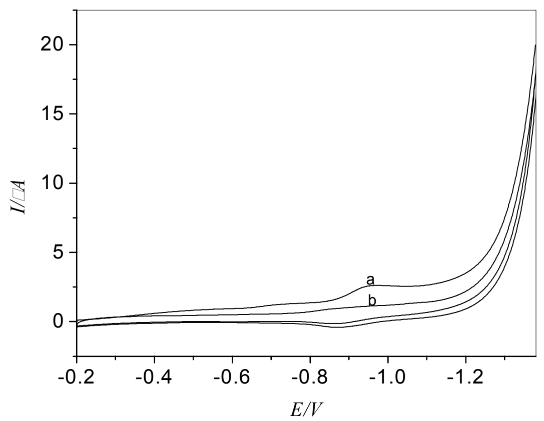


Figure 2: Electrochemical reductive desorption of MPA SAM from gold in deaerated 0.5M KOH solution at 20mV/s.First circle (a); scond circle (b).

By calculating, the estimated coverages of gold electrodes modified with pure MPA is 5.9×10^{-10} mol/cm^2. The calculated value for a packed alkylthiol ($\sqrt{3}\times\sqrt{3}$)/R30° overlayer structure at Au is 7.6×10^{-10} mol/cm^2 [7], which is in agreement with the value obtained for MPA.

The electrochemical response of EP immobilized on the modified electrodes

The reactivity of the immobilized EP on SAMs is towards the direct chemical coupling. The cyclic voltammetric response of EP in a PBS solution on a bare gold electrode was shown in figure 3. At the bare gold electrode, the cyclic voltammograms of EP demonstrated irreversible waves. According to Ref[8], the redox peak has been assigned to the redox transformation of EP hydroquinone/quinone. The instability of the oxidated form of EP, leading to the formation of intermediates, which can involve a ring-closure through the amine group, should be responsible for the irreversible electrochemical conversion. Peak a is the oxidation of EP to the open-chain quinone. Peak b is the reduction of this quinone, while peak c is the reoxidation of the cyclized product leucoadrenochrome to adrenochrome (SCHEME 1).

Pure MPA/SAMs were immersed in 0.1M PBS solution and the voltammograms obtained. As it can be observed in figure 4.2, the cyclic voltammogram of a MPA monolayer in PBS does not exhibit any redox process. But, a well-defined redox wave of EP covalenting attachment with MPA was observed at the modified electrode with ΔE_p (Figure 4) , the difference between peaks, was about 127mV. The formal potential of EP ($E_{1/2}$ vs Ag/AgCl) immobilied on the modified electrodes estimated was 0.20V. Undoubtedly it indicates that immobilization of EP on the electrode was accomplished. Furthermore, the observed peak reversibility of the immobilized EP, contrasting with the behavior on bare gold electrode in EP solution (Figure 3), is most probably due to the fact that amine group is linked to the SAM and therefore the closing of the ring through this group is no longer possible.

In order to further prove that EP were immobilized on MPA SAMs/Au through the covalenting attachment rather than static action. We also observed the cyclic voltammograms of EP in PBS (pH7.0)

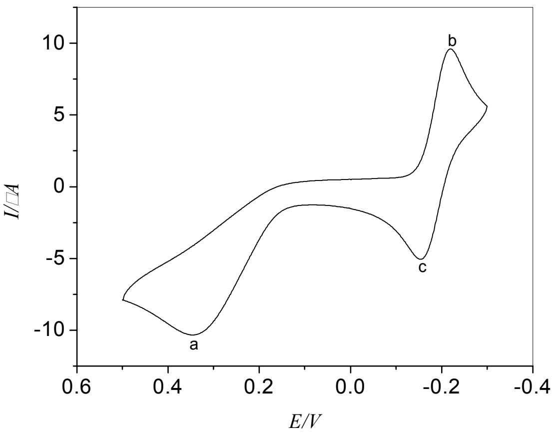


Figure 3: Cyclic voltammograms at a bare gold electrode for 1mM deaerated EP solution. 0.1M pH7.0 PBS. 0.1MKCl; scan rate: 50mV/s.

solution with MPA SAMs/Au and found the similar peak compared with bare gold electrode. Therefore, the results about EP immobilized on MPA SAMs/Au through the covalenting attachment could been gotten.

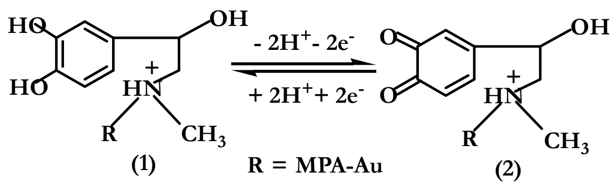
To verify whether the reversibility of EP is modified, the standard rate constant ks is determined at the self-assembled electrode. Plot E_{Pa} and E_{Pc} as the function of f lnν (ν: scan rate, mV s^{-1}). At scan rates (20~300 mV s^{-1}), the curves approach the straight line. Based on the slope and the intercept, the rate constant can be gotten according to following equations[9].

$$E_c = E^o - RT/\alpha nF \ln(\alpha nF\nu / RTk_s) \quad (1)$$

$$E_a = E^o + RT/(1-\alpha)nF\ln(1-\alpha)nF\nu/RTk_s \quad (2)$$

$$\log k_s = \alpha \lg(1-\alpha) + (1-\alpha)\lg\alpha - \lg(RT/nF\nu g) - \alpha(1-\alpha)nF\Delta E_p/2.3RT \quad (3)$$

The rate constants (ks) are calculated as 0.185 cm s^{-1} of EP immobilized at the self-assembled electrode,. According to the slope of the straight line, it is obtained that n = 1.9 and α = 0.42. This reveals the $2e^-$ transfer of EP at the electrode by one step. It is reported[10,11] that in the process of oxidization of DA, NE and EP by oxidizer, the free radical existing in the course of the electrode reaction of EP and the step of its forming decide the rate of the electrode reaction. But in our study, we didn't find the free radical at the redox of EP immobilized at the self-assembled electrode. So, the possible reaction mechanism of EP immobilized SAMs, we thought that peak a is the oxidation of EP to the quinone and peak b is the reduction of this quinone. Figure 4.1 was MPA SAM/Au in the same experimental condition. These results show that the electrochemical response of EP immobilized on the modified electrodes are promoted. The SCHEME of the reactions are expressed below (SCHEME 2):



SCHEME 2: The reaction mechanism of EP immobilized on the modified electrodes.

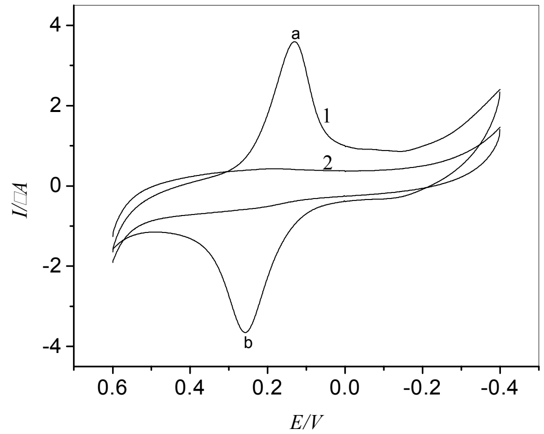


Figure 4: Cyclic voltammograms at a EP/MPA SAMs electrode (a) and at a MPA SAMs electrode (b). 0.1M pH7.0 air saturation PBS. 0.1MKCl; scan rate: 50mV/s.

When preparing mixed EP/SAMs with the purpose of diluting a given functional group on the surface, for a specific interaction, a common step is to check whether a solution proportion of 1:1, 1:4 of MPA:C_3SH yields similar surface fraction. Fig.5 shows that the peak currents of EP with changing the proportion of MPA on the films. Fig.5a was pure EP/MPA/Au in deaerated PBS (pH7.0, 0.1M, 0.1MKCl) solution. Figure 5b was 1:1 of MPA:C_3SH on electrode coupling EP in the same solution. Controlling the same condition and only changing the

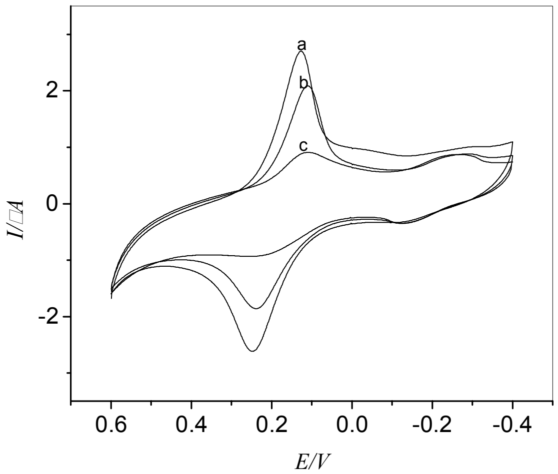


Figure 5: Cyclic voltammograms of EP immobilized at MPA/SAMs electrode (a); MPA: C_3SH (1:1) electrode (b); MPA:C_3SH(1:4) electrode (c). 0.1M deaerated pH7.0 PBS. 0.1MKCl; scan rate: 50mV/s.

proportion of MPA:C_3SH to 1:4, the voltammetric response of EP modified electrode was shown in figure 5c. With the proportion of MPA decreased on electrodes, the anodic and cathodic peak currents corresponding decreased, which was also in agreement with the value obtained for MPA or mixed MPA:C_3SH on gold electrodes. But from figure 5 we could find that the anodic and cathodic peak currents of EP immobilized pure or mixed SAMs didn't present the linear transformation. There are two possible reasons to explain this phenomenon. One is that MPA and C_3SH are short mercaptan molecule, There were many pinhole defects and callapsed sites[8] in pure or mixed monolayers and the electron could transfer through the pinhole defects or callapsed fields (Seen figure 3). The other is that the molecule dimension of EP is bigger than MPA's, so not all MP could combine with EP.

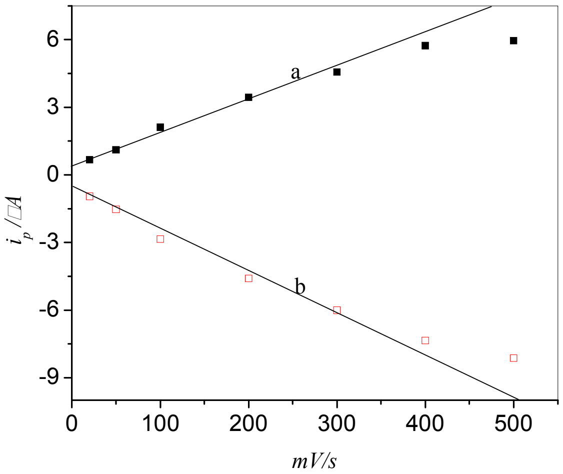


Figure 6: Plots of the anodic (a) and cathodic (b) peak currents against the sweet rate. 0.1M deaerated pH7.0 PBS. 0.1MKCl

Nevertheless, plots of the anodic and cathodic peak currents of EP immobilized pure SAMs against the sweet rate were linear over the range from 20 to 400 mV/s in the same solution. As demonstrated in the figure 6, electrode reaction is controlled by the surface of EP and also provides an additional support for the chemical attachment of the redox couple.

CONCLUSION

Immobilized epinephrine (EP) on SAMs/Au electrodes through the covalenting attachment, the electrochemical behavior of EP had been reviewed. Comparing with EP at bare gold electrode in solution, the electrochemical properties of EP changed greatly. A pair of well-defined redox waves of EP were gotten in PBS solution and the oxidation of EP was proved to be two electron transfer quasi-reversible process. This could provide one good chance to explore the electron transfer and the reaction mechanism about biologic molecules in life system[12].

ACKNOWLEDGMENTS

This work was supported by the Natural Science Foundation of China(No. 20275031, 20335030), The Teaching and Research Award Program for Outstanding Young Teachers in Higher Education Institutions of MOE P.R.C., The Key Laboratory of Polymer Materials of Gansu. The State Key Laboratory of Electroanalytical Chemistry (SKLEAC), Changchun Institute of Applied Chemistry, Chinese Academy of Sciences.

REFERENCES

[1] R.N.Adams; J.Pharm.Sci., **58(10)**, 1171 **(1969)**.
[2] M.D.Hawley, S.V.Tatawawadi, S.Piekarski, R.N.Adams; J.Am.Chem.Soc., **89**, 447 **(1967)**.
[3] A.Ulman; 'An Introduction to Ultrathin Organic Film', Academic Press, Boston, MA. **(1991)**.
[4] C.J.Miller, C.Pierre, M.J.Gratzel; J.Phys.Chem., **95**, 877 **(1991)**.
[5] M.D.Porter, T.B.Bright, C.E.D.Chidsey; J.Am.Chem. Soc., **109**, 3359 **(1987)**.
[6] H.O.Finklea, A.J.Bard, I.Rubinstein; 'Electroanalytical Chemistry: A Series of Advances', Marcel Dekker, New York, **19**, 109 **(1996)**.
[7] C.A.Widrig, C.Chung, M.D.Porter; J.Electroanal. Chem., **310**, 335 **(1991)**.
[8] H.M.Zhang, X.L.Zhou, R.T.Hui, N.Q.Li, D.P.Liu; Talanta, **56**, 1084 **(2002)**.
[9] E.Laviron; J.Electroanal.Chem, **101**, 19 **(1979)**.
[10] C.C.Felix, R.C.Sealy; J.Am.Chem.Soc., **103**, 2831 **(1981)**.
[11] B.Kalyanaraman, C.C.Felix, R.C.Sealy; J.Biol. Chem., **259**, 7584 **(1984)**.
[12] J.F.Cabrita, L.M.Abrantes, A.S.Viana; Electrochim. Acta, **50**, 2117 **(2005)**.