Study on the Molecular Recognition of Adrenaline by Supramolecular Complexation with Formamide

Tao Liu and Xiao-Wen Zheng

***Abstract*—Using cyclic voltammetry, we have investigated the electron transfer properties of supramolecular complexes of formamide (FA) with adrenaline (Ad) at graphite electrode and graphite electrode soaked in paraffine wax, respectively. The experimental results show that FA will affect the electron transfer properties of Ad. The formed supramolecular complexes by hydrogen bond (H-bond) interaction between FA and Ad will slow down the diffusion ability of adrenaline and make it hard to donate electron and be oxidized.**

***Index Terms*—Cyclic voltammetry, formamide, adrenaline.**

I. Introduction

The importance of the amide linkage is demonstrated by the fact that the amide peptide bond is the basic linkage in peptides and proteins. Its duality to function as both hydrogen bond (H-bond) donor and acceptor makes the linkage versatile in molecular assembly and recognition. For examples, the H-bonds among peptide bonds in proteins are the key driving forces for forming the organized α-helix and β-sheet secondary structures. The linkage also plays important roles in the pharmacophores of the antibacterial agents such as penicillins and carbacephems and has been utilized in designing enzyme inhibitors [1]-[4]. Formamide (FA) is one of the simplest molecules usually chosen as a model for studying biological systems exhibiting the peptide type of bonding and DNA structures. The characterization of the hydrogen-bonding interaction between formamide and water has been well studied by many theoretical calculations [5]-[9], while in the case of formamide-adrenaline complex there are a few investigations. Adrenaline (Ad) is a secreted hormone from the adrenal medulla, as an important catecholamine neurotransmitter in the mammalian central nervous system. Therefore, the investigation of the H-bond interaction between formamide and adrenaline must be very useful for studying biological systems exhibiting the peptide type of bonding and DNA structures.

II. Experimental

The pH 7.4 Krebs–Ringer phosphate buffer (KRPB) solution [10], consisting of 0.025 mol/L Na_2HPO_4, 0.13 mol/L NaCl, 4.9×10^{-3} mol/L KCl, and 2.5×10^{-3} mmol/L $MgSO_4$, mimics the physiological condition in human body. The reagent of adrenaline (>97%) was supplied by Fluka Co. (Sweden). The concentration of adrenaline aqueous solution was 3.0×10^{-4} mol/L. Other employed solutions were prepared with analytic grade reagents and doubly distilled water.

The electrochemical experiments were performed on an EG&G PAR M398 electrochemical impedance system with an M283 potentiostat/galvanostat. The three-electrode-system was used to carry out electrochemical tests. A graphite electrode served as a working electrode, a platinum wire served as a counter electrode, and a saturation calomel electrode (SCE) served as reference electrode. A luggin capillary was used to connect the reference and working electrodes. Highly pure nitrogen gas was passed through the solution for 15 min to remove dissolved oxygen in solution before measurements, and all measurements were carried out under nitrogen atmosphere at room temperature.

III. Results and Discussion

The cyclic voltammetry (CV) curves of adrenaline at graphite electrode in the solution with different concentration of FA are presented in Fig. 1. peak 1 of curve a corresponds to the oxidation peak of adrenaline. It can be seen that, with the addition of FA, the anodic peak current of peak 1 increases and reaches the maximum at 1:1 concentration ration of Ad with FA. To continue adding FA, the current of peak 1 will decrease instead. The phenomena can be interpreted as follows: the graphite electrode surface is loose and porous, which will provide strong absorption strength and big active surface area for electro-redox reactions. So small molecule, such as FA, will be absorbed into the micropores. Therefore, with the addition of FA at the beginning, the anodic peak current of peak 1 increases, which because the formation of the complex between Ad and FA combinding with H-bonds brings more Ad molecules into the micropores and makes the electro-redox reaction be easier. However, with the increase of the concentration of FA further, the H-bonds between Ad and FA will protect the phenolic hydroxyl group effectively and make it hard to donate proton, causing the anodic peak current of peak 1 decreases.

In order to prove above explication, the CVs of Ad on the graphite electrode (curve a) and graphite electrode soaked in paraffine wax (curve b) in KRPB are conducted and the results are shown in Fig. 2. The graphite electrode soaked in paraffine wax was fabricated according to the literature of [11]. It can be seen from that the peak currents on graphite electrode soaked in paraffine wax decrease obviously compared with those on graphite electrode, which because

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the mcropores are blocked by paraffine wax. So the explication above is reasonable.

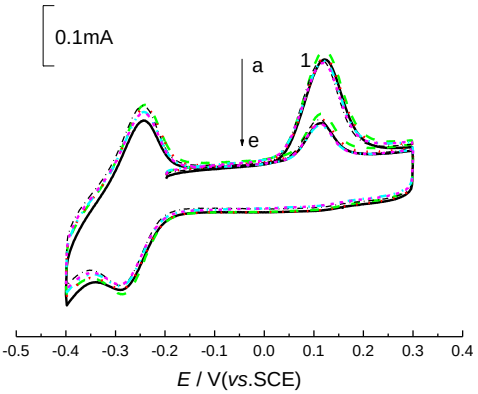

Fig. 1. Cyclic voltammograms of 3.0×10^{-4} mol/L adrenaline in KRPB (pH= 7.4) buffer solution using graphite electrode as a working electrode.

The voltammograms are obtained with a scan rate of 100 mV/s in the solution of adrenaline containing different concentration of FA. The cures of a, b, c, d, and e corresponds to the FA concentration of 3.0×10^{-4}, 6.0×10^{-4}, 0, 9.0×10^{-4}, and 1.2×10^{-3} mol/L, respectively. The peak 1 denotes the anodic peak.

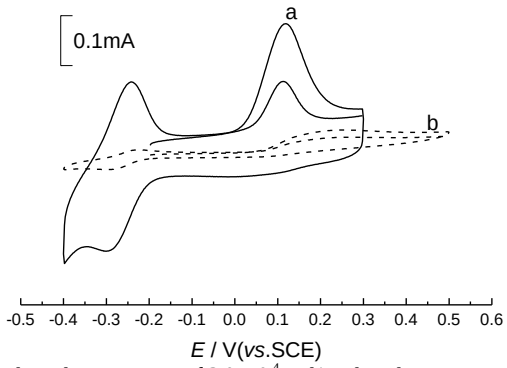

Fig. 2. Cyclic voltammograms of 3.0×10^{-4} mol/L adrenaline in KRPB (pH= 7.4) buffer solution with graphite electrode and graphite electrode soaked in paraffine wax as working electrode using a scan rate of 100 mV/s.

The cures of a and b denote the cyclic voltammograms with graphite electrode and graphite electrode soaked in paraffine wax, respectively.

The CVs of adrenaline on the graphite electrode soaked in paraffine wax in KRPB buffer solution containing different concentration of FA are also studied (Fig. 3). The results show that the anodic peak current of peak 1 decreases a little with the addition of FA. The phenomena demonstrate that FA inhibits the electron transfer of Ad weakly, which is consistent with the calculational result (see below) that there are weak H-bond interaction between FA and Ad, and the H-bonds will protect the phenolic hydroxyl group of Ad effectively and cause the oxidation reaction ability of Ad decrease.

The CV curves of 6×10^{-3} mol/L adrenaline at platinum electrode in the KCl-HCl solution (constant ionic strength (I=1) and constant PH value is 1) with different concentration of formic acid are presented in Fig. 4 showing peak 1 of curve a corresponds to the oxidation of adrenaline into adrenalinequinone (anodic peak), and peak 2 of curve a corresponds to the reduction of adrenalinequinone into adrenaline (canodic peak). It can be seen that with the addition of formic acid, the electron transfer ability of adrenaline decreases as follows: the anodic peak potential (E_{pa}) shifts positively, the canodic peak potential (E_{pc}) shifts negatively, the peak-to-peak potential separation between anodic and canodic peak potential (ΔE_p) increases, and the anodic and canodic peak current (i_{pa} and i_{pc}) decrease significantly. The results demonstrate the inhibition effect of formic acid on the electron transfer reaction of adrenaline, which has been verified by the fact that formic acid can form stable supramolecular complexes with adrenaline by hydrogen bond interaction and the formed supramolecular complexes will protect the phenolic hydroxyl groups of adrenaline and make it hard to donate H^+ and be oxidized.

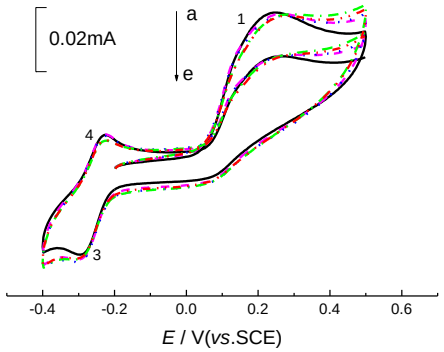

Fig. 3. Cyclic voltammograms of 3.0×10^{-4} mol/L adrenaline in KRPB（pH =7.4）buffer solution with graphite electrode soaked in paraffine wax as a working electrode.

The voltammograms are obtained in the solution with different concentration of FA and use a scan rate of 100 mV/s. The cures of a, b, c, d, and e denote the FA concentration of 0, 3.0×10^{-4}, 6.0×10^{-4}, 9.0×10^{-4}, and 1.2×10^{-3} mol/L, respectively. The peak 1 denotes the anodic peak.

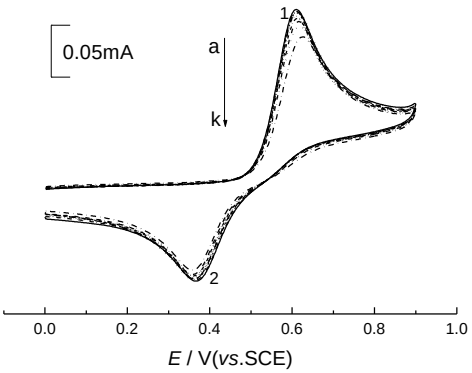

Fig. 4. CV curves of 6×10^{-3} mol/L adrenaline at platinum electrode in KCl-HCl (pH =1) solution with different concentration of formic acid. Scan rate: 100 mV/s. $C_{adrenaline}$:$C_{formic\ acid}$ (a) 1:0; (b) 1:1; (c) 1:2; (d) 1:3; (e) 1:10; (f) 1:20; (g) 1:50; (h) 1:100; (i) 1:200; (j) 1:500; (k) 1:750.

The CV curves of 6×10^{-3} mol/L adrenaline at platinum electrode in a series of KCl-HCl solution (constant ionic strength (I=1) of KCl) with different high concentrations of formic acid and different Ph values are shown in Fig. 5. It can be seen from Fig. 5 that with the concentration of formic acid increasing and the Ph value of solution decreasing, the peak-to-peak potential separation between anodic and canodic peak potential becomes larger, while the anodic and canodic peak current becomes smaller. In this experimental condition, there is a linear relationship between E_{pa} and i_{pa}

with Ph value.

In order to eliminate the influence of Ph values on the experimental results, we drew the CV curves of 6×10^{-3} mol/L adrenaline at graphite electrode in the HCl solution (constant ionic strength) with different Ph values (see Fig. 6). We can find that in the HCl solution without formic acid, ΔE_p decreases and i_p increases with the Ph value becoming smaller, which is contrary to the changing trend of ΔE_p and i_p for adrenaline in the solution with different high concentrations of formic acid and different Ph values. The phenomenon can be interpreted by the hydrogen bond interaction between adrenaline and formic acid, which will protect the phenolic hydroxyl groups of adrenaline and make it hard to donate H^+ and be oxidized. The effect of hydrogen bond interaction on the adrenaline is much larger than the effect of Ph value on it. Therefore, ΔE_p will decrease and i_p will increase with the Ph values increasing in the solution with different high concentrations of formic acid and different Ph values.

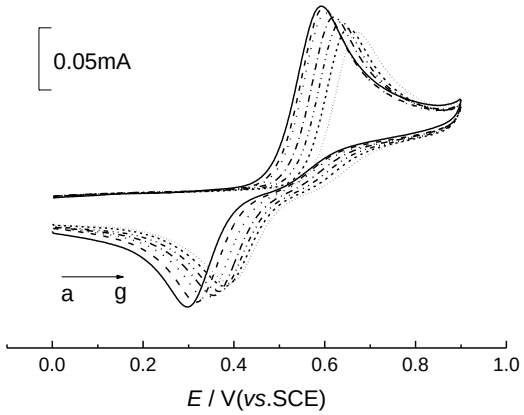

Fig. 5. CV curves of 6×10^{-3} mol/L adrenaline at platinum electrode in a series of KCl-HCl solution with different high concentrations of formic acid and different Ph values. Scan rate: 100 mV/s. pH: (a) 1.84; (b) 1.59; (c) 1.34; (d) 1.00; (e) 0.71; (f) 0.43; (g) 0.16. $C_{\text{formic acid}}$: (a) 0.47; (b) 1.17; (c) 2.33; (d) 4.66; (e) 6.99; (f) 9.34; (g) 11.66 mol/L.

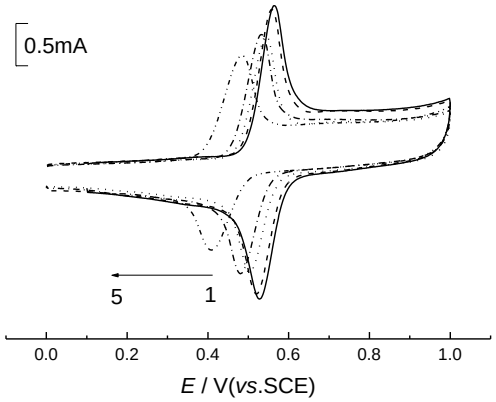

Fig. 6. CV curves of 6×10^{-3} mol/L adrenaline at graphite electrode in the HCl solution with different PH values. Scan rate: 100 mV/s. C_{HCl}: (1) 0.5; (2) 0.4; (3) 0.2; (4) 0.1; (5) 0.01 mol/L.

In this section, the KCl-HCl solution (constant ionic strength (I=1) and constant Ph value is 1) was also used as the studying medium as the experiment about acetic acid. The CV curves of 6×10^{-3} mol/L adrenaline at platinum electrode in the KCl-HCl solution with different concentration of acetic acid are presented in Fig. 7.

The changing trend is similar to the experimental phenomena of formic acid system. With the concentration proportion of acetic acid increasing, E_{pa} shifts positively, E_{pc} shifts negatively, ΔE_p increases, and i_p decreases. The results show that there is also inhibition effect of acetic acid on the electron transfer reaction of adrenaline. The hydrogen bond interaction will protect the phenolic hydroxyl groups of adrenaline and make it hard to donate H^+ and be oxidized.

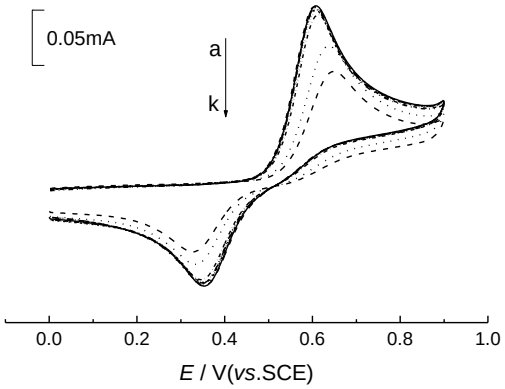

Fig. 7. CV curves of 6×10^{-3} mol/L adrenaline at platinum electrode in KCl-HCl (pH =1) solution with different concentration of acetic acid. Scan rate: 100 mV/s. $C_{\text{adrenaline}}$:$C_{\text{acetic acid}}$ (a) 1:0; (b) 1:1; (c) 1:2; (d) 1:3; (e) 1:10; (f) 1:20; (g) 1:50; (h) 1:100; (i) 1:200; (j) 1:500; (k) 1:1000.

IV. Conclusion

The electron transfer properties of supramolecular complexes of FA with Ad at graphite electrode and graphite electrode soaked in paraffine wax using CV have been investigated in this paper. Our experimental data show that FA affects the electron transfer properties of adrenaline. The supramolecular complexes of FA-Ad formed by H-bond interaction between FA and Ad will protect the phenolic hydroxyl groups of adrenaline and make them hard to donate electron and be oxidized.

Acknowledgements

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References

[1] A. Greenberg, C. M. Breneman, and J. F. Liebman, *The Amide Linkage: Structural Significance in Chemistry, Biochemistry, and Materials Science*, New York, USA: Willy, 2000.

[2] H. Gong, H. Zhou, and J. G. H. Hickford, "Diversity of the glycine/tyrosine-rich keratin-associated protein 6 gene (*KAP6*) family in sheep," *Mol. Biol. Rep*., vol. 38, pp. 31–35, 2011.

[3] D. B. Boyd, "Application of the hypersurface iterative projection method to bicyclic pyrazolidinone antibacterial agents," *J. Med. Chem.*, vol. 36, pp. 1443-1449, 1993.

[4] L. N. Jungheim, D. B. Boyd, J. M. Indelicato, C. E. Pasini, D. E. Preston, and W. E. Jr. Alborn, "Synthesis, hydrolysis rates, supercomputer modeling, and antibacterial activity of bicyclic tetrahydropyridazinones," *J. Med. Chem.,* vol. 34, pp. 1732-1739, 1991.

[5] F. J. Lovas, R. D. Suenram, and G. T. Fraser, "The microwave spectrum of formamide–water and formamide–methanol complexes," *J. Chem. Phys.*, vol. 88, pp. 722-729, 1988.

[6] J. F. R. Hinton and D. Harpool, "An ab initio investigation of (formamide)n and formamide-(water)n systems. Tentative models for the liquid state and dilute aqueous solution," *J. Am. Chem. Soc.*, vol. 99, pp. 349-353, 1977.

[7] J. C. Contador, M. L. Sanchez, M. A. Aguilar, and F. J. Valle, "Solvent effects on the potential energy surface of the 1:1 complex of water and formamide: Application of the polarizable continuum model to the study of nonadditive effects," *J .Chem. Phys.*, vol. 104, pp. 5539-5546, 1996.
[8] N. A. Besley and J. D. Hirst, "Ab Initio Study of the electronic spectrum of formamide with explicit solvent," *J. Am. Chem. Soc.*, vol. 121, pp. 8559-8566, 1999.
[9] Y. Shi, Z. Y. Zhou, and H. T. Zhang, "Density functional theory study of the hydrogen bonding interaction of 1: 1 complexes of formamide with glycine," *J. Chem. Phys. A*, vol. 108, pp. 6414-6420, 2004.
[10] H. Zheng, *Pharmaceutical Chemistry*, Beijing: People's Medical Publishing House, 2003.
[11] A. Szucs, G. D. Hitchens, and J. O. M. Bochris, "Electrochemical reactions of glucose oxidase at graphite electrodes," *J. Electroanal. Chem.*, vol. 275, pp. 133-148, 1989.

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