Spectroelectrochemical Studies on the Oxidation Pathway of Epinephrine

Suk Han KIM and In-Hyeong YEO

Department of Chemistry, Dongguk University, Seoul 100-715, Korea

Oxidation of epinephrine at a gold electrode was studied in acidic solutions by a near normal incidence reflectance spectroelectrochemical (NNIRS) system. Also, cyclic voltammetric(CV) data were used to interpret the oxidation pathway of epinephrine. Some spectroscopic results are in good agreement with the previous reports at a carbon paste electrode. However, assignment of each peak in the CVs is not always in good agreement with the past findings, especially the formation of dehydrated form of leucoadrenochrome is not likely to occur under acidic conditions

keywords Spectroelectrochemical, epinephrine, adrenochrome, adrenalinequinone, leucoadrenochrome

Electrochemical characteristics of catecholamine and its derivatives have been studied by many groups.¹⁻⁵ Epinephrine, one of hormones of the adrenal medullar, was found to have profound effects on the body metabolism. For many years, it is known that various effects caused by stimulation of sympathetic nerves were due to the release of epinephrine and its derivatives from the nerve.

The oxidation pathway of epinephrine is known to be involved in enzymatic and non-enzymatic processes. Due to easy oxidation of epinephrine under the physiological pH condition, the oxidation of epinephrine has not been clearly revealed and remains in a controversial matter. The first mechanistic study on the electrochemical oxidation of epinephrine was done at a planar carbon paste electrode by cyclic voltammetry and chronoamperometry.⁶ At carbon electrodes, oxidation of epinephrine proceeds through two electron process and the rate of one electron transfer is slower than that observed at metal electrodes. It was suggested that the over-all oxidation of epinephrine followed an ECC(electron transfer- chemical reaction- chemical reaction) mechanism, which was different from the ECE model in their previous paper⁷ although the two models give the same results qualitatively.

In this communication, the oxidation of epinephrine was studied by a NNIRS system and electrochemical techniques on a gold electrode in acidic solutions. Light absorbing species generated at the gold electrode by applying a potential reveal some new findings. Some of our results are in part consistent with the previous findings. The same and differences between

this study and the previous ones will be discussed based on *in situ* spectroscopic data recorded with electrochemical measurements.

Experimental

Reagents

Epinephrine obtained from Sigma Chemical Co. was used as received. Aqueous solutions were prepared by using purified water by a Milli-Q purification system. All inorganic salts for electrolytes were of reagent grade.

Apparatus

The spectroelectrochemical system, a near normal incidence reflectance mode, which is very similar to that of previously reported one⁸ was assembled in this laboratory. The schematic diagram of the experimental setup is shown in Fig. 1. The electrochemical components were just like an ordinary three electrode system. A high reflective gold electrode(Pine disk electrode) was used as a working electrode placing the optical fiber unit above it. The reference electrode put into a Luggin capillary was Ag/AgCl in a saturated KCl solution. All potentials are referred to this electrode. The counter electrode was a spiral type platinum wire. Electrochemical measurements were made under semi- infinite diffusion conditions or thin layer type by adjusting the distance between the working electrode and the optical probe unit. The whole part of the electrochemical cell was encapsulated into a light-tight box made of wood

and painted a black inside to prevent scattered radiation from outside.

The spectroscopic system consists of 150 W Xe lamp, holographic monochromator of 1200 lines/mm, a bifurcated optical fiber, and PMT as a detector of 185-870 nm range. The bifurcated optical probe unit was immersed into a solution of transparent silicone sealer in a tube, whose one of the end was sealed with a quartz slide for the spectral measurements.

Measurements

Cyclicvoltammograms of 3.3 mM epinephrine were obtained in 0.1 M H₂SO₄ and in ammonium acetate buffer solution of pH 3.0 and 4.8 at the high reflective gold electrode. The CVs were recorded under various scan rates, different potential windows and various starting potentials.

There is a series of measurements to get a series of spectra under different experimental conditions. The absorption spectra were recorded while the applied potential to the working electrode was stepped from a double layer potential region to a positive potential enough to oxidize the epinephrine. Also, absorption spectra were recorded at a certain wavelength while the applied potential of the working electrode was scanning to get a species selective absorption spectrum. After exhaustive electrolysis in the spectroelectrochemical cell (the applied potential for the electrolysis was 700, 650, 550 mV in each pH solution), a series of spectra was recorded with time at an open circuit potential or by applying carefully selected potentials. Therefore, the difference spectra could be obtained by subtracting from absorption bands at an applied potential to absorption bands at an open potential.

Results and Discussion

The interesting features of the CVs of epinephrine are in that the number and shapes of peaks are very much dependent on pH and working electrode materials. A CV of 3.3 mM epinephrine in 0.1 M H₂SO₄ solution at an Au electrode is shown in Fig. 2. The first oxidation peak at 570 mV in the forward scan and the corresponding reduction peak at 510 mV in the reverse scan were observed. The two peaks correspond to the oxidation and reduction of quinone and hydroquinone moiety of the epinephrine. This kind of behaviors was also observed at a planar carbon paste electrode by Hawley et. al.⁶

They presented a mechanism for the oxidation of epinephrine, which is schematically represented as

follows.

AD represents epinephrine whose name is also called adrenaline. ADQH and ADQ are the first oxidation product with N-protonated and deprotonated forms of adrenalinequinone, respectively. LAD and ADQC correspond to leucoadrenochrome and adrenochrome, respectively. IND represents the dehydrated form of LAD. As shown in the reactions above AD is oxidized to ADQH or ADQ depending on the pH of the solution. ADQ proceed a chemical reaction to form LAD, which is easily oxidized by ADQ and LAD can convert IND by losing water molecule.

In ammonium acetate buffer solution of pH 3.0, two cathodic peaks(C1, C2) and three anodic peaks(A1, A2, A3) as shown in Fig. 3a were observed. In pH 4.8 solution one cathodic peak(C1) and three anodic peaks(A1, A2, A3) as shown in Fig. 3b were

Fig. 1 Schematic diagram of experimental setup

Fig. 2 Cyclicvoltammogram of 3.3 mM epinephrine at an Au electrode in 0.1 M H₂SO₄. Scan rate; 50 mV/sec

observed. The A3 peak is responsible for the oxidation of epinephrine and C2 corresponds to the reduction of the oxidation products on the subsequent scan. In pH 4.8 solution it was observed that the cathodic peak corresponding to C2 in pH 3.0 solution disappeared and only one cathodic peak, C1, at a more negative potential appeared. This suggests that the first oxidation product undergoes chemical reaction quickly in higher pH solution and the product obtained following chemical step is more stable than the first oxidation product of epinephrine, ADQ or ADQH. The half-life of ADQ was estimated to be 0.7 s at a solution of pH 5.⁹ If potential scan starts from the potential below an onset potential of A3, then, the cathodic peak(C1) and the anodic peaks, A1 and A2, are not observed in the first scan. Once the applied potential is higher than that of first oxidation of epinephrine, then, the C1 is generated and the corresponding two anodic peaks appears in the second and more scans. This strongly implies that the A1 and A2 waves are due to reoxidation of the reduction products responsible for C1. It should be noted that the relative height of peaks C1 and C2 changes with scan rates. The peak, C2 is greater than C1 at a faster scan rate indicating that less time is given for occurring chemical reactions and the smaller C1 peak results.

A series of spectra was recorded when a positive potential or no potential was applied to monitor any light absorbing species as intermediates or products. When the epinephrine in 0.1 M H₂SO₄ is oxidized at 700 mV at a gold electrode, a recorded spectrum is shown in Fig. 4a which has two absorption bands at 290 and 380 nm. Shown in Fig. 4b appearing one absorption band at 290 nm is the spectrum when no potential is applied, i.e., an open circuit potential, in the presence of epinephrine. Therefore, it is believed that the first oxidation product, ADQH shows an absorption band at 380 nm due to $n - \pi^*$ transition of carbonyl group. The absorption maximum at 380 nm and smaller band at 525 nm indicates the formation of a new reaction product, ADQH.

If the applied potential is changed from 700 mV to an open circuit the spectral feature returns from 4a to 4b, indicating that the ADQH is unstable and goes quickly back to that of epinephrine through an rearrangement reaction in this condition. Therefore, it can be deduced that the heavily protonated quinones are hard to undergo cyclization reaction⁷ or be stabilized because of easy conversion to epinephrine.

When we ran the same experiments in pH 3.0 and 4.8 ammonium acetate buffer solution, different absorption bands were observed as shown in Fig. 5.

Fig. 3 Cyclic voltammograms of 3.3 mM epinephrine at an Au electrode (a) in pH 3.0 buffer solution (b) in pH 4.8 buffer solution. Scan rate; 50 mV/sec

Fig. 4 Absorption spectra recorded by applying (a) 700 mV, (b) no potential in 0.1 M H₂SO₄ containing 3.3mM epinephrine

Fig. 5 Absorption spectra recorded by applying potentials in pH 3.0 buffer (1) 200 mV after bulk electrolysis at 650 mV (2) 650 mV (3) no potential; (b) in pH 4.8 buffer, (1) 50 mV after bulk electrolysis at 550 mV (2) 550 mV (3) no potential.

Fig. 6 Differences in absorption spectra between a spectrum obtained at open circuit and a spectrum obtained at applied potentials (700 mV at pH 1.0, 650 mV at pH 3.0, 550 mV at pH 4.8) in various pH solutions.

When no potential was applied only one band at 290 nm responsible for the dihydroxylbenzene moiety appeared in various pH solutions. There are three absorption bands 315, 470, 525 nm, which are not observed at pH 1.0 solution when the potentials are applied. Also, absorbance of three bands is larger at pH 4.8 than at pH 3.0, indicating that the cyclized products through a chemical reaction form rapidly at higher pH solutions.

After bulk electrolysis in the same cell with a platinum gauze electrode, the recorded spectrum indicates that more than one products may be produced in the higher pH solution. Comparing all absorption peaks in pH 3.0 and pH 4.8 solution, there are at least two additional absorption bands which are strongly dependent on the pH of the solution. The peaks are of interest at 380 and 610 nm. The absorption band at 610 nm was observed at pH 3.0 solution only and very weak band at pH 4.8 and no band at pH 1.0 solution.

The absorption spectra are not likely to be changed with time after electrolysis at pH 4.8 at the open circuit. The strong absorption band at 610 nm (not shown) appeared when the potential was set to a negative potential than the C1 peak. The intensity of this band dramatically decreases by applying potentials greater than 50 mV. Therefore, this suggests that the A2 peak is due to the oxidation of a reduced form of ADQC, i.e., LAD. This is not in agreement with the previous papers. They assigned the peak as the oxidation of dehydrated form of LAD, i.e., IND; 5,6-dihydroxy-N-methylindole.

The difference spectra between the one obtained with an open circuit and the other obtained by applying an oxidation potential enough to oxidize the epinephrine in various pH solution are shown in Fig. 6. Formation of ADQC in higher pH solution was confirmed by two absorption bands at 315 and 470 nm, which agrees with a reported value of 302 and 485 nm of ADQC. The smaller absorption band at 380 nm in pH 4.8 solution than at more acidic solutions indicates that the ADQH converts quickly to the cyclized product of ADQC and/or LAD. Also, this assumption is also supported by absence of the C2 peak in Fig. 3b. Because the first oxidation product, ADQH, returns quickly to epinephrine in acidic environment the formation of ADQ form is not likely to occur and the LAD is difficult to be formed from the ADQH.

In conclusion, we demonstrate that our NNIRS system can be used to study electrochemical oxidation of epinephrine. It is not likely to produce the dehydrated form of LAD even in acidic condition. Further studies are under investigation to reveal the detailed mechanism on the oxidation of epinephrine.

Acknowledgment

This work was financially supported by Korea Science and Engineering Foundation (Contract No. 95-0501-05-01-3)

References

1. G. Dryhurst, "Comprehensive Treatise of Electrochemistry", Vol. 10, Chap. 2, Plenum Press, New York, 1985.
2. R. M. Wightman, L. J. May, and A. C. Michael, *Anal. Chem.*, 60, 769A (1988).
3. A. Sucheta and F. J. Rusling, *Electroanalysis*, 3, 735(1991).
4. M. R. Deakin, P. M. Kovach, K. J. Stutts, and R. M. Wightman, *Anal. Chem.*, 58, 1474 (1986).
5. X. Wu, L. Mu and W. Zhang, *J. Electroanal. Chem.*, 352, 295(1993).
6. M. D. Hawley, S. V. Tatawawadi, S. Piekarski, and R. N. Adams, *J. Am. Chem. Soc.*, 89, 447 (1967).
7. M. D. Hawley and S. W. Feldberg, *J. Phys. Chem.*, 70, 3459 (1966).
8. C-H. Pyun and S-M. Park, *Anal. Chem.*, 58, 251 (1986).
9. J. P. Skully and R. L. McCreery, *Anal. Chem.*, 52, 1885 (1980).