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Investigation of the reduction process of dopamine using paired pulse voltammetry

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Abstract

The oxidation of dopamine (DA) around +0.6V potential in anodic sweep and its reduction around −0.1V in cathodic sweep at a relatively fast scanning rate (300 V/s or greater) have been used for identification of DA oxidation in fast-scan cyclic voltammetry (FSCV). However, compared to the oxidation peak of DA, the reduction peak has not been fully examined in analytical studies, although it has been used as one of the representative features to identify DA. In this study, the reduction process of DA was investigated using paired pulse voltammetry (PPV), which consists of two identical triangle-shaped waveforms, separated by a short interval at the holding potential. Especially, the discrepancies between the magnitude of the oxidation and reduction peaks of DA were investigated based on three factors: (1) the instant desorption of the DA oxidation product (dopamine-o-quinone: DOQ) after production, (2) the effect of the holding potential on the reduction process, and (3) the rate-limited reduction process of DA. For the first test, the triangle waveform FSCV experiment was performed on DA with various scanrates (from 400 to 1000 V/s) and durations of switching potentials of the triangle waveform (from 0.0 to 6.0 ms) in order to vary the duration between the applied oxidation potential at +0.6V and the reduction potential at −0.2V. As a result, the ratio of reduction over oxidation peak current response decreased as the duration became longer. To evaluate the effect of holding potentials during the reduction process, FSCV experiments were conducted with holding potential from 0.0V to −0.8V. We found that more negative holding potentials lead to larger amount of reduction process. For evaluation of the rate-limited reduction process of DA, PPV with a 1Hz repetition rate and various delays (2, 8, 20, 40 and 80ms) between the paired scans were utilized to determine how much reduction process occurred during the holding potential (−0.4V). These tests showed that relatively large amounts of DOQ are reduced to DA during the holding potential. The rate-limited reduction process was also confirmed with the increase of reduction in a lower pH environment. In addition to the mechanism

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of the reduction process of DA, we found that the differences between the responses of primary and secondary pulses in PPV were mainly dependent on the rate-limited reduction process during the holding potential. In conclusion, the reduction process may be one of the important factors to be considered in the kinetic analysis of DA and other electroactive species in brain tissue and in the design of new types of waveform in FSCV.

Keywords

fast-scan cyclic voltammetry (FSCV); paired pulse voltammetry (PPV); rate-limited reduction process; kinetic analysis of DA; redox ratio

1. Introduction

Background subtraction fast-scan cyclic voltammetry (FSCV) in combination with carbon fiber microelectrodes (CFM) has been utilized *in vivo* for the detection of neurotransmitter release in brain with sub-second temporal resolution [1-5]. Conventionally, a triangle potential waveform has been used for DA detection, which consists of an anodic sweep from -0.4V to 1.0V and cathodic sweep from 1.0V to -0.4V with a 10Hz repetition rate [6, 7]. At relatively fast scan rates (300 V/sec and greater) the oxidation of DA adsorbed on CFMs occurs around 0.6V in the anodic sweep direction and reduction around -0.1V in cathodic sweep direction. These red/ox potentials have been used to identify DA using FSCV. The oxidation peak current is well known to change proportional to DA concentration in a biological concentration range [8]. In addition the extracellular concentration of DA in brain tissue has been utilized to calculate the adsorption rate and desorption rate of DA on CFM surfaces in the brain [9]. Thus, Michaelis-Menten kinetics used to model the rate of reuptake of DA from the extracellular space can be more accurately estimated by adjusting the DA adsorption rate with its oxidation peak [10, 11].

Whereas, compared to the oxidation peak of DA, the reduction peak has not been fully used in analytical *in vivo* studies, although it has been used as one of the representative features for identifying DA. The magnitude of the reduction peak of DA in FSCV is usually less than the oxidation peak. The magnitude of the reduction peak is typically around 60% of the oxidation peak magnitude with FSCV conventional triangle waveforms [9]. Bath et al. (2000) suggested that there are three possibilities to explain this observation. First, a percentage of dopamine-o-quinone (DOQ), as a result of DA oxidation, could instantly desorb from the CFM surface after DA oxidation and be electrochemically reduced back to DA in the time-frame of the scan [12]. Second, the reduction process may be rate-limited by the concentration of H^+ at the surface of CFM thereby relatively rate-limiting the reduction back to DA during the scan [9, 13, 14]. The reduction of DA proceeds by 2 individual H^+ , e-steps. The loss of the first 1 e- from the DA molecule may occur during the relatively fast voltage scan, while the second 1 e-reduction may subsequently occur between scans at the holding (starting) potential. Thirdly, a -0.4V holding potential may have an effect on the oxidative current via the electrostatic force exerted on the cationic species DA, thus delaying reduction [12, 15]. Despite these hypotheses, to our knowledge, there have been few

systematic studies examining these processes and presenting the quantification of the amount of reduction process during FSCV scan.

We have recently suggested that paired-pulse (scan) voltammetry (PPV) may minimize the confounding factors such as pH changes and transient effects at the CFM surface [16]. The scan waveforms are paired in doublets, two identical triangle-shaped waveforms, separated in time by a short interval at the holding potential. When the voltammogram for one of the pulses comprising a doublet is subtracted from the voltammogram of the other pulse, the effects of pH change can be eliminated. Although PPV was proposed for differentiating complex analytes, it can also be employed for the examination of the reduction process of DA using electrochemistry. That is, by varying the time between two applied scans, how much of the reduction process occurs during scans and at the holding potential between scans can be quantified.

In this study, the reduction process of DA was investigated using a triangle waveform and PPV. Especially, the discrepancies between the magnitude of the oxidation and reduction of DA were investigated based on three factors: (1) the instant desorption of the DA oxidation product (DOQ) after production, (2) the effect of the holding potential on the reduction process, and (3) the rate-limited reduction process of DA. The reduction process of DA in FSCV was investigated by varying pH, scan rate, and amplitude potential of the triangle waveform of FSCV, and the amount of reduction occurring during the holding potential between scans was evaluated with PPV by controlling the inter-pulse delay.

2. Experimental

2.1. Materials and Methods

2.1.1. FSCV waveform—Three types of potential waveforms were used in this study: a triangle waveform, a sawhorse waveform, and a paired-pulse waveform. The triangle FSCV waveform for the detection of DA consists of a triangle-shaped voltammetric pulse with the applied potential sweeps from -0.4 V to $+1.0$ V and back to -0.4 V, at a sweep rate of 400 V/s, while the electrode is held at a holding potential of -0.4 V between voltammetric pulses. The sawhorse waveform keeps the switching potential ($+1.0$ V) the same but holds there for some duration as shown in Fig. 3C [17]. PPV consists of triangle-shaped pairs of voltammetric pulses (primary and secondary pulse) with a specific time delay between the two pulses comprising each binary scans, repeated with a negative holding potential between the paired pulses [16]. The primary pulse is defined as the first pulse in the binary scans, and the secondary pulse as the following pulse. This allows two very different voltammograms (primary and secondary) to be obtained, each corresponding to the two pulses in the binary scans. In addition, it allows quantifying the reduction process occurring during scans by varying the time between two applied scans.

2.1.2. Data acquisition—All experiments were performed using the wireless instantaneous neurotransmitter concentration sensing system (WINCS) [18-21]. Briefly, WINCS incorporates front-end analog circuitry for FSCV, a microprocessor, and Bluetooth radio, all on a single rechargeable lithium-polymer battery-powered, multilayer, printed circuit board that is hermetically sealed in a polycarbonate case, permitting sterilization

using the STERRAD® gas plasma process. In FSCV mode, WINCS uses a transimpedance amplifier to convert current to voltage, and a difference amplifier to subtract a triangular waveform potential applied to a CFM prior to signal digitization. A digital-to-analog converter samples FSCV at a rate of 100 kilo-samples per second. Bluetooth 2.4-2.5 GHz digital telemetry is used to wirelessly communicate between WINCS and a base station computer running Windows XP. Custom software controls WINCS parameters and operation, such as data acquisition and transmission, the applied potential waveform, and data sampling rate. Data, in the form of a sequence of unsigned 2-byte integers, are saved to the base-station computer hard drive for offline processing. The acquired data are displayed in several graphical formats for nearly real time analysis by the Mayo Clinic engineered software (WINCSware) running on the base station computer.

2.1.3. Fabrication of carbon fiber microelectrodes—The carbon fiber microelectrode (CFM) was constructed by inserting a single carbon fiber ($d = 7 \mu\text{m}$) (cytec Thornel® T300) into a silica capillary and insulated with polyamide. The CFM was connected to the Nitinol wire (Fort Wayne Metals, Indiana, USA, Nitinol #1) with a silver-polyamide mixed paste. Nitinol wire was insulated with polyamide tubing. The exposed carbon fiber was trimmed to a final length around $100 \mu\text{m}$ using a scalpel [18]. The Ag/AgCl reference electrode was fabricated by chloriding a 31 gauge silver wire.

2.1.4. Flow injection apparatus—A flow-injection analysis system was used for *in vitro* measurements of DA and consisted of a syringe pump (Harvard Apparatus, Holliston, MA) that directed a buffer solution through a Teflon tube to a 6-port injection valve (Rheodyne, Rohnert Park, CA). The injection valve was controlled by a 12V solenoid and was used to introduce analyte from an injection loop in an electrochemical flow cell. A CFM was placed in a flowing stream of buffer and analyte was injected as a bolus. All chemicals were purchased from Sigma-Aldrich (St. Louis). The buffer solution was composed of 150 mM sodium chloride and 12 mM Trizma base at pH 7.4, and was pumped across the CFM at a rate of 2 ml/min [16].

3. Results and discussion

3.1. Examination of DA oxidation and reduction peaks with triangle waveform FSCV

When DA was measured in background subtracted FSCV with a triangle waveform, the DA oxidation peak current around +0.6 in the anodic sweep direction typically appeared larger than the reduction peak in the cathodic sweep direction as depicted in Fig. 1D. For the reduction peak of a voltammogram of DA in the flow cell, the peak current is governed by complex adsorption and desorption kinetics of DA and the corresponding DA oxidative product, DOQ. However, the electrode response can be simplified by determining only the desorption kinetics of DA and DOQ upon the removal of DA from the electrode vicinity following termination of the DA injection into the flow cell [9, 15]. Desorption kinetics for DA (1) and DOQ (2) can be mathematically described as:

$$\Gamma_{DA} = \Gamma_{DA}^o e^{-(k-1)\tau} \quad (1)$$

and

$$\Gamma_{DOQ} = \Gamma_{DOQ}^o e^{-(k_{-2}t)} \quad (2)$$

Where Γ_{DA}^o and Γ_{DOQ}^o are the initial coverage of DA and DOQ. And k_{-1} and k_{-2} are the DA desorption rate and DOQ desorption rate, respectively. The initial surface coverage for each successive iteration on the CFM can be determined by the final coverage of the previous step. Thus,

$$\Gamma_{DA} = \Gamma_{DA}^F e^{-N(k_{-1}\tau + k_{-2}t)} \quad (3)$$

where N is the cycle number since the termination of the DA injection into the flow cell and subsequent removal of DA from the CFM vicinity. As shown in Fig. 1C, the oxidation coverage of DA (Γ_{DA}) and the reduction coverage of DOQ (Γ_{DOQ}) decrease exponentially upon termination of the DA injection. The desorption processes occurred during the time period denoted by τ and t in Fig 1A. In this regard, it is crucial to consider that the initial coverage for each successive iteration (Γ_{DA}^o and Γ_{DOQ}^o) is determined by the final coverage of the previous step. Thus, at beginning of t , Γ_{DOQ}^o is equal to Γ_{DA} of the previous step and at beginning of τ , Γ_{DA}^o is Γ_{DOQ} of the previous step. Under these conditions, DA oxidation and reduction peak currents are dependent on the respective peak currents of the previous scan and desorption rate that are recorded following termination of the DA injection. To make sure that no additional DA is present to adsorb to the CFM surface, the response of ascorbic acid, which has been known as an analyte with no adsorption characteristics on carbon electrodes, was examined as shown in Fig. 1C. When the period after DA injection was examined, as shown in Fig. 1D, the reduction peak current was approximately half the amplitude of the oxidation peak current (red arrow). Most significantly, the oxidation peak current (blue arrow) was larger than the previous reduction peak even though there is no longer a supply of DA. This suggests that some further reduction of the DOQ to DA may occur between scans at the holding potential. Therefore, these findings suggest that the reduction process may be rate-limited by the concentration of H^+ at the CFM surface, as shown previously [9, 13, 14].

3.2. Effects of pH on the reduction of DA

In order to investigate the rate-limited reduction process by the concentration of H^+ in the electrolyte buffer solution, the oxidation and reduction responses of DA were evaluated with three different pH levels (pH 5.0, pH 6.0, and pH 7.0). All flow cell experiments were conducted with DA (3 μ M) using triangle waveform FSCV. As depicted in Fig. 2A, the oxidation peak at a lower pH (5.0) was larger than the one at the higher pH (7.0) level. More significant differences in responses were reductive responses of DA between these two pH levels. The magnitude of the reduction peak at pH 5.0 was significantly higher than the one recorded at pH 7.0. In addition, the ratio of reduction peak over oxidation peak was more than 80% at pH 5.0 as shown in Fig. 2C. Although the pH of the solution affects the DA response in a quite complex manner [13, 22], these pH-dependent responses are in accordance with previous studies [9, 13], which support the notion that at lower pH the increase in protons in the electrolyte medium results in an increase in the rate-limited two-

electron transfer process coupled to two-proton transfer steps. In addition, the potential of the reduction peaks for DA were shifted from a lower to higher voltage (Fig. 2B). The positive shift of the peak potential with acidification of the solution can be caused by quite complicated affects such as thermodynamic and kinetic effects [23]. With respect to the rate-limited reduction process, the thermodynamic and kinetic changes may lead to relatively faster and greater reduction of DOQ during the anodic sweep of the triangle waveform. Moreover, adsorption and desorption time responses for DA are much slower at lower pH levels due to these changes of reduction and oxidation kinetics (Fig. 2A).

3.3. Effects of concentration and scan parameters on the reduction of DOQ

In order to clarify the influence of scan parameters and DA concentrations on the reduction process, the responses of DA were measured by changing DA concentrations (from 1 μ M to 9 μ M), and scan rates (from 400V/s to 1000V/s) based on a triangle waveform consisting of -0.4 V holding potential and $+1.0$ V switching potential. It is well known that responses of adsorbed species in FSCV are proportional to the scan rate and to relatively low concentration ranges [8, 9, 24]. To examine the influence of these experimental parameters on the reduction process, the ratio of the reduction peak current over the oxidation peak current (redox peak ratio) was calculated. As shown in Fig. 3A, redox peak ratio did not significantly change across various concentrations of DA. In contrast, the redox peak ratio increased in a linear fashion with increases in scan rate (Fig. 3B). These results suggest that a percentage of DOQ could instantly desorb from the CFM surface after production. That is, the amount of DOQ desorption is proportional to the duration from $+0.6$ V (oxidation peak potential) of the anodic sweep to -0.2 V (reduction peak potential) of the cathodic sweep. Under the present conditions, the duration of a 400 V/s scan is 4.0 msec compared to 1.8 msec for a 1000V/s scan, a difference of 2.2 msec. Thus, the shorter duration 1000 V/s scan results in a higher percentage reduction of DOQ at the CFM surface compared to a 400V/s scan. To test the effect of the time between oxidation and reduction, the experiments were performed using a sawhorse waveform where the switching potential is extended as depicted in Fig. 3C. The durations keeping the switching potential the same were varied from 0 to 6ms. In a contrast with increases in DOQ reduction with faster scan rates, the redox ratio (both redox peak and redox area ratio) progressively decreased with increases in the longer holding time of the switching potential of the waveform due to the relatively longer duration between DA oxidation and DOQ reduction (Fig 3. D and E).

In addition, the influence of the holding potential to the reduction process was evaluated by changing the triangle waveform holding potentials from 0.0V to -0.8 V. As shown in Fig. 4A, the DA oxidation peak current progressively increased as the holding potential was set more negative, consistent with previously reported findings with FSCV of DA using extended scan waveforms [25]. In contrast, the redox ratio at an -0.8 V holding potential was lower than the ratio determined at -0.2 V (Fig. 4B), likely as a result of the rate-limited reduction process. Although the reduction peak current did not change as much as the oxidation peak current, the reduction process continued to the end of anodic sweep (around -0.8 V) of triangle waveform, as shown in Fig. 4A (red line voltammogram). To estimate the amount of a reduction process occurring during the holding potential, PPV was used with a 2ms gap between primary pulse and secondary pulse. As shown in Fig. 4C, both the

secondary DA peak currents and DA oxidation area compared to the primary DA peak currents and DA oxidation area increased as the holding potential was set more negative, suggesting that a relatively greater amount of DOQ reduction occurred at the lower negative holding potentials.

3.4. Examination of the DOQ reduction process during the potential holding time

PPV was used to confirm the occurrence of a rate-limited reduction of DOQ during the holding potential and its dependency on the concentration of H⁺ near the surface of a CFM. Although it has been previously shown that a reduction process occurs between voltammetric scans [9], it is difficult to quantify how much of the reduction process occurs during a scan using a conventional triangle waveform due to a fixed repetition time between scans. By varying the time between two applied scans in PPV, how much of the reduction process occurs during scans and at the holding potential between scans can be quantified. As shown in Fig. 5A, DA (3 μ M) voltammograms were acquired at a CFM in a flow cell using the PPV waveform with a 2ms delay, 400 V/s scan rate, +1.0V peak potential, and 10Hz repetition rate. For this PPV, τ_p , t_p , τ_s , and t_s are equal to 86.5ms, 4.5ms, 4.5ms and 4.5ms, respectively. As shown in Fig. 5B and 5C, the peak currents of primary voltammograms were comparatively higher than the peak currents of the secondary voltammograms, which can be explained by DA adsorption properties on CFMs [16]. Following the injection of DA, the kinetics of DA and DOQ were simplified by examining only desorption following the termination of the DA injection into the flow cell as described in the previous section. Under these conditions, the oxidation coverage of DA (Γ_{DA_p} and Γ_{DA_s}) and the reduction coverage of DOQ (k_{-1} and k_{-2}) decrease exponentially with desorption rates (and) in PPV waveform (Fig. 4A) and can be mathematically described as:

$$\Gamma_{DA_p} = \Gamma_{DA_p}^o e^{-(k_{-1}\tau_p)} \quad (4)$$

$$\Gamma_{DOQ_p} = \Gamma_{DOQ_p}^o e^{-(k_{-2}t_p)} \quad (5)$$

and

$$\Gamma_{DA_s} = \Gamma_{DA_s}^o e^{-(k_{-1}\tau_s)} \quad (6)$$

$$\Gamma_{DOQ_s} = \Gamma_{DOQ_s}^o e^{-(k_{-2}t_s)} \quad (7)$$

where $\Gamma_{DA_p}^o$, $\Gamma_{DOQ_p}^o$, $\Gamma_{DA_s}^o$, and $\Gamma_{DOQ_s}^o$ are the initial coverage values for DA and DOQ in the primary pulse and secondary pulse condition, respectively, and k_{-1} and k_{-2} are the DA desorption rate and DOQ desorption rate, respectively. Initial coverage values for each successive iteration can be determined by the final coverage of the previous step. Thus,

$$\Gamma_{DA_p} = \Gamma_{DA_p}^F e^{-N(k_{-1}\tau_p + k_{-2}t_p + k_{-1}\tau_s + k_{-2}t_s)} \quad (8)$$

and

$$\Gamma_{DA_{-s}} = \Gamma_{DA_{-p}}^F e^{-(k_{-2}t_p + k_{-1}\tau_s)} e^{-N(k_{-1}\tau_p + k_{-2}t_p + k_{-1}\tau_s + k_{-2}t_s)} \quad (9)$$

where N in this equation is the cycle number following the termination of DA injection and subsequent removal of DA from the CFM vicinity. To simplify the calculation of k_{-1} and k_{-2} , these values were estimated independently from responses from conventional triangle FSCV (two different repetition times: 10Hz and 30Hz) using eq. 3 as described in studies by Bath et al. [9, 15].

The DA oxidation peak currents of primary (red dotted line in Fig. 4C) and secondary (blue dotted line in Fig. 5C) voltammograms were simulated by using eq. 8 and eq. 9 with $k_{-1} = 1.47$, $k_{-2} = 4.01$, which were estimated from triangle waveform generated FSCVs. Times 86.5ms, 4.5ms, 4.5ms and 4.5ms were used as T_p , t_p , T_s , and t_s in the simulation. As shown in Fig. 5C, the DA oxidation peak current measured with the primary pulse (red solid line) matched with the simulation (red dotted line in Fig. 5C). However, there was a significant discrepancy between the magnitude of the measured (blue solid line in Fig. 5C) and simulated (blue dotted line in Fig. 5C) DA oxidation peak currents of the secondary pulse with the measured peak currents being relatively lower than the simulated values. It is important to note here, that the DA oxidation peak currents of the primary pulses were significantly larger than every preceding secondary pulse responses even though there was DA and DOQ desorption without a DA supply. This would suggest that the magnitude of the oxidation responses of secondary pulse cannot be explained entirely with DA and DOQ adsorption and desorption kinetics. Thus, it is conceivable that the differences in oxidation peak height responses between primary and secondary pulses in PPV may be accounted for by an amount of DOQ reduction to DA occurring at the holding potential between pulses.

To estimate the degree of reduction of the DOQ that occurs during the potential holding time PPV experiments were performed with various delays (gaps) between the pairs of pulses (2, 8, 20, 40 and 80ms). A long repetition time (1000ms, 1Hz) was used in these experiments to minimize the effect of the secondary pulse on the response recorded with the primary pulse in each subsequent scan. All data were acquired after termination of the DA injection so that only DOQ reduction during the delay time was considered in the absence of DA adsorption.

As shown in Fig. 6A and 6B, the DA oxidation peak current recorded in response to the primary pulse with an 80ms time gap PPV was comparable in magnitude to the DA oxidation peak current recorded in response to the primary pulse with a 2ms time gap PPV. As shown in Fig. 6C, increasing the delay between the paired pulses resulted in a progressive increase in the magnitude of DA oxidation peak current and area under the curve recorded in response to the second pulse. These findings support the notion that a significant amount of DOQ reduction takes place during the potential holding time.

3.5. Mechanism of paired pulse voltammetry

In our previous PPV study, we described the differences in DA responses between primary and secondary pulses based on characteristics of DA adsorption on CFMs [16]. As described in the section above, some of these differences may be accounted for by the different amounts of reduction of DOQ occurring during the potential holding time between scans.

This suggests that the difference voltammogram (primary minus secondary) can be enhanced by changing scan parameters that maximize the DOQ reduction process. For example, an enhancement in the DA responses of paired-pulse subtracted voltammograms can be obtained by simply increasing the switching potential of PPV from +1.0V to +1.5V (Fig. 7). The larger amount of DOQ that instantly desorbs after production at the primary pulse with a +1.5V switching potential, rather than +1.0V peak potential, is likely due to the longer duration between the DA oxidation peak potential and the DOQ reduction peak potential. This leads to a decrease in the response at the secondary pulse, thus resulting in greater magnitude in primary minus secondary voltammograms of DA oxidation, as depicted in Fig. 7B. It is interesting to note as well, that with PPV the reduction peak for primary minus secondary responses was reduced in magnitude and shifted in potential, suggesting that the magnitude of the reduction peak recorded at the primary pulse was comparable in magnitude to the reduction peak recorded at the secondary pulse and the reduction peak at the primary pulse was wider than the reduction peak at the secondary pulse. This may also be explained with a rate-limited reduction process. That is, in the short gap (2ms) in PPV, the source of the initial reduction peak is DOQ formed at the CFM surface by DA oxidation during the primary anodic sweep, whereas the source of the secondary reduction peak may be from residual DOQ remaining after the primary pulse (due to slow reduction) and from DOQ formed by DA oxidation during the secondary anodic sweep.

An additional point to be considered in Fig. 7 is that the CFM sensitivity to DA using a +1.5V switching potential showed only a relatively small increase compared to the electrode sensitivity observed using a +1.0V switching potential. In this regard, a previous study [25] reported an eight-fold enhancement in the sensitivity of CFMs to DA using an extended waveform (400V/s scan rate, +1.4V scan rate, and -0.6V holding potential), compared to a more traditional waveform (300V/s, +1.0V, and -0.4V), an effect that could be attributed to three scan parameters (scan rate, switching potential, and holding potential). The minor enhancement in the electrode sensitivity to DA, compared to this latter study, may be explained with the different scan parameters as well as the experimental procedure employed in our study. DA is also known to foul the surface of the electrode [26]. To avoid a DA fouling effect in our study, all experiments were performed after a few minutes of FSCV scans with a triangle waveform consisting of a +1.5V switching potential, which has the effect of resurfacing the electrode [27].

4. Conclusions

In this study, we determined the potential factors that may account for the magnitude of the reduction peak of DA relative to its oxidation using FSCV and PPV: (1) DOQ near instantaneous desorption after production, (2) the rate-limited reduction process, and (3) the effect of the holding potential on the reduction process. To simplify the examination, the reduction and oxidation responses of DA were determined immediately following termination of DA injection into a flow cell, thereby minimizing adsorption kinetics of DA. In summary, it appears that these two factors play a significant role in mediating the overall sensitivity of CFM to DA as assessed in experiments varying pH, scan rate, and peak potential in triangle waveform FSCV and PPV. In addition, the differences between

responses recorded with primary and secondary pulses in PPV can be accounted for by the DOQ reduction process that occurs during the holding potential between scans. In conclusion, the reduction of DOQ may prove to be an important additional factor in the kinetic analysis of DA using FSCV and PPV and in the design of new voltammetric waveforms in the future. In conventional FSCV the time-varying potential is a brief pyramidal waveform, typically repeated 10 times per second. The CFM is otherwise maintained at a specified holding potential with respect to the reference electrode. Here we introduce PPV where the waveforms are paired in doublets—two identical waveforms (“pulses”), separated in time by a short interval at the holding potential. The virtue of paired-pulse voltammetry is that it can discriminate analytes on the basis of their adsorption characteristics. When the voltammogram for one of the pulses comprising a doublet is subtracted from the voltammogram for the other pulse, the effects of pH change, for example, can largely be eliminated.

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Highlights

The reduction process of DA has not been fully examined in FSCV studies

The reduction process of DA was investigated using PPV

A part of DOQ could instantly desorb from the CFM surface after DA oxidation

The reduction process of DA was rate-limited processing

The reduction process might be one of important factors in the kinetic analysis of DA

Figure 1.

DA responses using triangle waveform in FSCV: **A.** Schematics of triangle waveform. **B.** A pseudo-color plot of conventional FSCV of 3 μ M DA recorded at a CFM in a flow cell. **C.** The time responses of oxidative peak current. Red arrow represents peak DA oxidation current just after the end of the DA injection (black dashed line) and blue arrow at the next scan. The response of ascorbic acid is shown (red dotted line) to make sure that no additional DA is present to adsorb to the surface. **D.** Voltammograms at two consecutive scans. Red and blue arrows point to the DA oxidation peaks for these two scans.

Figure 2.

The effects of pH on DA oxidation and reduction processes. All flow cell experiments were conducted with DA (3 μ M) in varying pH of buffer. **A.** The oxidation and reduction peak current responses over time at pH 5.0 and pH 7.0. **B.** DA voltammograms with pH 5.0, pH 6.0 and pH 7.0. **C.** The ratio of reduction peak current over oxidation peak current with respect to the pH levels.

Figure 3.

The ratio of DOQ reduction peak current over DA oxidation peak current (redox peak ratio) depending on the (A) concentration of DA, and (B) scan rate, and (C) a sawhorse waveform used for evaluating the effect of the time between oxidation and reduction, (D) resulting voltammograms with different switching potential durations, and (E) redox peak ratio and the redox area ratio depending on the switching potential durations.

Figure 4.

The effect of holding potentials on DA oxidation and reduction **A.** The voltammograms depending on the holding potentials (0.0, -0.2, -0.4, -0.6, and -0.8V) using a triangle waveform. **B.** The ratio of DOQ reduction peak current over DA oxidation peak current, **C.** The peak current ratio and the oxidation area under the curve between primary pulse and secondary pulse in PPV. PPV was applied at a 1Hz repetition rate to estimate the amount of the reduction process occurring during the holding potential (2ms gap), +1.0V peak potential, and three different holding potentials (0.0, -0.4, and -0.8V).

Figure 5.

Examination of the DOQ reduction process using PPV. **A.** The PPV scan waveform. **B.** Primary and secondary pseudo-color map. **C.** The oxidation and reduction peak current responses over time. **D.** Four primary (red voltammograms) and secondary (blue voltammograms) oxidation and reduction peak responses recorded following the termination of the DA injection into the flow cell

Figure 6.

The effect of gaps (2, 8, 20, 40 and 80ms) on the 3 μ M DA redox responses recorded in response to the secondary pulse in PPV. PPV was applied at a 1Hz repetition rate, -0.4 V holding potential, and $+1.0$ V peak potential. **A.** Primary and secondary cyclic voltammograms with a gap of 2ms. **B.** Primary and secondary cyclic voltammograms with a gap of 80ms. **C.** The oxidation peak current ratio and the oxidation area under curve between primary pulse and secondary pulse.

Figure 7.

PPV responses with +1.0V and +1.5V switching potentials (2ms gap). PPV responses to DA (3 μ M) at a switching potential of +1.0V (A) and at a switching potential of +1.5V (B). The differences between primary pulse and secondary pulse responses at +1.5V switching potential were higher compared to those recorded with a switching potential of +1.0V.