The Interference of Ascorbic Acid in Sensitive Detection of Dopamine by a Non-Oxidative Sensing Approach

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ABSTRACT

Determination of Dopamine electrochemically by direct oxidation is difficult due to the co-oxidation of ascorbic acid present in high concentration in biological fluids in the same potential window. In this report, we present a non oxidative approach to electrochemically detect dopamine with high selectivity and sensitivity using our newly developed poly (anilineboronic acid) composite with single walled carbon nanotubes. The mechanism of interference by ascorbic acid was studied and the results showed that ascorbic acid interacted with poly (anilineboronic acid) both chemically and electrocatalytically due to its multi-functional diol motif. An association constant between ascorbic acid and phenylboronic acid was determined to quantitate the strength of the interaction between the two molecules. By depositing a layer of permselective ionomer Nafion on top of the composite, the sensor can selectively detect dopamine in pM~ nm range. The high sensitivity along with the improved selectivity of this approach might have potential application toward molecular diagnosis of Parkinson's disease.

Keywords: Polyaniline; carbon nanotubes; ascorbic acid; interference; neurotransmitter, dopamine

1 INTRODUCTION

Dopamine (DA) is an important neurotransmitter in the mammalian central nervous system and its deficit results in brain disorders such as Parkinson's disease (PD) and Schizophrenia [1]. As Parkinson's disease is characterized by the severe depletion of DA, from the in vivo dopamine pool, the ability to sensitively and selectively measure the concentration of neurotransmitter dopamine could potentially be used in the molecular diagnosis of PD. Tremendous effort has been made to develop sensors to detect dopamine *in vivo*. However, determination of DA electrochemically by direct oxidation method is difficult due to the presence of ascorbic acid (AA) present in biological fluids. It can be oxidized at the same potential window. Furthermore, all the electrochemical techniques, suffered from another common problem, i.e. the oxidation products of dopamine could react with ascorbic acid in the sample, which results in severe interference of the detection. As the concentration of AA is relatively higher than that of DA in biological samples (4-5 orders of magnitude higher than DA), high sensitivity and selectivity are equally important for in vivo measurement of DA. In our recent work, we reported a non-oxidative approach to detect dopamine electrochemically in the presence of AA with high selectivity and sensitivity [2]. In this report, we report the mechanism of AA interference on the composite sensor by studying both the chemical and electrochemical interactions between the two species. For the first time the association constant between ascorbic acid and boronic acid groups was experimentally determined to quantitatively describe the interaction between two species.

2 EXPERIMENTAL SECTION

2.1 Materials

Purified single-walled carbon nanotubes were purchased from Carbon Nanotechnologies, Inc. Houston, TX. Single-stranded DNA with sequence d (T)30 was purchased from Integrated DNA Technologies, Inc. Coralville, IA. 3-Aminophenylboronic acid hemisulfate salt, 3-hydroxytyramine hydrochloride (dopamine), L-ascorbic acid, potassium fluoride, potassium dihydrogen phosphate, potassium hydrogen phosphate, sodium phosphate, magnesium chloride, sodium chloride, Alizarin Red S, disodium EDTA, Nafion® perfluorinated ion exchange resin (5 wt.% in mixture of lower aliphatic alcohols and H2O), and all other chemicals were of analytical grade purity and were used as received from Aldrich Chemicals Inc., Milwaukee, WI. All solutions were prepared using nanopure water (18.2 MΩ) (Barnstead). The bundled single-walled carbon nanotubes were dispersed into water using the method described by Zheng et.al[3]. The self-doped polyaniline/carbon nanotube composite was fabricated on the gold electrode surface by following the procedure described in our recent work [2].

AA solutions were prepared immediately before use in the containers protected from light by aluminum foil in order to avoid photoinduced oxidation.

2.2 Electrochemical Measurements

All electrochemical measurements and electrochemical polymerization of 3-aminophenylboronic acid and electrochemical characterization of the resulting films were carried out by following the method described in our pervious work [3] at a CH Instrument 750 series electrochemical station. All the potentials quoted in this work are in terms of the Ag/AgCl scale.

2.3 Fluorescence Binding Assay

The binding affinity between the boronic acid group and dopamine or ascorbic acid was determined by following the protocol established by Springsteen and Wang [4]. The binding of the diol to the PBA decreases the concentration of the PBA-ARS complex, and the concomitant decrease in the fluorescence signal is monitored. The binding constant between the PBA and the diol (DA and AA in this work) was calculated by determining the concentration of PBA displaced from the PBA-ARS complex upon addition of various concentrations of the diol. PBA and ARS solutions with concentration 2mM and 9 µM respectively, 9 µM ARS and 2 mM PBA, and 9 µM ARS and 2 mM PBA with a range of DA or AA concentrations were prepared in 0.10 M phosphate buffer (PBS) (pH 7.4). They were allowed to react for 5 minutes at room temperature before performance of the fluorescence experiments. The solutions were excited at 468 nm and the fluorescence intensities were monitored at the emission wavelength of 588-590 nm. Equations used to calculate the binding constants are shown below:

$$Q = \frac{[\text{ARS}]}{[\text{ARS-PBA}]} \qquad (1)$$

$$\frac{[\text{diol}]}{P} = \frac{K_{eq}}{K_a} Q + 1 \qquad (2)$$

$$P = [\text{diol}_o] - \frac{1}{QK_{eq}} - \frac{[\text{ARS}_o]}{Q+1} \qquad (3)$$

where, K_{eq} is the association constant of the ARS-PBA complex, K_a is the association constant of the boronic acid–diol complex, $[\text{diol}_o]$ is the total diol concentration, $[\text{ARS}_o]$ is the total ARS concentration, Q is the ratio of the concentration of uncomplexed ARS to complexed ARS (Equation 1), and P is defined by Equation 3. The K_a of the boronic acid–diol complex was determined plotting [diol]/P vs. Q, and dividing K_a by the slope of the plot, as per Equation 2. The fluorescence emission spectra were obtained at a Cary Eclipse fluorescence spectrophotometer (Varian).

3 RESULTS AND DISCUSSION

3.1 Detection of Dopamine

Fig 1a shows the stable CV curves of the PABA/ssDNA/SWCNTs composite before and after adding different concentrations of dopamine in PBS solution (pH 7.4). The two redox couples centered at 0.25 V and 0.45 V (vs. Ag/AgCl), corresponding to the transition of the polyaniline backbone from the fully reduced leucoemeraldine state to the partially oxidized emeraldine salt state, and from the emeraldine salt state to the fully oxidized pernigraniline state, respectively.

Upon addition of dopamine the faradic current (at E=0.45 V) of the composite decreases and this decrease is linear with increasing concentration of dopamine (Figure 1b). This suggests that direct oxidation of dopamine did not occur. Instead, the diol of dopamine chemically binds to the boronic acid groups along the polyaniline backbone [2]. As the native polyaniline is not conductive or electrochemically active in neutral pH solutions, the electrochemical activity and the conductivity of polyaniline towards higher pH is due to the strong intra- or inter-chain tetrahedral boron–nitrogen interactions in the boronic acid moities; which stabilize the protonated emeraldine form to some extent at pH 7.4. The high affinity binding between dopamine and boronic acid affects the polyaniline backbone in different and seemingly divergent ways that require delineation. On one hand, the conversion of the boronic acid to the boronate ester complex along the polyaniline backbone interrupts the intra- or inter-chain tetrahedral boron–nitrogen interactions, which decreases the self-doping and therefore the electrochemical activity in neutral solutions. Furthermore, the steric effect of the formed anionic ester also hinders the electrochemistry and conductivity of the polyaniline backbone. This is because oxidation and reduction of polyaniline during cyclic voltammetry are accompanied by conformational changes of the polymer backbone, which become less energetically favorable as large molecules are introduced along the backbone

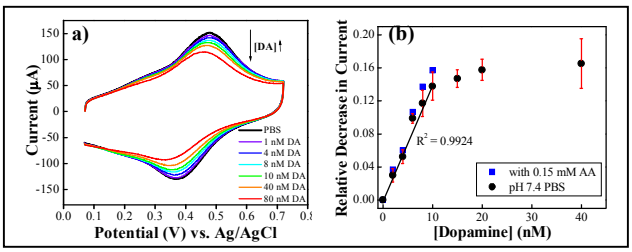

Fig 1(a) Cyclic voltammograms of the composite in PBS and in the presence of different concentrations of dopamine; (b) Correlation curves for the detection of dopamine on the electrode modified with ssDNA/SWNT/PABA/ Nafion composite in the absence (•) and presence (▪) of 0.15 mM AA (n=3). Potential scan rate: 100 $mV \cdot s^{-1}$.

On the other hand, formation of the boronate complex leads to an increase in the electron donating ability of the boron in the boronate substitutent groups. This ability is expected to stabilize the acid form of the quinone diimine group along the polymer backbone, a trademark of the conductive form of polyaniline (emeraldine salt), which means an enhanced self-doping ability. Therefore, the electrochemical activity of the polyaniline backbone should be enhanced upon binding. It becomes apparent that these two effects on the electrochemical activity of the polyaniline backbone offset one another. Upon binding of dopamine, we found that the redox current decreased,

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suggesting that the steric effect of the formed anionic ester played the more important role. The resulting ester hindered the electrochemical activity of the polyaniline backbone.

3.2 Interference by Ascorbic Acid

Ascorbic acid is the most severe interferent in the determination of DA in electrochemical sensors. Like dopamine, ascorbic acid contains one planar diol, and can be oxidized in the same potential window as dopamine. We found that the mechanism of interference is very different from those approaches that rely on the direct oxidation of the dopamine at the electrode. Instead of regenerating dopamine, the ascorbic acid reduced the fully oxidized polyaniline backbone and electrocatalytically binds with boronic acid groups through its plannar di-ols as dopamine does (Scheme 1). In the detection scheme described here-in, direct oxidation of dopamine on the electrode and its consequent regeneration by AA were avoided.

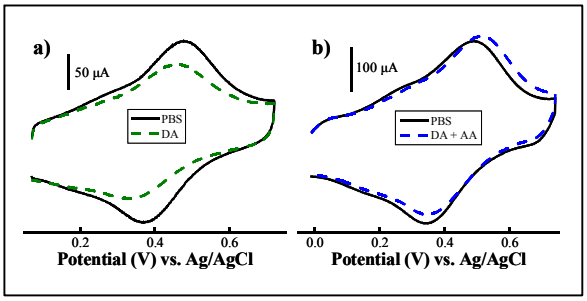


Figure 2. Cyclic voltammetric curves of the ss-DNA/SWNT/PABA modified Au electrodes in pH 7.4 PBS and upon addition of (a) 10 nM dopamine and (b) 10 nM dopamine and 0.15 mM ascorbic acid

Contrary to the PABA report [5], we found that AA interference results in an increase of the oxidation current and positive shift of the oxidation potential (Fig 2). To understand the interference mechanism by AA we studied the electrochemical behavior of the PABA/ssDNA/SWNT composite upon introducing AA to the electrochemical cell alone. We found an extremely large initial oxidation current and a decrease of the corresponding reduction current of the polyaniline backbone (Fig 3). This is typical electrochemical response characteristic of electrocatalytic reduction of the polyaniline backbone by AA. Briefly, polyaniline is oxidized to its fully oxidized form, pernigraniline, when sweeping the potential in the positive direction in the CV experiment. Due to the strong reductive ability of the AA, the fully oxidized pernigraniline is reduced to the fully reduced state of the polymer backbone, leucoemeraldine, in the presence of AA, which becomes available for oxidation again in a larger quantity thereby giving rise to the large catalytic oxidation current during the subsequent oxidation wave. In our experiment, further cycling caused the oxidation current to decrease rapidly and then stabilize at a value close to that before addition of AA (Fig 3). Note that the electrocatalytic reduction of the polyaniline backbone with ascorbic acid usually did not cause an oxidation potential shift and a decrease in the oxidation current with cycles. Both phenomena were observed in this detecting platform, indicating that another chemical process also occurred during the electrocatalytic process. As can be seen in Fig. 2b, both the current and the potential increase with increasing levels of AA. In fact, we found a linear, monotonic correlation between potential increase of the CV curve and the concentration of AA which demonstrates that at higher concentrations binding of the diol to the PABA causes a positive shift in oxidation potential of the PABA. Therefore, the positive shift of the oxidation potential and the decrease of the oxidation current may be understood as a result of the formation of boronate ester complexes between AA and the boronic acid groups in the PABA/SWNT composite.

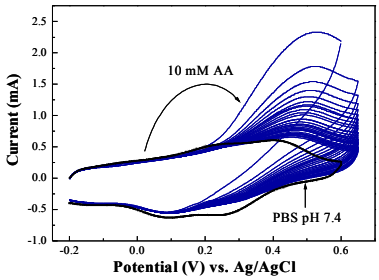


Figure 3. CVs of the composite in PBS (—) and upon addition of 10 mM ascorbic acid (—) the first CV upon addition of AA is the tallest one, and each successive cycle yielded a smaller curve

3.3 Fluorescence Binding Assay

AA's ability to electrocatalytically reduce the PANI backbone has been studied previously, which explains the extremely large oxidation current and the subsequent decrease in reduction current in the CV curves of the composite upon addition of AA. However, the positive shift in potential, suggests that some of the AA also binds to the boronic acids of the polymer: this is contrary to a previous report. Previously, it is reported that ascorbic acid up to 20-fold the concentration of dopamine did not interfere with electrochemical detection of dopamine [6], which does not support our data (the comparison is reasonable because the repeating unit of the PABA in the composite is essentially phenylboronic acid). To resolve these contradictory results we studied the affinities of AA and DA to boronic acid groups of PABA using the fluorescence binding assay protocol developed by Wang and co-workers [4]. The fluorescence emission spectra obtained for the AA system (Fig. 4a) are very similar to the emission spectra obtained for the DA system (Fig. 4c), except for the large difference in the concentration ranges of the species used to elicit the result. The binding assay requires that a certain percentage of the ARS-PBA complex become unbound and that a certain percentage of the PBA bind to the diol of interest – the extent to which the PBA binds the diol after disassociating with the ARS dye results in the factor Q, (Equation 1). In fact, the Q value must be obtained in the approximate range of 0.5-2.5. The concentration range of the diol required to obtain Q values in that range depends on the binding constant of the PBA-diol complex. The AA

concentration range is almost 250-fold larger than the DA concentration range. This difference can be seen in the [diol]/P vs. Q graphs (Fig. 4b, 4d), obtained using Equations 2 and 3. The [diol]/P values are much larger for AA than DA. The association constants for the PBA-DA complex and the PBA-AA complex were obtained from the slopes of these graphs.

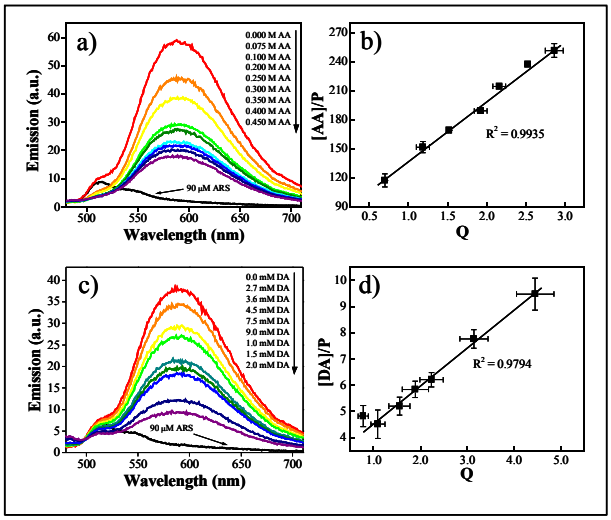
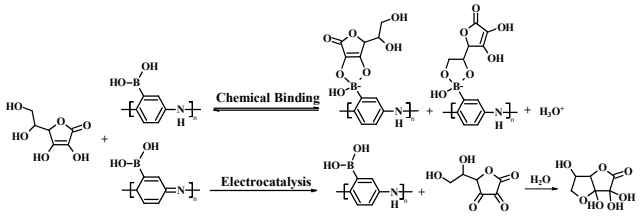


Figure 4. Fluorescent binding assay results of the affinity between PBA and AA and between PBA and DA: a) Fluorescence emission curves of the PBA-ARS complex upon titration with a range of AA concentrations; b) Linear correlation between [AA]/P and Q c) Fluorescence emission curves of the PBA-ARS complex upon titration with a range of DA concentrations; d) Linear correlation between [DA]/P and Q.

Experimental determination of the binding affinity of AA and PBA in 0.1 M phosphate buffer (pH 7.4) showed the binding constant to be 21 ± 1.8 M^{-1} (mean ± SEM), which is approximately 40-fold lower than that of PBA and DA (830 ± 42 M^{-1}). We used phenylboronic acid (PBA) instead of 3-aminophenylboronic acid (the exact repeating unit of the polymer) to eliminate any possible florescence quenching due to PET by the amine groups. Since, the concentration of AA is three or four orders of magnitude higher than the concentration of DA in physiological samples, large amounts of AA could bind to the boronic acid groups along the polyaniline backbone under physiological conditions. Therefore, the interference by AA toward the detection of dopamine is a two-pronged problem in this system. On one hand, the electrocatalytic reductive ability of AA caused a large increase of the oxidation current of the polyaniline backbone, and on the other hand AA chemically bonded to the boronic acid groups, which induced a decrease of the oxidation current and a positive shift of the oxidation potential. The net effects of these two divergent factors determine the degree of AA interference on the detection of DA. The chemical and electrochemical interactions between PABA and AA are summarized in Scheme 1.



Scheme 1. Molecular basis for the interactions between PABA and AA

3.4 Elimination of the Ascorbic Acid Interference

Since AA interacts with the polymer through its plannar diol, the strategy to eliminate the interference is to use charge discriminating membranes to preferentially accumulate he positively charged dopamine (pKa 8.9) and reject the negatively charged ascrobate (pKa 4.2) at the electrode surfaces in physiological pH. In our Previous work, we used a layer of negatively charged perfluorosulfonated polymer Nafion, which electrostatically rejected the ascrobate anion and provides a transport channel for positively charged dopamine [2], thus eliminating the interferences as shown in Figure 1b.

In summary, the interference mechanism is found to be very different from the approaches relying on direct oxidation of dopamine at the electrode. The ascorbic acid electrocatalytically reduced the fully oxidized polyaniline backbone and it was also able to bind to the boronic acid groups through its planar diol as dopamine does, although its binding affinity is lower. These results suggest that it is very necessary to understand the correlation between the structure of a diol and its binding affinity for a given boronic acid for developing boronic acid based biosensors.

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