Interference of Ascorbic Acid in the Sensitive Detection of Dopamine by a Nonoxidative Sensing Approach

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The electrochemistry of a poly(anilineboronic acid)/carbon nanotube composite was studied in the presence of dopamine and ascorbic acid. To understand the binding affinity of dopamine and ascorbic acid to the boronic acid functional groups in the composite, the association constants between the diol groups in dopamine and ascorbic acid and the boronic acid were experimentally determined using a fluorescence-based binding assay. The results demonstrate that ascorbic acid could severely interfere with the detection of dopamine in nonoxidative boronic acid-binding approaches: Ascorbic acid was able to electrocatalytically reduce the fully oxidized polyaniline backbone during the electrochemical oxidation process; similarly to dopamine, ascorbic acid was also able to bind to the boronic acid groups through its planar diol group even though the binding affinity is much lower. The examination of the dopamine transduction mechanism and ascorbic acid interference mechanism in this nonoxidative approach will benefit the design of future boronic acid-based sensors.

Introduction

Most of the current detection techniques for in vivo detection of dopamine exploit the ease of oxidation of the neurotransmitter. However, the direct oxidative approaches suffer from a common problem: the oxidation product of dopamine can react with ascorbic acid present in samples and regenerate dopamine, which severely limits the accuracy of detection. In a previous study, we reported a nonoxidative approach to electrochemically detect dopamine with extremely high sensitivity.[1] Dopamine concentrations as low as 1 nM were detected with cyclic voltammetry and 40 pM with differential pulse voltammetry. This approach takes advantage of the high performance of our newly developed poly(anilineboronic acid) (PABA)/carbon nanotube (CNT) composite. The binding of dopamine to the boronic acid groups of the polymer with high affinity affects the electrochemical properties of the polyaniline backbone, which act as the transduction mechanism of this nonoxidative dopamine sensor. The unique reduction capability and high conductivity of single-stranded DNA functionalized single-walled CNTs greatly improved the electrochemical activity of the polymer in a physiologically relevant buffer, and the large surface area of the CNTs significantly increased the density of the boronic acid receptors. Since direct oxidation of dopamine on the electrode was not involved in this sensing approach, its related problems, such as regeneration of dopamine by the ascorbic acid, were avoided. The high sensitivity along with the improved selectivity of this sensing approach may hold great promise for molecular diagnosis of Parkinson's disease.

However, we found that ascorbic acid (AA) also interfered with this dopamine detection approach, which was contrary to the previous report that used PABA alone to detect dopamine.[2,3] In our prior study,[1] the AA interference was effectively eliminated by deposition of a thin layer of ion-exchange polymer Nafion on top of the PABA/CNT composite, but the mechanism of AA interference in this detection approach was not fully studied. Toward the goal to develop a dopamine sensor for in vivo and in vitro applications it is essential to fully understand the dopamine transduction mechanism and AA interference mechanism of this nonoxidative approach, which is the focus of this work.

Experimental Section

Reagents. Purified single-walled CNTs 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, 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), which was also used to rinse and clean samples after polymerization and before any characterization.

Dispersion of Single-Walled Carbon Nanotubes in Solution. The bundled single-walled CNTs were dispersed into water using the method described by Zheng and co-workers.[4,5] Briefly, 1 mg of purified HiPco single-walled nanotubes was suspended in 1 mL of aqueous DNA solution (1 mg mL^{-1} dT(30), 0.1 M NaCl). The mixture was kept in an ice−water bath and sonicated (Branson, 2510) for 90 min. After sonication, the samples were centrifuged (Eppendorf 5415 C) for 90 min at 16 000*g* to remove insoluble material, leaving DNA-dispersed nanotube solutions at a mass concentration in the range of 0.2−0.5 mg/mL.

Au Substrates. The Au substrates were prepared by sputtering high-purity Au onto cleaned Si(100) wafers with a Ti adhesion layer (100 nm Au and 10 nm Ti). The resulting gold substrates were cleaned with freshly made piranha solution (98% H_2SO_4/30% H_2O_2, 2:1 v/v, 90 °C, 3 min (***Caution:*** *piranha solution should be handled with extreme care*), and rinsed with water, and finally rinsed with ethanol. Then the

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substrates were immersed into an ethanol solution of 20 mM 2-aminoethanethiol for 24 h, rinsed with ethanol and nanopure water, and dried with a flow of N_2. Modification of the electrode with the cysteamine self-assembled monolayer promoted adsorption of the negatively charged ssDNA/SWNTs.

Electrochemical Measurements. Electrochemical polymerization of 3-aminophenylboronic acid and electrochemical characterization of the resulting films were carried out at a CH Instrument 750 series electrochemical station. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were conducted using a homemade Teflon cell (0.25 cm^2 area) with the modified gold substrate as the working electrode, a platinum wire as a counter electrode, and a Ag wire as a quasi-reference electrode. (The quasi-reference electrode was calibrated against the more widely used Ag/AgCl/saturated KCl reference, and all the potentials quoted in this work are in terms of the Ag/AgCl scale.)

Synthesis of Nanocomposite. The self-doped polyaniline/CNT composite was fabricated on the gold electrode surface by following the procedure described in our recent work.[6] Briefly, 4 μL of the dispersed ssDNA/SWNT solution was cast on top of the Au electrode modified with the 2-aminoethanethiol monolayer and allowed to dry at room temperature to form an ssDNA/SWNT film. Poly(anilineboronic acid) was then deposited onto the modified Au substrate by sweeping the electrochemical potential from −0.16 to 0.94 V (vs Ag/AgCl) in 0.05 M 3-aminophenylboronic acid monomer, 0.04 M KF, 0.5 M H_2SO_4. It was reported that the presence of F^- in the monomer solution could decrease the potential required for the polymerization of 3-aminophenylboronic acid;[7] thus, its addition minimized overoxidation of the polyaniline backbone. After the second cycle, the polymerization potential was decreased to 0.89 V to further reduce the possibility of overoxidizing the polymer backbone. Repeated cycling of the potential resulted in continuous deposition of PABA onto the electrode surface. The resulting deep green PABA/ssDNA/SWNT composite on the electrode was first stabilized in 0.5 M H_2SO_4 and then in 0.01 M phosphate-buffered saline (PBS, pH 7.4) by sweeping the potential between −0.21 and 0.71 V (in H_2SO_4) and 0.04 and 0.79 V (in PBS) until the CV curves were stabilized. Compared to the neat PABA, we found that the composite has greatly enhanced electrochemical stability mainly due to the electrocatalytic reduction ability of ssDNA-functionalized CNTs.

Results and Discussion

Electrochemistry of the PABA/SWNT Composite in the Presence of Dopamine. After stabilization of the composite film in 0.01 M PBS (pH 7.4), dopamine with different concentrations was added into the electrochemical cell. After each addition, the potential was swept between 0.04 and 0.79 V until the CVs were stable, and the electrochemical current of the polyaniline backbone was measured. Figure 1 shows the stable CV curves of the PABA/ssDNA/SWNTs composite before and after adding a range of dopamine concentrations into the PBS solution (pH 7.4). The CV curves show two redox couples centered at 0.25 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.

Addition of dopamine did not affect the redox peak potentials of the polyaniline backbone. However, the faradic current of the composite's anodic peak at 0.45 V decreases with increasing concentration of dopamine, which suggests that direct oxidation

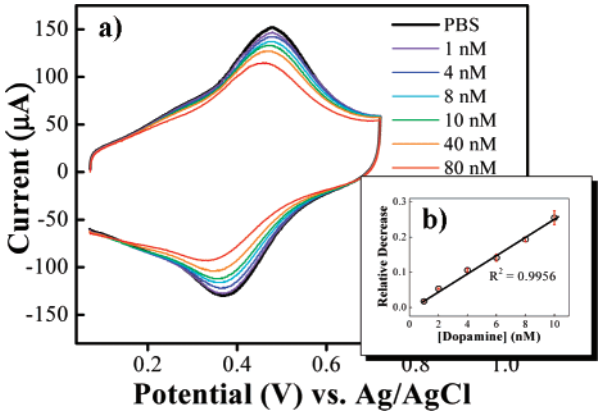
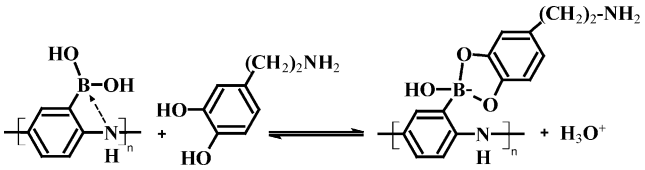


Figure 1. (a) Cyclic voltammograms of the composite in PBS and in the presence of different concentrations of dopamine. (b) A correlation curve of the data presented in panel a (n = 5). Potential scan rate: 100 mV·s^{-1}.

SCHEME 1: Complexation between Dopamine and Poly(anilineboronic acid)



of dopamine on the electrode did not occur. Rather, the diol of dopamine chemically bound the boronic acid groups along the polyaniline backbone—this interaction has been well-studied for diols like saccharides, and the complex is a negatively charged tetrahedral boronate ester (Scheme 1).[8−10] In the case of the polymer, the boronate esters form along the polymer backbone, and they influence the electrochemical activity of the polyaniline backbone .[7]

It is well-known that native polyaniline is not conductive or electrochemically active in neutral pH solutions.[3,6] The boronic acid moieties extend the electrochemical activity and the conductivity of polyaniline toward higher pH due to the strong intra- or interchain tetrahedral boron−nitrogen interactions which stabilize the protonated emeraldine form at pH 7.4.[11,12] This self-doping process preserved to a large extent the electrochemical activity and conductivity of polyaniline in neutral pH solutions.[13]

The high affinity binding between dopamine and boronic acid can affect the electrochemistry of the polyaniline backbone in different and seemingly divergent modes and, thus, requires clarification. On one hand, the conversion of the boronic acid to the boronate ester complex along the polyaniline backbone interrupts the intra- or interchain tetrahedral boron−nitrogen interactions, which *decreases* the self-doping and therefore the electrochemical activity of the polymer in neutral solutions. Furthermore, the steric effect of the formed anionic ester also hinders the electrochemical activity of the polyaniline backbone.[14] This is because oxidation and reduction of polyaniline during CV are accompanied by conformational changes of the polymer backbone, which become less energetically favorable as large molecules are introduced along the backbone.

On the other hand, formation of the boronate complex eliminates the electron-withdrawing nature of the boron's vacant p-orbital and, instead, leads to an increase in the electron-donating ability of the boron in the boronate substitute groups. Increasing the electron-donating ability of the substitute 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. The net effect depends on the relative magnitude of the influences.

These multifaceted effects on electrochemical activity were observed recently in a boronic acid-substituted bipyridine Fe(II) complex.[14] Nicolas et al. found that the apparent formal potential of the ferrocene/ferrocenium redox couple decreased and the redox current increased upon formation of the electron-donating boronate anion structure with F^-, whereas with fructose an increase of the formal potential and decrease of redox current were observed. The authors ascribed these contrary changes to the different steric effects imparted by the F^- and fructose to the pyridine backbone in spite of their similar abilities to convert the boronic acid groups from electron-withdrawing to electron-donating substituents. Upon binding of dopamine, we found that the redox current decreased, 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, which is in agreement with the report by Nicolas et al.[14]

It was reported that formation of the anionic ester could reduce the K_a of the protonated quinone diimine groups in the polyaniline backbone, and reduction of the K_a caused a positive shift in the potential of the electrochemical conversion of emeraldine to pernigraniline. Shoji and Freund developed a PABA-based potentiometric sensor for detection of saccharides using the potential shift of PABA as the transduction principle.[7,15] Therefore, we expected a decrease of the oxidation current and a concomitant positive shift in the peak potential upon dopamine binding. However, our data does not show appreciable shifts in E_{pa} upon dopamine binding. This is likely due to the low concentration of dopamine (nanomolar range) used in this study: the formation of a small amount of anionic ester could not induce an observable potential shift. In fact, both a decrease in the faradaic current of the anodic peak as well as an increase in the potential of the anodic peak was observed when 1 μM dopamine was added to the electrochemical cell.

Interference by Ascorbic Acid. Ascorbic acid is the most severe interferent in the determination of dopamine (DA) in electrochemical sensors. It coexists with DA in the extracellular fluid of the central nervous system, and its concentration is 3 to 4 orders of magnitude higher than that of DA.[16,17] Like dopamine, ascorbic acid contains one planar diol, and it can be oxidized in the same potential window as dopamine. Modification of the electrodes to promote the oxidation of dopamine (to separate the oxidation potential of the two molecules) and use of dopamine-specific enzymes (to selectively oxidize dopamine) did not eliminate interference from AA. This is because the oxidized dopamine product, dopamine-*o*-quinone, can be reduced by the AA so that dopamine can be regenerated and become available once more for electrochemical oxidation at the electrode surface. In the nonoxidative detection scheme described herein, dopamine was not directly oxidized on the electrode. Therefore, the problem of regeneration by AA was inherently avoided.[16,18] Indeed, it was reported that the AA interference was largely eliminated at a PABA-electrode dopamine sensor due to the high binding affinity between dopamine and the boronic acid moieties of PABA.[3] To evaluate the selectivity of the present PABA/ssDNA/SWNT system for in vivo detection of DA in the nanomolar range, CV was used to study the electrochemical response of the PABA/ssDNA/SWNT composite to a mixture of DA and AA.

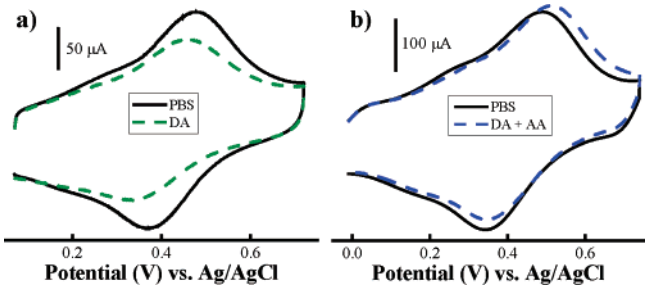


Figure 2. Cyclic voltammetric curves of the ss-DNA/SWNT/PABA modified Au electrodes in pH 7.4 PBS and upon addition of (a) 10 nM dopamine and (b) 10 nM dopamine and 0.15 mM ascorbic acid. Potential scan rate: 100 $mV \cdot s^{-1}$.

Surprisingly, we found that AA still significantly interferes in the detection of dopamine but with a different interference mechanism compared to oxidative dopamine sensors. Figure 2 shows that addition of 0.15 mM AA to 10 nM dopamine resulted in an increase of the oxidation current and positive shift of the oxidation potential (0.15 mM AA was used in this study because it is close to the physiological level in extracellular spaces of brain.[19]) To understand the interference mechanism by AA we studied the electrochemical behavior of the PABA/ssDNA/SWNT composite upon introducing AA alone to the electrochemical cell, and the results are displayed in Figure 3a. It is clear that introducing AA to the electrochemical cell triggered an extremely large initial oxidation current and a decrease of the corresponding reduction current of the polyaniline backbone. This is a typical electrochemical response characteristic of electrocatalytic reduction behavior of AA toward the polyaniline backbone.[20] Briefly, polyaniline was 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 was reduced to the fully reduced state of the polymer backbone, leucoemeraldine, which became available again for oxidation in a larger quantity, thereby giving rise to the large electrocatalytic oxidation current during the subsequent sweep in the positive potential direction. In our experiment, further cycling caused the oxidation current to decrease rapidly, and then the current stabilized at a value slightly higher than before addition of AA (Figures 2b and 3a). 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.[20] Both phenomena were observed in this process, indicating that another chemical process occurred along with the electrocatalytic process.

Shoji and Freund reported that the oxidation potential of the PANI backbone shifts positively when a diol binds to the boronic acid groups along the backbone of PABA.[7,15] 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. By adding different concentrations of AA into the electrochemical cell, we found that both the oxidation current and the potential increased (after the CV curves were stabilized, Figure 3b) and the positive potential shift increased monotonically as a function of the AA (Figure 3c). These results are consistent with the reports by Freund et al., suggesting that binding occurs between ascorbic acid and the boronic acids on the PABA. The formation of the anionic ester between AA and the boronic acid

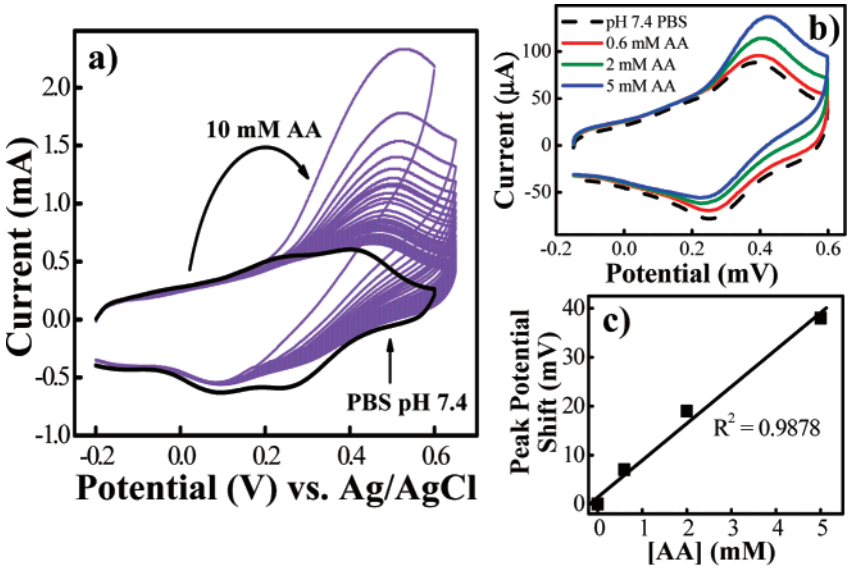
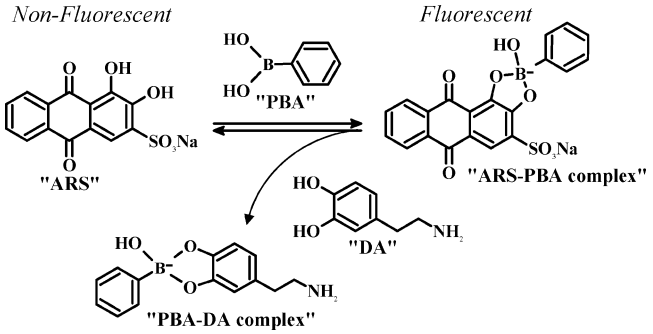


Figure 3. Influence of AA alone on the PABA/SWNT composite sensor: (a) CVs of the composite in PBS (black) and upon addition of 10 mM ascorbic acid (violet); the first CV upon addition of AA is the tallest one, and each successive cycle yielded a smaller curve; (b) titration of the composite with AA, ranging from 0.6 to 5 mM; (c) a graph of the shift in peak potential of the composite's CV upon addition of AA as a function of the concentration of AA. Potential scan rate: 100 mV•s^{-1}.

groups in the PABA/SWNT composite reduced the K_a of the protonated quinone diimine groups in the polyaniline backbone, and reduction of the K_a caused a positive shift in the potential of the electrochemical conversion of emeraldine to pernigraniline. To further confirm this conclusion and to quantify the binding affinities of dopamine and ascorbic acid with the boronic acid groups along the polyaniline backbone, a fluorescence binding assay was utilized to measure the association constants.

Fluorescence Binding Assay. Springsteen and Wang developed a general method for measuring association constants of diol−boronic acid complexes under physiological pH solutions.[21,22] This protocol is a three-component competitive assay containing a fluorescent reporter dye, alizarin red S (ARS), phenylboronic acid (PBA), and the diol-containing compound of interest. ARS has a diol group and is able to form a boronate ester with PBA. The free ARS is only weakly fluorescent because the excited-state proton transfer from the phenol hydroxyl group of ARS to the ketone oxygen results in the fluorescence quenching. The fluorescence of ARS increases upon formation of the boronate ester with PBA because the fluorescence quenching mechanism is removed. The binding constant between the diol of interest and PBA is determined based on the competitive binding of the ARS and the diol to PBA (Scheme 2). When the diol binds to the PBA, it disrupts the ARS−PBA complex, thereby decreasing the concentration of the ARS−PBA complex, as well as the fluorescence signal of the solution. This protocol requires knowledge of the binding constant between the PBA and the ARS, which can be determined using the Benesi−Hildebrand method.[23] The binding constant between PBA and the diol (DA and AA in this work) is calculated by determining the concentration of PBA displaced from the PBA−ARS complex upon addition of various concentrations of the diol.[22] Note that we could not directly measure the binding constants of DA or AA to the boronic acid groups in the PABA composite using this method because of the possible quenching ability of the CNTs in the composite and the relative difficulty in determining the concentration of the boronic acid moieties in the composite. (This concentration is required in the calculations for this protocol.) Considering that the repeating unit of the PABA in the composite is essentially

SCHEME 2: Three-Component Fluorescence Protocol for Determining the Binding Constant between Boronic Acids and Diols Using the Reporter Dye ARS



PBA, the measured binding constant between PBA and DA (or AA) could indicate the relative binding strength of the DA (or AA) to the boronic acid groups in the PABA composite, although the absolute values may be different.

Solutions of 9 μM ARS, 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 (pH 7.4). They were allowed to react for 5 min 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 use to calculate the binding constants are shown below:[24]

$$Q = \frac{[\mathrm{ARS}]}{[\mathrm{ARS{-}PBA}]} \tag{1}$$

$$\frac{[\mathrm{diol}]}{P} = \frac{K_{eq}}{K_a}Q + 1 \tag{2}$$

$$P = [\mathrm{diol_o}] - \frac{1}{QK_{eq}} - \frac{[\mathrm{ARS_o}]}{Q+1} \tag{3}$$

where K_{eq} is the association constant of the ARS−PBA complex (determined by the Benesi−Hildebrand method), K_a is the

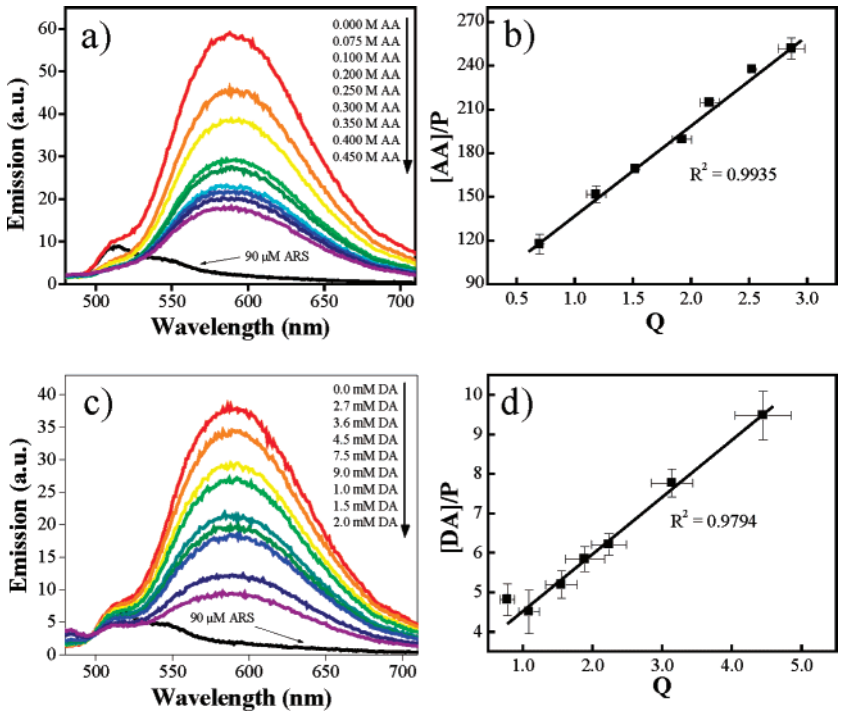


Figure 4. Fluorescent binding assay results of the affinity between PBA and AA and between PBA and DA: (a) fluorescence emission curves of the PBA−ARS complex upon titration with a range of AA concentrations; (b) linear correlation between [AA]/*P* and *Q* (see the Experimental Section for explanation); (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*.

association constant of the boronic acid−diol complex, [$diol_o$] is the total diol concentration, [ARS_o] is the total ARS concentration, *Q* is the ratio of the concentration of uncomplexed ARS to complexed ARS (eq 1), and *P* is defined by eq 3. The K_a of the boronic acid−diol complex was determined by plotting [diol]/*P* vs *Q* and dividing K_a by the slope of the plot, as per eq 2. The fluorescence emission spectra were obtained at a Cary Eclipse fluorescence spectrophotometer (Varian).

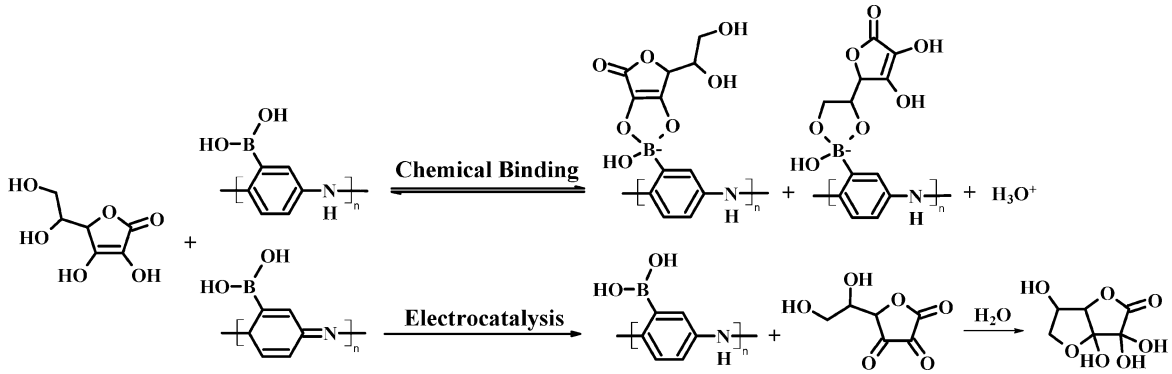
Figure 4c shows the fluorescence of the ARS−PBA complex upon addition of different concentrations of dopamine. The fluorescence decreases as a function of the dopamine concentration, as expected. We calculated the binding constant between PBA and dopamine as 890 ± 42 M^{-1} (mean ± SEM). Springsteen and Wang determined the binding constant between PBA and catechol to be 830 M^{-1}. As catechol is very structurally similar to dopamine (although it does not contain an ethylamine group like dopamine), the small difference in their K_a values is understandable. However, it is necessary to mention the possibility that the existence of free amine group in dopamine may quench the fluorescence of the ARS−PBA complex due to the lone electron pair on the amine nitrogen.[25] This would result in an apparently larger binding constant compared to catechol, which only contains a diol group.[22]

To elucidate the influence of the amine group in dopamine on the fluorescence signal during the binding constant measurement, a control experiment was performed with tyramine. Tyramine is also a neurotransmitter, and it has a very similar molecular structure as dopamine but without a diol group to bind boronic acid: rather, it possesses a single alcohol group and an ethylamine group in the para position. We utilized the same fluorescence binding assay to study how the amine group interacts with the PBA−ARS complex by monitoring the fluorescence signal upon addition of different concentrations of tyramine into the PBA−ARS complex solution (Supporting Information). It is obvious that addition of tyramine barely changed the fluorescence signal of the PBA−ARS complex, suggesting that possible quenching of the ARS−PBA complex by the free amine groups of dopamine, resulting in an overestimate of the calculated PBA−DA binding constant, is negligible. It was also reported that amine groups can interact with boronic acid through a B−N dative bond. The tyramine control experiment result also overrules additional dopamine binding to the boronic acid through a B−N dative bond in this concentration range. Therefore, we conclude that dopamine interacts with boronic acid groups mainly through the formation of a boronate ester with the diol group.

Chemical Interaction between AA and the Boronic Acid Group. The ability of AA to electrocatalytically reduce the polyaniline backbone has been studied previously, but AA may also bind to the boronic acid groups of the PABA, indicated by the positive shift of the oxidation potential of the polyaniline backbone. This type of interaction, however, is contrary to the previous reports.[2,3] Strawbridge et al. developed an electrochemical catecholamine detection method based on the ability of a diol to selectively form a boronate ester with PBA.[2] The formed boronate ester could be oxidized at a very different potential from that of "free" dopamine or ascorbic acid. They demonstrated that ascorbic acid up to a 20-fold higher concentration than dopamine did not interfere with the electrochemical detection.

We utilized the fluorescence-based binding assay described above to study the binding affinity of ascorbic acid to PBA. The fluorescence emission spectra obtained for the AA system (Figure 4a) are very similar to the emission spectra obtained for the DA system (Figure 4c), except that much higher concentrations of AA were used to elicit *Q* values in the range of 0.5 and 2.5. This is because 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 is described by the factor *Q* (eq 1). The concentration range of the diol required to obtain

SCHEME 3: Molecular Basis for the Interactions between PABA and AA



Q values in the 0.5−2.5 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 (Figure 4, parts b and d), obtained using eqs 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.[24]

The binding affinity of AA to PBA in 0.1 M phosphate buffer (pH 7.4) showed the binding constant to be $21 \pm 1.8\ M^{-1}$ (mean ± SEM), which is approximately 40-fold lower than the DA association constant ($890 \pm 42\ M^{-1}$). Considering that the concentration of AA is 3 or 4 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 nonoxidation approach. 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 3, which may serve as a molecular paradigm for the interference of AA toward other PABA-, PANI-, and boronic acid-based sensors.

Although we still did not understand yet why the current approach is contradictory to the previous reports about AA interference, we speculate that one of the most important reasons is the extremely high sensitivity provided in the current sensing approach, which "detected" the previously undetectable AA, leading to the observed interference. Finally it is important to mention that a freshly prepared ascorbic acid solution is required to study the interference effect of AA. We noticed that the AA solutions that were used approximately 1 day after preparation did not demonstrate interference. We understand this is because AA is a reducing agent and is not stable in solution.[26] In vivo AA is protected by chemical interactions with physiological proteins, but in vitro AA is susceptible to oxidation, which is not surprising considering that the foremost chemical role of vitamin C is as a reducing agent. It is reported that measurable oxidation of AA occurs within hours.[26] The oxidized product of AA is dehydroascorbic acid,[27] which is not electrochemically active, and its binding to boronic acid is extremely weak.[28]

Conclusions

In this report the electrochemistry of PABA/CNT composite in the presence of dopamine and ascorbic acid was discussed. For the first time, the binding affinity of dopamine and ascorbic acid to the boronic acid functional group was experimentally determined using a fluorescence-based binding assay. We found that ascorbic acid was able to severely interfere with the electrochemical detection of dopamine in the nonoxidative detection approach, which was contrary to the previous reports. However, the interference mechanism of ascorbic acid in this nonoxidative electrochemical detection approach is 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 did, although the binding affinity is much lower. Even though in our previous report the interference of ascorbic acid was effectively eliminated by coating a thin layer of Nafion on top of the PABA/CNT composite, a clear understanding the dopamine transduction mechanism and AA interference mechanism in this nonoxidative approach interference mechanism is essential to eliminate other interferences toward in vivo and in vitro detection of dopamine. Furthermore, as boronic acid has been widely used as a recognition motif in the construction of sensors for saccharides, nucleotides, and antibody mimics targeted on cell-surface carbohydrates, this study will also benefit the design of a wide variety of biosensors with high sensitivity and selectivity.

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Supporting Information Available: Additional information (the fluorescence binding assay result with tyramine) as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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