Fabrication of a Boronic Acid-Surface and InSitu Optical Detection of Catecholamines∗

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We fabricated a boronic acid-surface on a flat silica surface for optical detection of recognition of catechol structure. The boronic acid-surface was obtained by chemical reaction between 4-carboxyphenylboronic acid (CPB) and the primary amine group of 3-aminopropyltriethoxysilane (APS) modified onto the silica surface. The UV-vis spectroscopic and the X-ray photoelectron spectroscopic (XPS) measurements indicated that 90% of the primary amines of APS reacted with CPB. Recognition of the catechol structure on the CPB surface was observed with in-situ time dependent measurement using optical configuration of surface plasmon spectroscopy. Injection of phosphate buffers containing 1.0 mM dopamine (DA) and catechol, respectively, at pH 7.0 allowed increase of the reflectivity, suggesting complexation between DA or catechol and the boronic acid of CPB. On the other hand, increasing of the reflectivity (∆R) in the case of tyramine, which is very similar in chemical structure to DA but has no catechol structure, was quite small. The ratio of ∆R between DA and tyramine (DA/tyramine) was experimentally estimated to be 5.5, which briefly shows that the CPB-modified surface can recognize catechol structure. The ∆R behaviors of DA differed from the pH of buffers. At pH 8.1, polymerization of DA was observed on the CPB-modified surface while no recognition at pH 6.0. The efficient pH for the 1:1 complexation between DA and boronic acid of the CPB-modified surface is thought to be around 7.0. [DOI: 10.1380/ejssnt.2009.571]

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I. INTRODUCTION

The complexation between boronic acids and diols is well known (Fig. 1) and has been taken in separation and assay of sugars, enzymes, nucleotides, and catecholamines [1–7]. One of the most important factors for complexation and disassociation is pKₐ of the boronic acid. The pKₐ of boronic acid depends on the own chemical structure [8, 9]. If the pKₐ value is controlled, more sensitive and efficient separation can be performed. Phenylboronic acid derivatives are attractive materials in terms of control of pKₐ and many kinds of the derivatives are available commercially. In addition, immobilization onto silica and polymer matrices is carried out in simple ways.

Analysis of catecholamines is required as a part of physical and psychological health care. This is because catecholamines are deeply linked to cardiovascular disorders such as hypertension and myocardial infarction (MI) [10] and psychological stress as a trigger of MI [11–13]. In conventional analysis of catecholamines, high-performance liquid chromatography (HPLC) with electrochemical and fluorescent detection has been commonly employed [14–18]. However, the HPLC technique is a high sensitive method which requires complicated instruments and specially-trained operators. Recently, surface-based sensing methods combined with surface chemical modification and measuring techniques like quartz crystal microbalance (QCM), electrochemistry, and UV-vis spectroscopy have been devised for analysis of catecholamines [19–21]. We have also searched the surface-based methods and reported fluorescent detection via surface chemical reaction of catecholamines with a probe material modified onto a fused silica glass surface [22]. This method is simple because post-labeling process can be omitted. However, the signal-to-noise ratio (S/N) was quite small due to overlapping of fluorescences from the fluorophores and the fused silica substrate.

In this study, we applied combination of surface chemistry and plasmonics for detection of catecholamines. Firstly, we fabricated a phenylboronic acid-surface by surface chemical reaction for recognition of catechol structure and analyzed the resulting functional surface by UV-vis spectroscopy and X-ray photoelectron spectroscopy (XPS). Next, in order to confirm the ability of recognition

FIG. 1: Reversible complexation pathways of a diol and a boronic acid.
on the phenylboronic acid-surface, catechol, dopamine, and tyramine as analytes were tested. The pH effecting on the recognition behaviors of dopamine were also considered. The recognition behaviors of the analytes onto the phenylboronic acid-surface were observed using the technique based on surface plasmon resonance (SPR). The technique provides high sensitive detection of changes of thickness and/or refractive index on the surface without special labeling such as fluorescent labeling. Moreover, in-situ observation of changes on the surface can be also performed so that we can follow kinetics of complexation. Our final target is to build an analyzing system combined with the “catecholamines chip” and the technique of surface plasmon spectroscopy. We expect this study to lead to building the analyzing system.

II. EXPERIMENTAL

A. Chemicals

3-Aminopropyltriethoxysilane (APS) 1 was purchased from Aldrich. 4-(4,6- Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinum chloride (DMT-MM) 2 [23] was purchased from Sigma. 4-Carboxyphenylboronic acid (CPB) 3, dopamine hydrochloride (DA) 4, catechol 5, and, tyramine hydrochloride 6 were purchased from Tokyo Chemical Industry Co., Ltd. Chemical structures were shown in Fig. 2.

B. Process for fabrication of boronic acid surfaces

Flat fused silica glass was used as a substrate except for SPR measurement. Before surface modification, the fused silica substrate was immersed into a solution of hydrogen peroxide (30%) and concentrated sulfuric acid (95%) in the ratio 3:7 (Piranha solution) for 30 min, rinsed with water, and dried in a stream of nitrogen. As a solution for activation of the fused silica surface, 1 mM solution of acetic acid in methanol (94% by volume) was mixed with 1% APS and 5% deionized water. The substrate was immersed into the APS solution for 2 h at room temperature. The APS-treated substrate was rinsed twice with methanol in order to remove excess APS and baked at 120°C for 5 min. For SPR measurement, a high refractive index glass (S-LAH66, OHARA, n =1.769 at 632.8 nm) was used. Chromium (1 nm), gold (47 nm), and chromium (1 nm) were thermally deposited in turn onto the S-LAH66 glass under high vacuum. Subsequently, silica (18 nm) was deposited onto the outer chromium layer by sputtering. The APS modification was also carried out on the resulting silica surface. The APS-modified substrate was immersed into a 100 mM phosphate buffer (PB) at pH 7.0 containing 1.0 mM CPB and 10 mM DMT-MM as a condensing agent at room temperature (Fig. 3). Then the substrate was rinsed with water twice and dried by the stream of nitrogen gas.

C. Measurements

XPS spectra were collected by a PHI 5600ci X-ray photoelectron spectrometer using a monochromatic Al Kα source (1486.5 eV, 100 W) with a take-off angle of 75°. The binding energy values were referenced to the Si2p peak in fused silica set at 103.3 eV [24]. UV-vis absorption was measured using a Hitachi U-4100 spectrophotometer (reference: air). SPR measurement was performed using the “Kretschmann-Raether configuration” [25]. The substrate coupled with a prism (S-LAH66) via an index-matching oil was mounted on a flow cell with the volume of 1.0 ml and placed on a goniometer controlled by a personal computer. A linearly p-polarized He-Ne laser beam at 632.8 nm was used as an incident beam, which was mechanically chopped in conjunction with a lock-in amplifier before incidence into the prism. In the angular scan, the reflection light from the substrate was detected by a photodiode and recorded as a function of the incident angle. Complexation of DA onto the CPB-modified surface was observed in real time by recording changes in the reflectivity at a fixed angle (kinetic scan). The CPB-modified surface was exposed to a 100 mM PB at least for 30 min for obtaining stable baseline without circulation of the 100 mM PB before injecting the sample solution containing DA to the flow cell. 100 mM PBs at pH 6.0, 7.0, and 8.1 containing 1.0 mM DA were prepared as the sample solutions just before injection to the flow cell. In addition, PBs at pH 7.0 containing 1.0 mM catechol and...
tyramine, respectively, were also injected for comparison of complexation behaviors. All the sample solutions or PBs for rinse were injected at a rate of 2.0 ml/min. The solutions in the flow cell were completely replaced with the sample solutions or PBs for rinse by injecting 4.0 ml. The kinetic scans were carried out without circulation of the sample solutions or PBs for rinse.

III. RESULTS AND DISCUSSION

A. Fabrication of boronic acid surface

To observe the reaction of CPB onto the APS-modified surface, UV-vis spectra of the surfaces were measured after reaction (Fig. 4). The absorption band due to the phenyl group of CPB was observed at 220-260 nm in 2 h of reaction time. The absorbance increased with reaction time, which suggests that the reaction of CPB with the primary amine of APS should proceed. Then the absorbance at 235 nm virtually became leveling off after 8 h of reaction time (Fig. 4, insert). The reaction of CPB onto the APS-modified surface seems to be approximately completed in 8 h. Next, in order to confirm the binding between CPB and APS, XPS spectra of the surfaces were measured. Obviously, the high resolution B1s spectrum of the surface reacted with CPB after 16 h of reaction time indicated the existence of boron atoms while no peak observed from the APS-terminated surface (Fig. 5(a)). The high resolution N1s spectra showed the existence of nitrogen atoms in both surfaces. Both spectra have two peaks attributed to neutral nitrogen (lower binding energy) and ionized nitrogen (higher binding energy), respectively. After reaction of CPB, the peak attributed to neutral nitrogen slightly shifted higher as compared with the APS-terminated surface (from 399.3 eV to 399.7 eV), suggesting changes in the bonding state of neutral nitrogen atoms (Fig 5(b)). Probably APS and CPB formed amide bonding. Surface concentration (Γ) of CPB binding with the primary amine of APS can be estimated using the following formula:

$$\Gamma = \frac{AN_A}{2\varepsilon} \times 10^{-3}, \quad (1)$$

where A is the absorbance at 235 nm (the both sides were modified.), ε is is Avogadro’s number [26]. The surface concentration of CPB modified onto the APS-terminated surface is calculated to be $2.5 \times 10^{14}/\text{cm}^2$ (molecular area of ∼ 0.40 nm²). In XPS measurement, the B/Si ratio in the CPB-modified surface was found to be 0.057 while the N/Si ratio in the APS-terminated surface was 0.064, implying $2.8 \times 10^{14}/\text{cm}^2$ of surface concentration of APS (molecular area of ∼ 0.36 nm²) [27]. It is considered that about 90% of APS molecules on the glass surface reacted with CPB.

B. Catechol selectivity on the CPB-modified surface

It is reported that a 1:1 complex between phenylboronic acid and catechol forms at neutral pH [8, 28]. Here, in order to observe recognition of catechol on the CPB-modified surface, PBs containing 1.0 mM catechol, DA, and tyramine at pH 7.0, respectively, were injected into the flow cell and the reflectivity changes (∆R) were monitored using SPR technique (Fig. 6). In the case of catechol, the ∆R slowly increased and the increase seems to be saturated temporarily at ∼ 3500 s. Then the ∆R steeply increased from 3700 s but decreased to ∼ 0.0066 by rinse with the PB. The behavior of ∆R suggests slow formation of the 1:1 complex between the boronic acid and catechol. The decrease of ∆R to ∼ 0.0066 after rinse shows removal of the excessively adsorbed-catechol and that the 1:1 complex formed in the first saturation at ∼ 3500 s is maintained. After injection of DA, ∆R increased and was saturated at 2500 s (∆R ∼ 0.0065), which suggests that formation of the boronic acid-DA complex reached the equilibrium state. After 2800 s, ∆R slightly increased but decreased to ∼ 0.0065 by rinse with the PB. Probably the slight increase of ∆R is attributed to physical adsorption of excess DA. The difference of the complexation rates between catechol and DA may bring about changes in the apparent pKₐ of boronic acid due to the difference of chemical structure between catechol and DA. In the report by Singhal et al., the apparent pKₐ value of phenylboronic acid was lowered by the presence of catechol, L-dopa, and DA [9]. Especially, in the presence of L-dopa and DA owning amino groups, the pKₐ value of phenylboronic acid tends to be lower than that in the presence of catechol. Additionally, Niwa et al. also reported the apparent lowering of pKₐ of phenylboronic acid derivatives in the presence of amino groups [29]. The apparent lowering of pKₐ allows complexation at the lower pH. In our experiment, 7.0 of the pH might be the efficient value for complexation between DA and the boronic acid, leading to the faster complexation of DA on the CPB-modified surface as compared with catechol. The efficient pH for complexation between catechol and the CPB-modified surface may be more than 7.0.

On the other hand, the behavior of ∆R after injection of tyramine is similar to that after injection of DA. How-
FIG. 5: XPS spectra of the APS- and CPB-terminated surfaces on the fused silica glass substrates. (a) High resolution B 1s XPS spectra. (b) High resolution N 1s XPS spectra.

FIG. 6: *In situ* kinetic measurement during reaction with 1.0 mM catechol (○), 1.0 mM DA (●), and 1.0 mM tyramine (▲) in 100 mM PB at pH 7.0.

However, the saturated ∆R is quite smaller. The detail of binding state of tyramine onto the boronic acid is not clear at this moment. The ratio of ∆R (DA/tyramine) at pH 7.0 was estimated to be 5.5 from the kinetic scans, which briefly shows that the CPB-modified surface has the catechol selectivity.

C. Effects of the pH on complexation behaviors of DA

For observation of the pH which effects on complexation behaviors of DA with the CPB-modified surface, the phosphate buffers at pH 6.0, 7.0, and 8.1 containing 1.0 mM DA were injected into the flow cell (Fig. 7). At pH 7.0, the behavior of ∆R showed complexation on the CPB-modified surface as mentioned in previous section. At pH 6.0, ∆R temporally increased just after injection of DA but became approximately zero at the end. DA injection at pH 6.0 allowed formation of no complexes. The pKₐ of boronic acid of CPB modified onto the APS-terminated surface would be more than 6.0 at least.

At pH 8.1, ∆R steeply increased just after injection of DA and then continued to increase at a constant rate until rinse with PB. Also when monitoring for 3 h after injection of DA at pH 8.1, the reflectivity continued to increase until rinse (Fig. 8, insert). After rinse the SPR angle shifted high and the minimum reflectivity increased (Fig. 8). Shifting to a higher angle is derived from the increase of the thickness on the surface. The factor of increase in the minimum reflectivity is thought as light absorption by the adsorbed layer. In case the layers with absorption band at irradiated light were deposited onto the surface (polyaniline on the gold surface etc.), the minimum reflectivity tend to increase [30]. This is because the imaginary part of the dielectric constant of the layer, which is related to the optical absorption,
changes to nonzero value. According to the report by Lee et al., DA polymerizes in a buffer solution at pH 8.5 and the polymerized dopamine adheres to many kinds of materials [31]. Actually, the color of our sample solution at pH 8.1 containing 1.0 mM DA changed to dark brown in around 10 minutes of dissolving DA in the PB. Subsequently formation of the precipitation and adherence of the dark-brownish film on the wall of the glass vessel were observed. After the CPB-modified glass substrate was immersed into 1.0 mM DA solution at pH 8.1 for 3 h, the edge of absorption was at more than 800 nm in the UV-vis spectrum of the substrate (Fig. 9). The differential spectrum, which was obtained by subtraction of the spectrum of the CPB-terminated substrate from that of 3 h immersion into the DA solution at pH 8.1, clearly presents the existence of absorption at 632.8 nm (Fig. 9, insert). In the differential spectrum at pH 7.0, no remarkable increase of absorption was observed at long wavelengths like 632.8 nm. Therefore, it is supposed that the monotonic increase of the $\Delta R$ at pH 8.1 is caused by polymerization of dopamine on the CPB surface or adsorption of the polymerized dopamine offshore.

IV. CONCLUSION

We fabricated the CPB-modified surface onto silica surfaces by surface chemical reaction for detecting the recognition of catechol structure. The ability of recognition for catechol structure was confirmed by observation of change in $\Delta R$ using SPR technique. It is considered that the efficient pH for complexation with the CPB-modified surface varies according to the kind of catechol derivatives. For example, DA, which owns the amino group, forms the complex with the boronic acid at lower pH as compared with catechol. When DA was injected at pH 8.1, monotonic increase in $\Delta R$ was observed and the $\Delta R$ was not saturated. The angular scan in SPR setup and UV-vis spectrum of the surfaces after 3 h of injection showed possibility of polymerization of DA. In this study, we realized high sensitivity of the SPR technique by observing complexation of the small molecules such as catechol and DA. Currently, we have searched the detection limit and observation of selectivity. Additionally, we have prepared a new probe material including phenylboronic acid structure and improved the selectivity for catecholamine structure, not only catechol structure.

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FIG. 8: Angular scan curves before (○) and after (●) reaction with 1.0 mM DA in the PB at pH 8.1 for 3 h.

FIG. 9: UV-vis absorption spectra of the CPB-modified substrates after reaction with 1.0 mM DA in the PB at pH 7.0 (green line) and 8.1 (black line) for 3 h. The red spectrum is the CPB-terminated substrate without reaction with DA. The insert is the differential spectra which were subtracted from the green and black spectra to the red spectrum.

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