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Boronic Acid-Based Electrochemical Sensors for Detection of Biomolecules

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Boronic acids can form reversible covalent bonds with 1, 2- or 1, 3-diols to generate five or six-membered cyclic complexes. Thus, boronic acid functionalized compounds and materials have attracted much attention in both chemistry and biology as the recognition motif for chemo/biosensing and enrichment and separation of biomolecules. In this contribution, we addressed the recent progress of boronic acid-based electrochemical sensors for the detection and immobilization of diol-containing biomolecules including dopamine and glycoproteins. This work will be valuable for the development of novel boronic acid-based electrochemical sensors.

Keywords: Boronic acid; electrochemical sensors; dopamine; glycoproteins; diol

1. INTRODUCTION

Boronic acids can form reversible covalent bonds with 1, 2- or 1, 3-diols to generate five or six-membered cyclic complexes. Boronic acid functionalized compounds and materials have thus been widely used as the recognition motif for chemo/biosensing and enrichment and separation of diol-containing biomolecules, such as sugars, dopamine and glycoproteins. Based on the boronic acid-diol interaction, extensive efforts have been focused on the development of colorimetric, fluorometric and electrochemical sensors for determination of diol-containing compounds. In the past, many review articles and books concerning boronic acid-based optical and electrochemical sugar sensors have been published [1-5]. However, to the best of our knowledge, there is no review on the boronic acid-based electrochemical sensors for the detection and immobilization of dopamine and glycoproteins (e.g. glycosylated enzymes and andtibodies). In this review, we addressed the recent development about that.

2. DOPAMINE SENSORS

2.1 Boronic acid-modified carbon materials for detection of dopamine

Dopamine (DA), one of the most important neurotransmitters with aromatic diol group, is derived from the amino acid tyrosine. It is widely distributed in the central neural system, brain tissues and body fluids of mammals and plays pivotal roles in the function of the central nervous, renal, hormonal and cardiovascular systems. Concentration change in dopamine has been associated with various diseases and disorders, such as Parkinson's disease, Alzheimer's disease, Huntington's disease, epilepsy, pheochromocytoma and neuroblastoma [6]. Dopamine can be easily oxidized electrochemically at electrodes. The development of voltammetric methods for dopamine determination in human fluid such as urine and serum has received considerable interest. However, some biochemical compounds such as ascorbate acid and uric acid, the levels of which are 100-1000 times higher than that of dopamine, are oxidized at nearly same potential. The overlap of their voltammetric responses makes the sensitive and selective detection of dopamine highly difficult. Phenylboronic acid can form stable boronate ester bond with dopamine, and the resulting complex was employed to develop electrochemical or electronic sensors for dopamine detection. For example, Strawbridge et al. reported the electrochemical detection of dopamine in solution based on the formation of boronate ester between dopamine and boronic acid [7]. The boronate ester is electrochemically oxidized at potential considerably more positive than that necessary to oxidize dopamine itself, indicating that the formation of the neutral ester is thermodynamically favourable. Moreover, the oxidized ester tends to revert back to (oxidised) catechol and the phenylboronic acid. The method is selective to dopamine at the presence of excess ascorbic acid (around 20-fold).

To avoid the interference of biochemical compounds, boronic acid functionalized electrodes have also been constructed for dopamine detection based on the formation of boronate ester. In 2003, Fabre et al reported a dopamine sensor with an interdigitated microarray electrode coated with poly(aniline boronic acid) [8]. Under physiological conditions, the covalent anchoring of the aromatic diol to the immobilized boronic acids on electrode resulted in a decrease in the electrical conductivity of functionalized polyaniline. The conductivity changes were detected by the decrease in the drain current between the two arrays at a constant offset potential. With this method, dopamine can be detected at physiological pH in the presence of excess ascorbic acid. Moreover, they found that the electrode can be easily regenerated in acidic medium owing to the pH-sensitive boronic acid-dopamine complexation equilibrium. In 2005, Mathiyabrasu et al. investigated the behaviour of a poly (aniline boronic acid) modified glassy carbon electrode (GCE) for the detection of dopamine in the presence of excess of ascorbic acid using cyclic voltammetry and differential pulse voltammetry [9]. They found that the poly(aniline boronic acid) favours dopamine oxidation through ester formation with boronic acid motif. However, ascorbic acid oxidation is also promoted by polyaniline backbone through the involvement of ascorbic acid in the redox of polyaniline. Nafion is known to filter out negatively charged species like ascorbate anion (pKa = 4.10) through electrostatic repulsion and can be used to reduce the interference from the ascorbate anion. Their results also demonstrated that Nafion incorporated poly(aniline boronic acid) film was suitable for the selective determination of dopamine

in the presence of ascorbic acid. The selectivity was contributed to the accumulation of dopamine on the electrode surface through formation boronate ester and the suppression of ascorbic acid oxidative current through charge discrimination by Nafion.

Carbon nanotubes are generally considered to be ideal electrode materials due to their outstanding characteristics of high conductivity, chemical inertness, large surface area, low mass density and good biocompatibility. Wu et al. reported the sensitive detection of dopamine with boronic acid functionalized multi-walled carbon nanotubes (MWNTs)-modified glass electrode in the presence of excess ascorbic acid [10]. They suggested that dopamine can be selectively picked out from the mixtures of dopamine and excess ascorbic acid via the recognition binding between the boronic acid group and dopamine. Due to the high conductivity and finely-dispersed recognition sites on the MWNTs-modified electrode, the detection limit was far below 0.05 μM. Importantly, the MWNTs-modified GC electrode can be regenerated by acid treatment in 0.5 M HCl solution with almost no loss of activity, and therefore is potentially recyclable.

The products of dopamine oxidation can react with ascorbic acid present in samples and regenerate dopamine again. Thus, the detection of dopamine by the oxidation itself severely limits the accuracy. In 2007, Ai et al. developed a nonoxidative approach to detect electrochemically dopamine with high sensitivity and selectivity [11]. This approach takes advantage of the high performance of poly(anilineboronic acid)(PABA)/carbon nanotube composite and the excellent permselectivity of the ion-exchange polymer Nafion (Fig. 1). The binding of dopamine to the boronic acid groups of the polymer with large 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 carbon nanotubes greatly improved the electrochemical activity of the polymer in physiological buffer, and the large surface area of the carbon nanotubes largely increased the density of the boronic acid receptors. Note that without the coating of Nafion, ascorbic acid could severely interfere with the detection of dopamine in such nonoxidative boronic acid-binding approaches since ascorbic acid can electrocatalytically reduce the fully oxidized polyaniline backbone during the electrochemical oxidation process and bind to the boronic acid groups through its planar diol group [12].

Molecularly imprinted polymers offer more reproducible, stable, and robust films for sensor applications. Recently, Hong et al. developed a sensor for the detection of dopamine with the molecularly imprinted polymeric films consisting of co-polymerization of 3-acrylamidophenylboronic acid, acrylamide, and N,N’-methylenebisacrylamide [13]. The interaction of dopamine-boronic acid and the negative charge on the molecularly imprinted polymeric film allowed excellent selectivity against ascorbic acid and other structurally similar interferents. To improve the sensitivity, a thin layer of multiwalled carbon nanotubes was deposited on the gold electrode surface prior to electropolymerization, and a detection limit to ~20 nM was achieved.

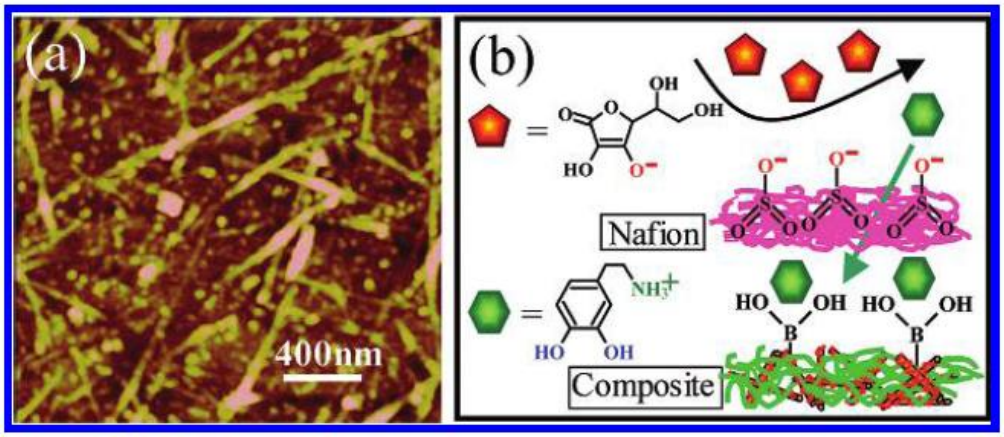

Figure 1. (a) Tapping mode atomic force microscopic image of the ssDNA/SWNT/PABA nanocomposite, fabricated by electropolymerization of 3-aminophenylboronic acid in the presence of the ssDNA-wrapped, single-walled carbon nanotubes. (b) A schematic drawing of the layer-by-layer dopamine sensor on a gold electrode. The top layer is Nafion, which electrostatically repels ascorbate away from the electrode surface. Dopamine penetrates through this layer to the bottom layer of ssDNA/SWNTs/PABA to bind with the boronic acid groups. (From ref. [11], with permission. Copyright E 2007 American Chemical Society).

2.2 Self-assembled monolayers-covered gold electrodes for detection of dopamine

Self-assembled monolayers (SAMs) are an inexpensive and versatile surface coating for molecular recognition for sensors. SAMs on gold have been used frequently for controlling the non-specific adsorption of biomolecules [14]. Shervedani et al. reported the detection of dopamine with the boronic acid-modified gold electrode [15]. The sensor was fabricated by coupling 4-formylphenylboronic acid onto the cysteamine SAMs gold surface. The interaction of boronic acid and dopamine induced the increase of faradaic impedance and the decrease of current of the modified electrode in $[Fe(CN)_6]^{3-/4-}$ solution. A linear response in the range of 5.0×10^{-9} to 5.0×10^{-6} M doapmine was obtained by cyclic voltammetry. Recently, Casalini et al. described a potentiometric sensor based on Electrolyte-Gated Organic Field-Effect Transistor (EGOFET) for ‘‘in vitro’’ detection of dopamine [16]. The sensing element of this device resides at the Au gate–aqueous solution interface also composed by cysteamine and 4-formylphenyl boronic acid. The covalent and selective adsorption of dopamine induces a surface dipole potential which shifts the electrode work function and modulates the double layer capacitance. Such device is capable to detect dopamine up to pico-molar concentration. These two works above mentioned presented new methods for dopamine detection. However, the selectivity and interference were not evaluated because other diol-containing compounds such as sugars can also bind to boronic acid groups on electrode.

Sandwich-type biosensor is one of the major analytical techniques for sensitive and selective detection of biological species and has found wide applications in clinical diagnosis, biomedical research, food quality control and environmental monitoring. Our group recently reported a sandwich-type biosensor for dopamine detection [17]. Specifically, 4-mercaptophenylboronic acid (MBA)-modified gold electrode was used to capture dopamine through the formation of stable boronate ester

bond (Fig. 2). The anchored dopamine was then derivatized with biotin for the attachment of ferrocene(Fc)-capped gold nanoparticle/streptavidin (Fc-capped SA-AuNPs) conjugates. The voltammetric responses were found to be proportional to the concentrations of dopamine ranging from 0.5 to 50 nM. A detection limit of 0.2 nM was achieved. The method is new, but it cannot be used to detect dopamine in real body fluid samples because of the interference from other biological species, such as glucose. Thus, new chips are needed for selective capture of dopamine in the sandwich-type biosensor. In another study, we found that dopamine can be selectively captured by nitrilotriacetic acids-Fe(III)-covered electrode and glucose shows no interference in the detection [18].

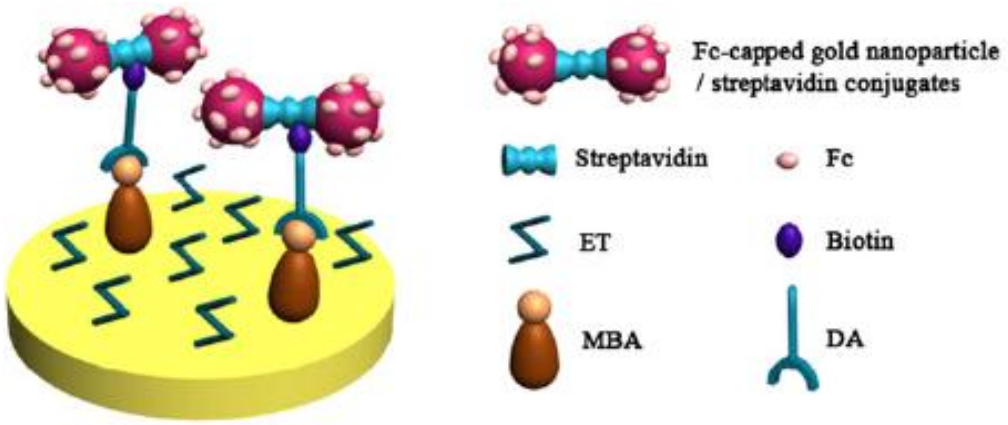

Figure 2. Schematic representation showing the capture of DA by the immobilized MBA and the detection of DA by attachment of Fc-capped SA-AuNPs conjugates. (From ref. [17], with permission. Copyright E 2013 Elsevier Science B. V.).

3. GLYCOPROTEINS SENSORS

Many proteins can be glycosylated *in vivo* and *in vitro* to form glycoproteins, including many enzymes and antibodies; the immobilization and recognition of glycoproteins based on the boronic acid–diol interaction has thus attracted much attention. The current progress in the immobilization and recognition of glycoproteins through the formation of boronate ester bond is discussed herein.

3.1 Immobilization of glycosylated enzymes for electrochemical sensing

Enzyme immobilization is one of the key factors in constructing high-performance enzyme biosensors on solid or colloidal substrates. Willner's group was the first to introduce a method for the immobilization of enzymes through the boronic acid-diol interaction [19, 20]. They constructed electrically contacted enzyme-electrodes by using a phenylboronic acid ligand as the building block for the association of FAD or $NAD(P)^+$ cofactors (Fig. 3). The surface-reconstitution of apoflavoenzymes (e.g. apo-glucose oxidase) on the phenylboronic acid-FAD monolayer yields an aligned and electrically contacted glucose oxidase (GOx) electrode. Similarly, the cross-linking of affinity complexes generated between PQQ-phenylboronic acid-FAD and $NAD(P)^+$ interfaces and the

biocatalysts GOx and malate dehydrogenase (MalD) or lactate dehydrogenase (LDH) yields integrated electrically contacted FAD/NAD(P)$^+$-dependent enzyme-electrodes [20]. The MalD enzyme-electrode exhibited a turnover rate of 190 s^{-1}, whereas the LDH enzyme-electrode revealed a turnover rate of 2.5 s^{-1}. At the same time, Abad et al. also reported the covalent immobilization of glycosylated proteins, glycoprotein horseradish peroxidase (HRP), through the direct binding of carbohydrate moieties to boronic acid groups on alkanethiol SAMs gold surface [21].

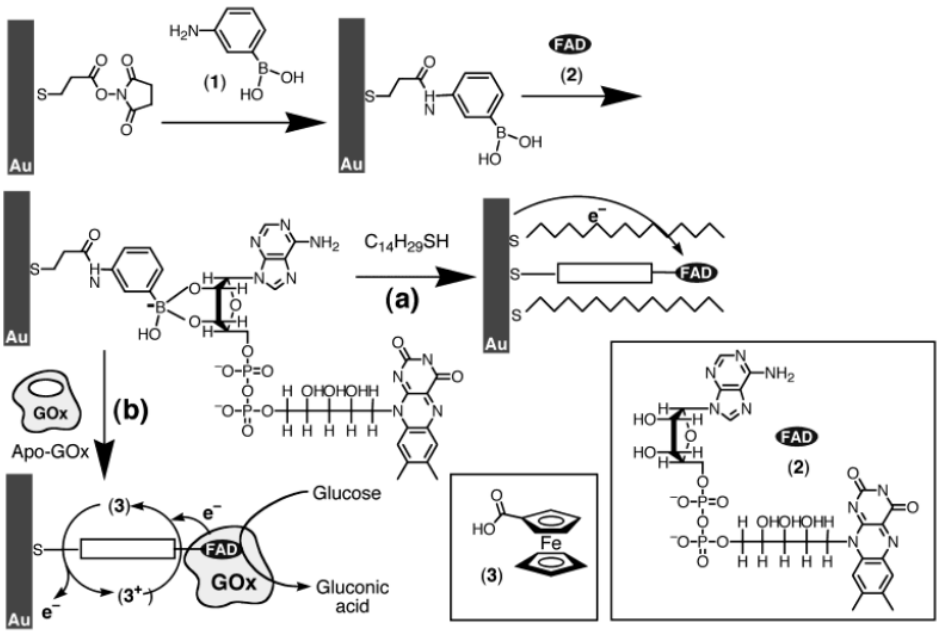

Figure 3. Covalent Coupling of FAD Cofactor to a Phenylboronic Acid-Functionalized Au-Electrode: (Route a) Formation of a Mixed FAD/Long Chain Thiol Rigidified Monolayer; (Route b) Reconstitution of Apo-GOx on an FAD-Functionalized Electrode. (From ref. [20], with permission. Copyright E 2007 American Chemical Society).

In 2005, Ma et al. studied the immobilization of glycosylated enzymes, such as glucose oxidase, horseradish peroxidase, dehydrogenase, on boronic acid-modified glassy carbon electrode based on the interaction of boronic acid and carbohydrate moiety within the enzymes [22]. For the electrode modification, 4-aminomethylphenylboronic acid was covalently grafted on a glassy carbon electrode by amine cation radical formation in the electrooxidation process of the amino-containing compound. The adsorptions of three kinds of enzymes were investigated by cyclic voltammetry and electrochemical impedance spectroscopy. At the same time, Liu et al. fabricated a phenylboronic acid self-assembled layer on glassy carbon electrodes for the recognition of glycoprotein peroxidase, where 3-aminophenylboronic acid is covalently bound to the electrochemical pretreated electrode surface with glutaraldehyde linkage (Fig. 4) [23]. The specific binding of glycoprotein peroxidase with the self-assembled layer was studied using horseradish peroxidase (HRP) as a model glycoprotein. Furthermore, they investigated the specific and non-specific binding of glycoproteins on the glassy carbon electrode covered with the thin film of poly(aniline boronic acid) [24]. The specific interaction between boronic acid and glycosylation sites of the glycoproteins including HRP and GOx is

reversible since it can be released in acidic solutions and can also be split by sugars. Their results also demonstrated that the poly(aniline) and poly(aminobenzoic acid) without the boronate group can also bind with HRP. Based on the reversible binding between enzyme-conjugated prostate-specific antibody (HRP-anti-PSA) and the phenylboronic acid SAMs on gold electrode, they also developed an immunosensor for the detection of prostate specific antigen (PSA) [25]. After incubating an HRP-anti-PSA modified electrode in a PSA solution, a decrease in the electrocatalytic response of the HRP-anti-PSA modified electrode to the reduction of H_2O_2 is observed. The photometric activity assays show that this decrease of the electrocatalytic response arises from the formation of immunocomplexes of HRP-conjugated anti-PSA. Moreover, besides electrochemical techniques, the interaction of glycoproteins and boronic acid-modified surface was also confirmed by surface plasmon resonance (SPR). For example, De Guzman et al. suggested that glycoproteins can be selectively captured by the carboxymethyl dextran substrate coupled with the boronic acid derivative, 4-[(2-aminoethyl)carbamoyl]phenylboronic acid [26]. They found that glycoproteins can be readily released from the AECPBA surface using borate buffer.

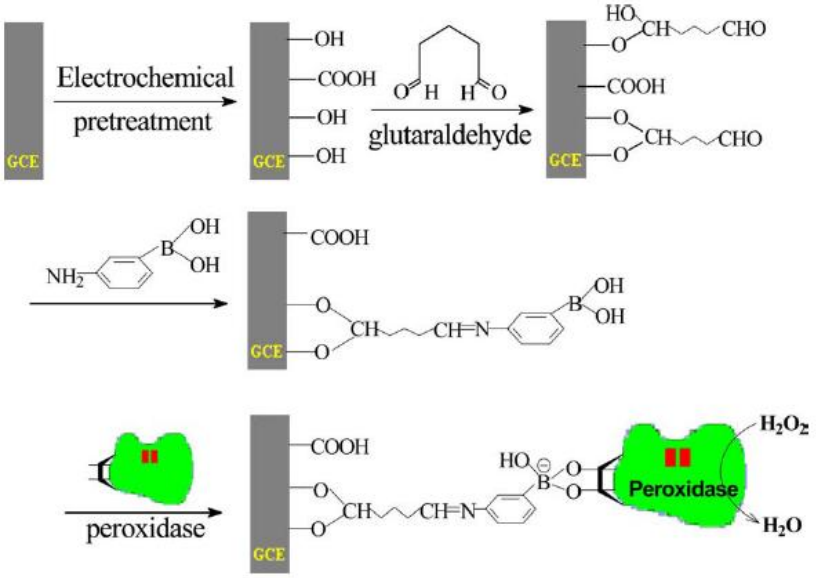

Figure 4. Preparation of enzyme modified electrode. (From ref. [23], with permission. Copyright E 2005 Elsevier Science B. V.).

Nanomaterials have attracted more and more attentions in the field of electrochemistry as electrode modified materials due to their high surface area, high adsorptivity and excellent catalytic capability. Villalonga et al. reported the oriented immobilization of HRP on gold electrodes with an electropolymerized matrix of Au nanoparticles, followed by modification with 2-mercaptoethanesulfonic acid, 3-mercaptophenyl boronic acid and p-aminothiophenol [27]. The electrode exhibited a rapid response within 8 s and a linear calibration range from 5 μM to 1.1 mM H_2O_2. Recently, Huang et al. reported a new protocol for efficient immobilization of a glycoprotein enzyme based on the interaction of boronic acid and diol groups within the enzyme [28, 29]. The electrode was prepared by casting a mixture of GOx and anilineboronic acid followed by a $NaAuCl_4$ solution to an Au-plated Au electrode surface. Chitosan was then cast onto the GOx–

poly(anilineboronic acid)–AuNPs bionanocomposite. In the proposed method, the small-sized Au nano or Au subnanostructures can form near/on the enzyme molecule, which greatly promotes the electron transfer of enzymatic reaction and enhances the amperometric responses.

3.2 Immobilization of antibodies for the capture of analytes

A common approach towards developing immunoassays is to attach antibodies onto the surfaces of assay devices via a solid support. When directly adsorbed onto surfaces, however, antibodies generally adopt random orientations and therefore, often fail to exhibit their immunoaffinity. To preserve the antigen binding activity of antibodies, there is an urgent need to develop specific and novel linking chemistries for attaching the antibodies to the solid surfaces in an oriented manner. Antibodies are glycoproteins belonging to the immunoglobulin superfamily. There are many reports on the immobilization based on the reversible interaction between boronic acid and carbohydrate within the constant domain, fragment crystallizable, of the antibody. For example, Liu's group reported a reusable electrochemical immunosensor for carcinoembryonic antigen on 3-aminophenylboronic acid-modified SAMs gold electrode [30]. In this work, the resulting boronic acid coating layer can specifically bind with the enzyme-conjugated carcinoembryonic antibody. The attached enzyme-conjugated antibody on the electrode surface could catalyze the reduction of hydrogen peroxide in the presence of thionine. After the formation of the immunocomplex between carcinoembryonic and the antibody, the access of the active center of HRP to thionine was partially inhibited. The assembly and regeneration process of the immunosensor was also investigated by cyclic voltammetry, electrochemical impedance and surface plasmon resonance. In the same way, they reported the detection of α-fetoprotein (AFP) with the enzyme conjugated AFP antibody [31]. The whole assay process including incubation, detection and regeneration of the electrode could be completed in 35 min. Avian leukosis virus (ALV) is a common avian retrovirus associated with neoplastic diseases. Wang et al. reported the electrochemical detection of Subgroup J of ALV (ALV-J) on glassy carbon electrodes modified with a film of poly(3-thiophene boronic acid), gold nanoparticles and grapheme [32]. In this work, the ALV-J antibody was immobilized onto the modified electrode through the covalent interaction between the boronic acid and the glycosyl groups of the antibody. The binding of ALV-J to the anchored antibody resulted in a decrease in the rate of charge transfer between the electrode and the redox probe and an increase in the electron transfer resistance.

Recently, Ho et al. compared the performances of two immobilization methods on screen-printed graphite electrodes (SPGEs) [33]. The first approach involves the deposition of gold nanoparticles (AuNPs) onto the SPGE and subsequent adsorption of monovalent half-antibody (monoAb) fragments of the anti-biotin Ab via Au–thiol bonds. For the second technique, 3-aminophenylboronic acid (APBA)-presenting SPGE surface was prepared for binding with the carbohydrate unit of the anti-biotin Ab. The results demonstrated that the sensitivity of the Ab/APBA/SPGE biosensor was ca. 250 times higher than that of the monoAb/AuNP/SPGE system. Furthermore, Moreno-Guzmán et al. reported the competitive electrochemical immunosensors for the detection of adrenocorticotropin (ACTH) and cortisol [34, 35]. In their works, aminophenylboronic

acid-modified dual screen-printed carbon electrodes were prepared for the oriented immobilization of ACTH and cortisol antibodies through the interaction of boronic acid and glycosylation sites of the antibodies (Fig. 5). The competitive immunoassays between the antigen and the biotinylated hormone for the binding sites of the immobilized antibody were performed. The electroanalytical response was generated by using alkaline phosphatase-labeled streptavidin or alkaline phosphatase labeled cortisol and 1-naphtyl phosphate as the enzyme substrate. The developed immunosensor was applied to a human serum sample with good results.

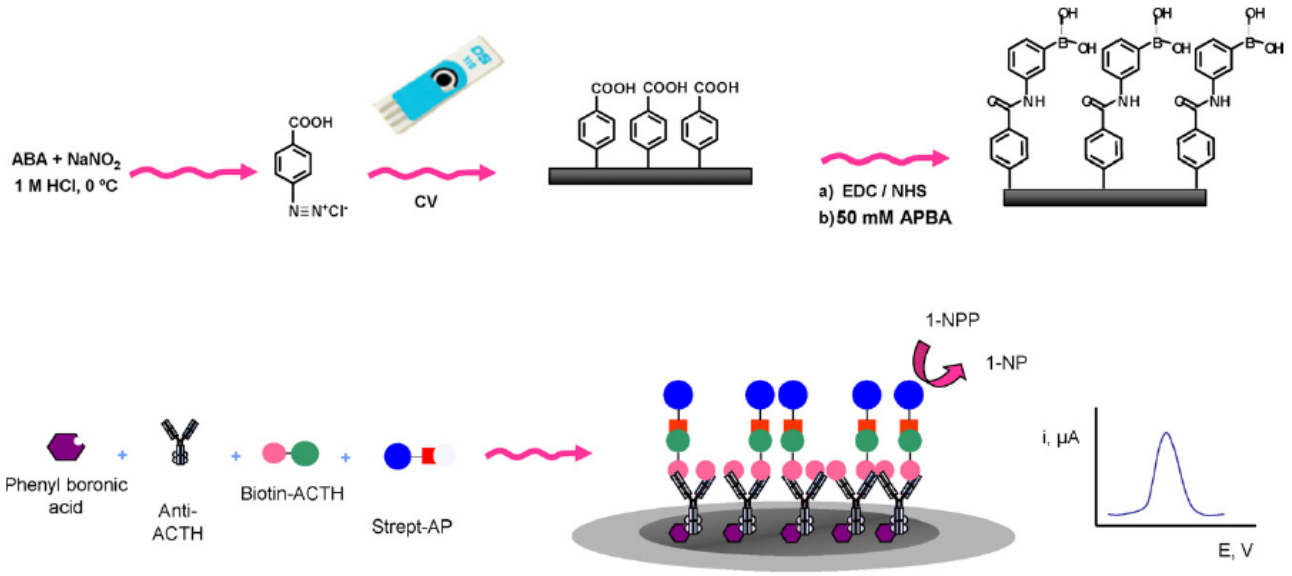

Figure 5. Schematic display of the reactions and protocols involved in the development of disposable electrochemical immunosensors for ACTH using screen-printed electrodes modified with phenylboronic acid. (From ref. [34], with permission. Copyright E 2012 Elsevier Science B. V.).

3.3 Detection of glycoproteins

Glycated hemoglobin (HbA1c), formed by the nonenzymatic attachment of glucose to the *N*-terminal valine of one or both of the hemoglobin beta-chains to form a stable ketoamine, has become an important clinical marker for lifetime healthcare. Measurement of HbA1c is now a common tool for monitoring long-term glycemic control of patients with diabetes. In 2010, HbA1c was added to the diabetes diagnosis criteria by American Diabetes Association. Based on the interaction between boronic acids and sugars, several groups developed the electrochemical sensors for the detection of HbA1c. For example, ferroceneboronic acid (FBA), an electrochemically active ferrocene derivative, can be used for the specific recognition of carbohydrates or glycoproteins through their interaction with boronic acid. Liu et al. reported the electrochemical biosensors for the determination of HbA1c in human whole blood with FBA probes [36]. In this work, hemoglobin was immobilized on a zirconium dioxide nanoparticle modified pyrolytic graphite electrode (PGE) in the presence of didodecyldimethylammonium bromide (DDAB). FBA was specifically bound to fructosyl residue of the HbA1c. The square wave voltammetric response of the bound ferroceneboronic acid reflects the

amount of glycated hemoglobin at the surface. However, this method needs the separation of sample to remove the plasma. Halámek et al. in the same group suggested that the complex of HbA1c and ferroceneboronic acid can be captured by the deoxycholate (DOCA) modified gold electrode [37]. Importantly, the electrode can be regenerated by pepsin digestion of the deposited hemoglobin and the sensor can be re-used more than 30 times. Furthermore, Park et al. reported the detection of HbA1c with thiophene-3-boronic acid (T3BA)-covered gold electrode by the electrochemical impedance spectroscopy [38]. The rate of charge transfer between the electrode and the redox probe is shown to be modulated by the amount of HbA1c in the matrix hemoglobin solution due to the blocking effect caused by the specific binding of HbA1c with boronic acid. The selective capture of HbA1c by the boronic acid SAMs was also confirmed by quartz crystal microbalance, atomic force microscopy, and EIS experiments.

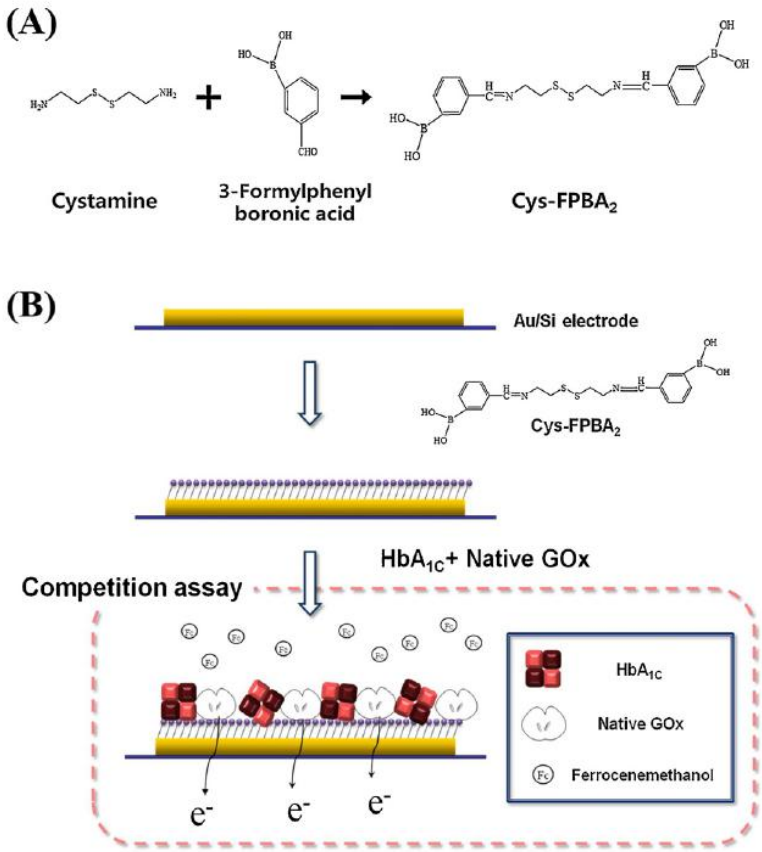

Figure 6. (A) Synthesis of Cys-FPBA2. (B) Schematic illustration of the competition assay between HbA1c and native GOx. (From ref. [39], with permission. Copyright E 2012 Elsevier Science B. V.).

Moreover, the electrodes modified with boronic acid-functionalized poly(amidoamine) G4 dendrimer have also been used for the attachment of HbA1c in Yoon's group [40]. To confirm the HbA1c binding to the boronic acid layer, periodate-treated glucose oxidase (GO*x*) was backfilled to the remaining amine groups of dendrimer on the electrode surface and quantified electrochemically. Subsequently, they developed an electrochemical HbA1c biosensor for diagnosing diabetes in whole human blood based on the competitive binding reaction (Fig. 6) [39]. HbA1c and GOx at a

predetermined concentration were reacted through a competition onto the boronate-modified electrode, allowing HbA1c to be detected linearly within a range of 4.5 – 15% of the separated hemoglobin sample (HbA1c/total hemoglobin). However, all of these methods may not be regarded to be specific for glycated HbA1c in blood because boronic acids can also bind to other glycated proteins. Thus, the pretreatment of blood sample is required in these works.

Sandwich-type electrochemical affinity biosensor is one of the major analytical techniques for sensitive and selective detection of proteins as well as small biomolecules. In this format, the crucial step is the capture (selectivity) and identification (sensitivity) of analytes. Regarding selectivity, the specific binding between the target analytes and the immobilized biomolecules on electrode, such as antigen–antibody, DNA–DNA and protein–nucleic acid, has been extensively used to capture analytes. The increasing demand for detection of ultralow amount of analytes is pushing the enhancement of detection sensitivity by selecting different signal amplification strategy. Nanoparticles (AuNPs) coated with boronic acid groups have been widely used for the specific capture and purification of glycoproteins [41-44]. For example, magnetic beads have been used for protein separation because they provide a simple and fast procedure for separating reacted protein from the rest of the reaction mixture by using an external magnet; Zhou et al. reported the selective separation of glycopeptides and glycoproteins using aminophenylboronic acid-functionalized magnetic nanoparticles [41]. Qi et al reported the selective enrichment of glycopeptides and glycoproteins with Fe_3O_4@C@Au magnetic microspheres functionalized with 4-mercaptophenylboronic acid [43]. Mesoporous silica holds the attractive features of high surface area and large accessible porosity. Xu et al. reported the enrichment of glycopeptides with boronic acid functionalized mesoporous silica at the first time [44]. In comparison to direct (traditional) analysis, this method enabled 2 orders of magnitude improvement in the detection limit of glycopeptides. The unbiased nature of organo-boronic acid groups also made this method applicable to all kinds of glycopeptides regardless of their sizes, structures, and hydrophilicities.

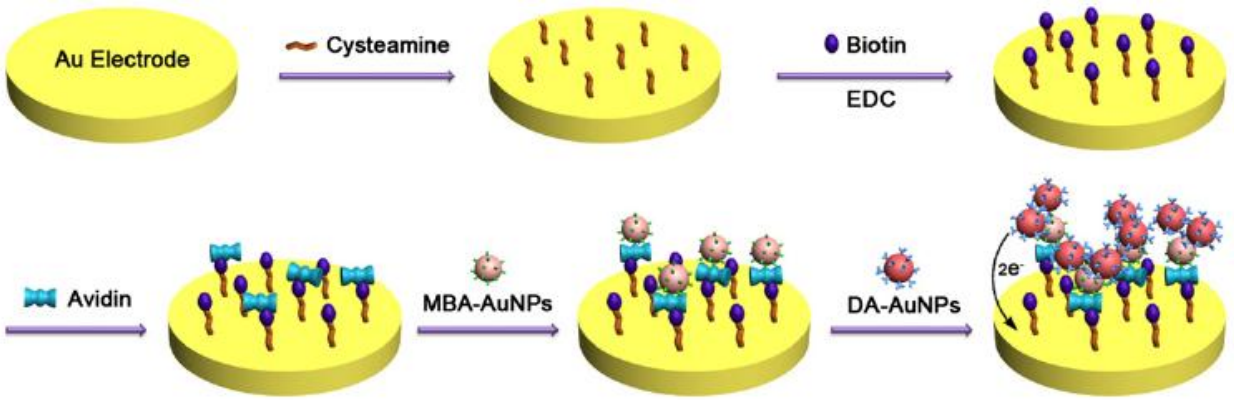

Figure 7. Schematic illustration of the strategy of avidin detection using MBA-AuNPs and DA-AuNPs. (From ref. [45], with permission. Copyright E 2013 Elsevier Science B. V.).

Recently, our group developed a dual-amplified sandwich-type electrochemical biosensor for the detection of glycoproteins at low levels using 4-mercaptophenylboronic acid (MBA)-capped

AuNPs (MBA-AuNPs) and dopamine (DA)-capped AuNPs (DA-AuNPs) [45]. The sandwich-type system was formed by specific recognition of the biosensor surface-confined elements to glycoproteins through the formation of tight covalent bonds between the boronic acids of MBA-AuNPs and diols of glycoproteins (Fig. 7). This step is followed by the successive attachment of MBA-AuNPs and DA-AuNPs via the interaction of boronic acids with DA tags. The method is highly sensitive because a MBA-AuNP can capture more than one DA-AuNP and each DA-AuNP contains a large number of electrochemically active DA molecules. To demonstrate the amenability of our method to other glycoproteins analysis, we tested prostate specific antigen (PSA, the most common serum marker for diagnosing prostate cancer). In this work, PSA was captured by the single strand DNA aptamer (5'-HS-$(CH_2)_6$-ATTAAAGCTCGCCATCAAATAGC-3'). The detection limit was estimated to be 50 fM. Moreover, this biosensor obviates the need of expensive and less stable antibody conjugates for the recognition of captured glycoproteins.

4. CONCLUSION

Boronic acid-modified electrodes have been successfully used for the development of electrochemical sensors for detection of biomolecules. Self-assembled monolayers and polymer films are deposited on the electrode surface for this purpose. The oxidation product of dopamine can react with ascorbate acid present in sample and regenerate dopamine that becomes available again for oxidation, which severely limits the accuracy of detection. A nonoxidative approach based on the interaction of dopamine and poly(anilineboronic acid) can solve this problem. Carbon nanotubes can improve the detection sensitivity because of their outstanding characteristics of high conductivity, chemical inertness, large surface area, low mass density and good biocompatibility. Nafion can filter out negatively charged species like ascorbate anion through electrostatic repulsion; thus, Nafion incorporated boronic acid film was suitable for the selective determination of dopamine in the presence of ascorbic acid.

Glycosylated enzymes and antibodies can be immobilized onto the boronic acid-modified electrodes through the formation of reversible boronate ester covalent bonds. The electrodes can be regenerated with HCl, sugars and borate buffer. However, such immobilization method may not be suitable for the direct assay of real sample since the interaction between boronic acid and diol groups within glycoproteins can be split by sugars in blood. Boronic acid derivatives bearing redox-active moieties, such as ferrocene, can be used for the recognition of glycoproteins, in which the redox potential and/or current are dependent on the concentration of glycosylated glycproteins. Moreover, for the development of electrochemical sensors, boronic acid-modified nanoparticles can be used for both the recognition of captured glycoproteins and the attachment of electroactive diol compounds for signal output through the formation of boronate ester covalent bonds. Boronic acid-based sandwich-type electrochemical sensors would be useful alternatives for the sensitive and selective detection of biomolecules without sample pretreatment.

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