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Multivalent interaction-based carbohydrate biosensors for signal amplification

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ABSTRACT

Multivalent interaction between boronic acids immobilized on quartz crystal microbalance (QCM) sensor surface and the carbohydrates modified Au-nanoparticle (AuNP) has been demonstrated for the development of a sensitive carbohydrate biosensor. Briefly, a boronic acid-containing polymer (boropolymer) as multivalent carbohydrate receptor was oriented immobilized on the cysteamine coated electrode through isourea bond formation. Carbohydrates were conjugated to AuNPs to generate a multivalent carbohydrates moiety to amplify the response signal. Thus, the binding of the carbohydrate conjugated AuNPs to the boropolymer surface are multivalent which could simultaneously increase the binding affinity and specificity. We systematically studied the binding between five carbohydrates conjugated AuNPs and the boropolymer. Our studies show that the associate constant (K_a) was in the order of fucose < glucose < mannose < galactose < maltose. A linear response in the range from 23 μM to 3.83 mM was observed for mannose conjugated AuNPs and the boropolymer recognition elements, with the lower detection limit of 1.5 μM for the carbohydrate analytes. Furthermore, the multivalent binding between carbohydrates and boronic acids are reversible and allow the regeneration of boropolymer surface by using 1 M acetic acid so as to sequentially capture and release the carbohydrate analytes.

1. Introduction

Carbohydrates and glycoconjugates play essential roles in biology, chemistry and material sciences (Ashwell and Morell, 1974). Understanding the role of carbohydrates in various physiological processes and their biological functions requires methods to measure and characterize molecular interactions between carbohydrates and their corresponding receptors. Unlike proteins and DNAs, most of carbohydrates are complex, irregular and have only one kind of functional group (hydroxy) with different stereochemistry, the hydroxylated exteriors of the carbohydrates may blend well with water, making the recognition of different carbohydrates in aqueous solution highly challenging (Klein et al., 2005). Additionally, carbohydrates lack of chromophores or flurophores in their structure and their detection by optical methods is very difficult. In the past decades, various indirect and direct methods including electrochemical (Deo and Wang, 2004), refractive index (Jakeway and de Mello, 2001), mass spectrometry (Harvey, 1999), light scattering (Wei and Ding, 2000), chiroptical (Droz et al., 2001), pre- and post-column derivatization reactions for optical detection (UV–vis, fluorescence) (Ahn and Yoo, 1999; Honda, 1996) post-column enzyme reactor (Marko-Varga et al., 1990), etc. were demonstrated. However, many of mentioned methods are either complex or require the use of expensive instruments. Recent years, carbohydrate biosensors based on enzymatic catalysis (Baldwin, 1999; Fink et al., 2009; Szabo et al., 1996), lectin recognition (Kobayashi et al., 2005; Nagase et al., 2003), microbial interaction (D'Souza, 2001; Held et al., 2002) have been demonstrated and they show great promise. Nevertheless, carbohydrate biosensors using enzyme, lectin or microbial recognition motifs have short lifetime due to the lack of stability of the proteins and cells, thus require replenishment.

To address the low stability of those natural recognition motifs described above, the development of synthetic molecular receptors in recognition of carbohydrates such as boronic acid (Tony et al., 1996; Zhang et al., 2006) have gained significant momentum in recent years. Boronic acid, first reported by Lorand and Edwards in 1959, is known to form covalent bonds between its 1,2- or 1,3-diol group and the *cis*-diol group of the carbohydrates under alkaline conditions (Lorand and Edwards, 1959). Boronic acid has been demonstrated to selectively and reversibly interact with carbohydrates to form esters in aqueous media (James, 2007), which

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supports their use in the development of sensors for selective and continuous carbohydrate monitoring. A variety of boronic acid-based host compounds have been prepared and evaluated as synthetic carbohydrate-binding receptors by immobilizing them on either metallic particles or electrodes for fabricating carbohydrate and glycoprotein sensors. For example, coupling 3-aminophenylboronic acid with dithiodialiphatic acids on either Au-colloids or electrodes (Kitano, 1999), phenylboronates-oxyrane mixed functional monolayer (Abad et al., 2002) and aminophenylboronic acid monolayer on Au-electrode (Zayats et al., 2002) have been reported for electrochemical sensing of carbohydrates or glycoproteins. However, these methods provide only a two-dimensional display of monomeric boronic acids and give rise to the low detection sensitivity.

Furthermore, the interaction of carbohydrates with target proteins is not a simple monomeric binding event, but an oligo- or polymeric binding mechanism in the biological processes. For example, the binding of a trivalent oligosaccharide ligand to its asialoglycoprotein cell surface receptor occurs with a binding constant of $10^8\,M^{-1}$ even though the binding constant of corresponding monovalent interaction is only $10^3\,M^{-1}$(Mathai et al., 1998). We have focused on the development of label free affinity carbohydrate-based biosensors by using label free transducers such as quartz crystal microbalance (QCM) transducers (Shen et al., 2007; Zhang et al., 2003). Interfacial mass changes due to the binding between a ligand (boronic acid derivatives) immobilized on a solid support and the analyte (carbohydrates) can result in changes in the QCM oscillation frequency. However, QCM measures only those materials that are acoustically coupled to the sensor surface. It is binding-based detection rather than proximity-based detection (e.g. surface plasmon resonance, SPR) and requires the ligand–analyte complex to be rigid (e.g. strongly attached on the sensor surface). Additionally, QCM is a mass sensor. The higher the molecular weight of the target analytes and the higher density of recognition elements immobilized, the bigger the mass change and the bigger signal. The usual big size of ligands such as lectins plus the small molecular weights of carbohydrates especially monosaccharide makes it difficult to obtain an observable signal by QCM due to the low densities of recognition elements immobilized and the low mass of the analytes. Therefore, exploring the characteristics of the polyvalent interactions of carbohydrate in nature, we designed the boronic acids polymers that are capable of reversible formation of covalent bonds with the diol functionalities of carbohydrates in the form of cyclic esters. Moreover, the self-assembled carbohydrates on the Au-nanoparticles (AuNPs) were used to amplify the mass of the carbohydrate analytes as well as to generate a multivalent carbohydrate moiety. Our approach allows both the ligands (boronic acids) and analytes (carbohydrates) to be multivalent so that the interaction of the carbohydrates and the ligands are simultaneously amplified multiple times. Specifically, a boronic acid-containing polymer (boropolymer) with multivalent boronic acids appending onto polyacrylamides backbone and with a *O*-cyanate (OCN) chain-end functional group (Fig. 2) was synthesized. The OCN group is known to form isourea bond with amine group and thus facilitates an oriented immobilization onto amine functional group decorated surfaces. On the other hand, the challenge of the small molecular weight of saccharides was addressed by the multiple copies of saccharides immobilized on the AuNPs carriers. AuNPs are ideal carriers in our carbohydrates sensor systems for several reasons: first, AuNPs can be easily prepared in a wide range of sizes; second, the specific activities of carbohydrates can be retained when coupling carbohydrates to AuNPs (de la Fuente and Penades, 2006); third, the carbohydrates–AuNPs conjugates can provide amplification of the detection signal due to the large surface/volume ratio. The binding between the polyvalent boropolymers and carbohydrates–AuNPs conjugates was characterized by the QCM, Ultraviolet-visible (UV) Spectroscopy, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).

2. Experimental

2.1. Materials

Cysteamine, bovine serum albumin (BSA) and sodium citrate dihydrate were purchased from Aldrich–Sigma Co. Hydrogen tetrachloroaurate trihydrate was purchased from Acros Organics. PEG-thiol was obtained from Nektar. The AuNPs (mean size: 15 nm, 30 nm, 50 nm) were purchased from Ted Pella Inc. Au nanorods (width: 7 nm, length: 25 nm) were prepared via seed-mediated growth procedure according to the literature (Busbee et al., 2003) and characterized by TEM. Bi-carbonate buffer (pH 8.3) was prepared using 0.1 M $NaHCO_3$ and 0.5 M NaCl, then adjust the pH to 8.3. Unless otherwise indicated, all chemicals, reagents and solvents were obtained from Sigma and used as supplied without further purification.

2.2. Preparation of carbohydrates–AuNPs conjugates

Five kinds of thiolated carbohydrate derivatives, namely mannose, galactose, glucose, fucose and maltose thiols were synthesized. The preparation, purification and characterization of all the functionalized carbohydrate thiols are described in details in the supplementary information. The structures of these five carbohydrate thiols consist of three components. The first is the carbohydrates linked through their respective reducing end; the second is the poly(ethylene glycol) (PEG; $[OCH_2CH_2]_nOH$, $n=3$) chain; and the third component is the alkyl portion terminated with a thiol group, which can adsorb strongly on the Au surface via Au–S bond. Carbohydrates–AuNPs conjugates were made by coupling each carbohydrate thiol to the AuNPs via self-assemble process. 0.5 mg of those synthesized carbohydrates were dissolved in the 0.1 mL AuNPs (15 nm, 30 nm, 50 nm) and Au nanorods (width: 7 nm, length: 25 nm) solution separately, then stand for 24 h with thorough mixing to allow self-assembly of carbohydrate thiolates on the AuNPs surfaces. The carbohydrates–AuNPs conjugates were evaluated using UV-spectrophotometer (Cary 100, Bio).

2.3. Synthesis of boronic acid-containing polymer

Boropolymer was prepared through arylamine initiated cyanoxyl-mediated free radical polymerization, which has been previously demonstrated as a straightforward approach to synthesize chain end functionalized polymers in our laboratory (Hou et al., 2004; Sun et al., 2002a,b). Briefly, the boropolymer was synthesized by a copolymerization between acrylated phenylboronic acid and acrylamide initiated by cyanoxyl radicals, which was generated by reaction between cyanate anions from a sodium cyanate aqueous solution and aryl-diazonium salts prepared in situ through a diazotization reaction of arylamine in water. Detail synthesis has been described in another paper (Chalagalla and Sun, 2010). Structure of boropolymer is shown in Fig. 2.

2.4. Oriented immobilization of boropolymer

Oriented immobilization of boropolymer on Au-electrode was done by coupling the cyanate functionalized boropolymer to amine terminated SAM modified Au-electrode. Nonpolished AT-cut Au quartz crystals (10 MHz, area 0.23 cm^2) were mounted in a custom-made Kel-F cell sealed with two Viton O-rings. Prior to the

modification process, the Au coated quartz crystal was cleaned by sequential immersion in a concentrated nitric and sulfuric acid mixture, biological grade water and ethanol in series for 3 times to remove impurities, and then dried with nitrogen. After the complete cleaning, amine-active self-assembled monolayer was formed by immersing one side of the Au coated quartz crystal or Au-plate in 10 mg/mL cysteamine solution in ethanol at 4 °C for over night. The Au-electrode surface was then rinsed with ethanol and biograde water to remove the weakly adsorbed cysteamine. Finally 20 μL 4 mg/mL boropolymer was added to the cysteamine modified surface for additional 10–12 h. The coupling between amine and cyanatefunctional groups of boropolymer will allow oriented immobilization of boropolymer on the cysteamine modified electrode as shown in Fig. 2.

2.5. UV-spectroscopy

UV–vis absorption measurements were performed using a Cary 100 Bio UV–vis spectrophotometer at room temperature. Quartz cuvette with an optical light path of 1 mm was used to contain the sample while collecting the spectra in the wave length range from 400 to 800 nm.

2.6. QCM measurement

The boropolymer modified Au quartz electrode was mounted in the Kel-F cell, and the QCM cell filled with 1 mL of bi-carbonate buffer (pH 8.3) was placed in a Faraday cage at room temperature. Agilent 4395A was used to measure the frequency and damping resistance changes of the QCM simultaneously. Contents of the QCM cell were continuously stirred before, during, and after the addition of the analyte, which was added to the cell in certain volumes of carbohydrates–AuNPs conjugates. The relationship between the change in resonant frequency (ΔF) resulting from a change in mass (Δm), was given by the Sauerbrey's equation, $\Delta F = -2\Delta n F_0^2(\mu_q \rho_q)^{-1/2}\ A^{-1}$. Where n is the overtone number, μ_q is the shear modulus of the quartz (2.947×10^{11} g cm^{-1} s^{-2}), and ρ_q is the density of the quartz (2.648 g cm^{-3}). According to the Sauerbrey's equation, our fitted frequency change of 1 Hz corresponds to a mass change of 1 ng for the 10 MHz quartz crystal used in this work.

2.7. Electrochemical characterization

CV and EIS were carried out with a potentiostat–galvanostat (Princeton Applied Research PARSTAT 2263). A three-electrode system with a bare or modified Au-plate electrode, a platinum wire counter electrode and a Ag/AgCl reference electrode (with saturated KCl) was employed. All experiments were performed in Kel-F cell filled with 2 mL of 0.1 M KCl and 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ at room temperature. CV was scanned at 50 mV/s. EIS was obtained with ac amplitude of 10 mV, and frequency from 100 mHz to 100 mHz. The bias potential was set at open circuit potential.

3. Results and discussion

3.1. Investigation of carbohydrates conjugated AuNPs

Since the carbohydrates were chemically modified with thiol groups that can covalently attach to the Au surface, the carbohydrates can therefore coat on the AuNPs surface via self-assemble process. The carbohydrates were conjugated to the AuNPs as outlined in Fig. 1. This process was characterized by UV–vis Spectroscopy based on the unusual dependence of the optical and electronic properties on the AuNPs size and shape (Nath and Chilkoti, 2002; Liu et al., 2009). The UV–vis spectrum of 15 nm AuNPs shows a plasmon absorption peak at 520 nm. Then this peak was shifted to 526 nm after carbohydrate binding (Fig. 1). The 6 nm red-shift in the peak plasmon absorption is related to a change in the local dielectric constant around the AuNPs as a result of the adsorption of carbohydrates onto the AuNPs.

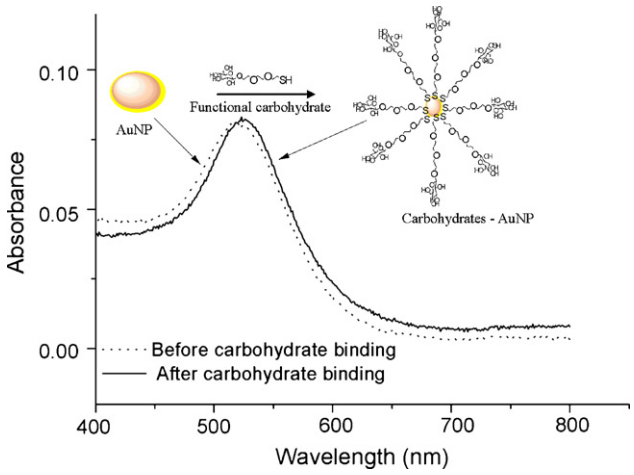

Fig. 1. UV–vis spectra of carbohydrate stabilized AuNPs (15 nm) solution before (dot line) and after addition of mannose (solid line).

The concentration of AuNPs was measured by UV–vis and calculated as 1.9 nM for 15 nm AuNPs, 0.23 nM for 30 nm AuNPs and 0.10 nM for 50 nm AuNPs, according to Beer's law by using known values of the extinction coefficients for AuNPs (4.2×10^8 L M^{-1} cm^{-1} at λmax_{520} for 15 nm AuNPs, 3.7×10^9 L M^{-1} cm^{-1} at λ_{528} for 30 nm AuNPs and 1.5×10^{10} L M^{-1} cm^{-1} at λ_{540} for 50 nm AuNPs) (Demers et al., 2000; Jin et al., 2003). The final concentrations of carbohydrates were 16 mM for fucose derivative, 15 mM for mannose, galactose, glucose and 10 mM for maltose. Thus, we intentionally have carbohydrate concentrations three or four fold higher than that of the AuNP carriers to ensure the each carbohydrate–AuNP conjugate has the maximum surface coverage of carbohydrates on the AuNP surface, so that a quantitative analysis of carbohydrate–boropolymer binding can be achieved.

3.2. Characterization of boropolymer modified electrode

A SAM of cysteamine was formed on the Au surface of the sensor chip via Au–thiol chemistry. The resulting sensor surface with terminal amino groups was then coupled with a cyanate chain-end functionalized boropolymer to form a SAM of boronic acid. The strong and specific cyanate–amine reaction facilitates highly oriented assembly of boronic acids on the sensor surface (Santra et al., 2001). This modification of the Au-electrode is schematically shown in Fig. 2. Cyclic Voltammetry Characterizations of the bare Au, cysteamine modified Au, and boropolymer and cysteamine modified Au-electrodes were obtained in the presence of 5 mM $Fe(CN)_6^{3-/4}$ redox probe as shown in Fig. S1. Compared with bare Au-electrode, the peak currents reduced after the electrode was modified with cysteamine, indicating that the cysteamine was attached on the Au surface and formed an electron-transfer barrier to the $Fe(CN)_6^{3-/4-}$ redox active couple. Formation of the boropolymer monolayer on Au-electrode led to further decrease of peak currents due to electrostatic repulsion between $Fe(CN)_6^{3-/4-}$ and boropolymer, as boronic acid is in an anionic form in alkaline solution (pH 8.3). This result confirmed the presence of boropolymer on the electrode surface. The same results were also obtained by EIS (Fig. S1, inset).

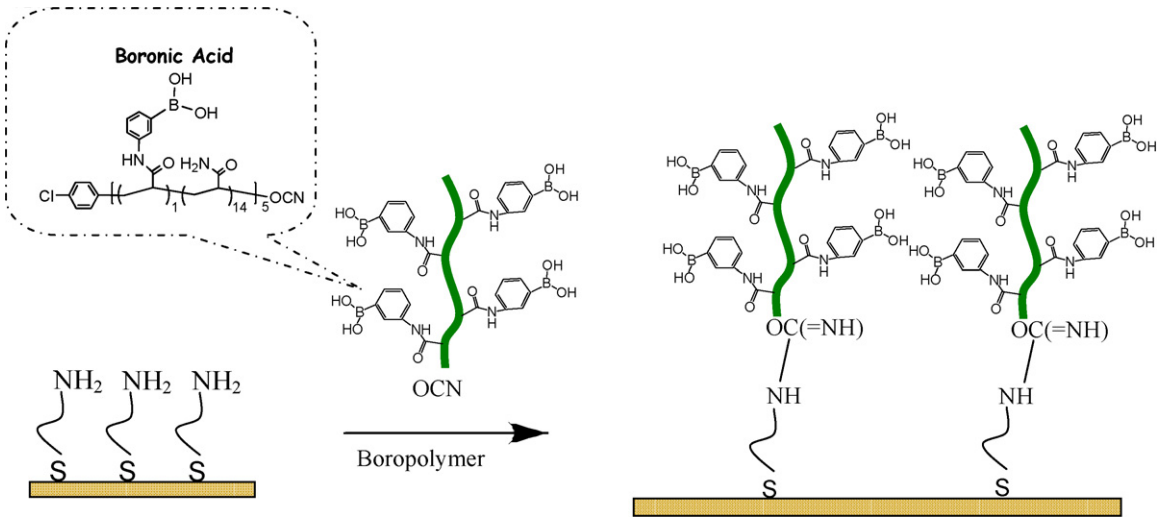

Fig. 2. Schematic representation of oriented immobilizing boropolymer onto cysteamine-functionalized Au-electrode surface.

3.3. Evaluation of carbohydrate and boropolymer interactions

Monosaccharide is small in size, therefore it is very difficult to obtain high sensitivity of its measurement using mass sensors such as QCM. As shown in Fig. 3, the frequency decrease was only about 7 Hz when 0.1 mg/mL free mannose thiols was added to the boropolymer modified Au sensor surface (curve a, upper). However, when treatment of the resulting solution with amplification probe-AuNPs (15 nm), it yielded a frequency decrease of about 32 Hz (curve a, lower), indicating that AuNPs would conjugate with functional mannose and the binding of mannose–AuNP conjugations offered a significant amplification of carbohydrates–boronic acid binding. But addition of AuNPs alone on the boropolymer modified Au sensor surface almost did not give rise to the frequency change (curve c). To evaluate the degree of non-specific interaction between the modified surface and carbohydrate, we performed parallel experiment on the Au surface that was only modified with cysteamine. The frequency response of addition of the 0.1 mg/mL of mannose thiols was found to be 2 Hz (curve b). It confirmed that carbohydrate binding was mainly attributed to the carbohydrates interaction with the boronic acid groups of the boropolymer. These results also revealed that the carbohydrates–AuNPs conjugation amplified the signal of carbohydrate-boropolymer interaction and allowed carbohydrate sensing to be highly specific and sensitive.

To investigate the affinity of the carbohydrates–AuNPs conjugates with boropolymer immobilized on the Au sensor surface, five kinds of carbohydrates, mannose, fucose, galactose, glucose, and maltose derivatives conjugated with AuNPs were prepared in the identical ways. Fig. 4 shows these carbohydrates–AuNPs conjugates binding with boropolymers. As expected, all the boropolymer modified Au quartz crystal electrodes showed significant frequency changes upon the addition of the functionalized carbohydrates–AuNPs conjugates, and the frequency decrease is concentration-dependent. Since monosaccharides are similar in size and molecular weight and our experimental conditions ensured the maximum surface coverage of carbohydrates on the AuNP surface, we can reasonably assume that the number of monosaccharide immobilized on the AuNP are similar. Thus we will be able to obtain the associate constants (K_a) of the binding between carbohydrates–AuNPs and boropolymer using Eq. (1). Association

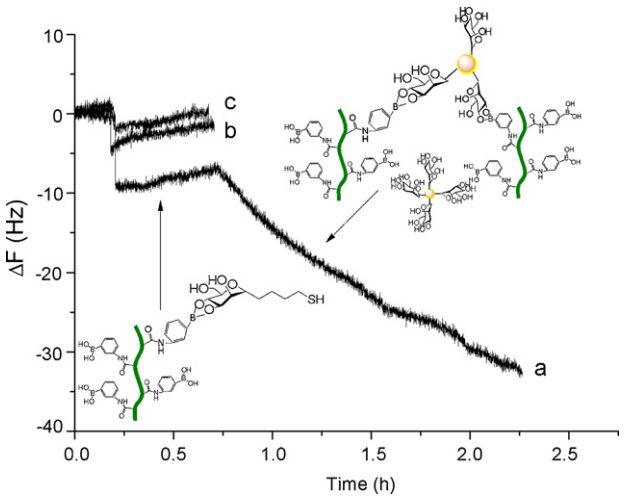

Fig. 3. Frequency change vs. time curves of boropolymer–cysteamine modified Au quartz exposed to 0.1 mg/mL mannose thiols and 20 μL AuNPs in series (a), cysteamine modified Au quartz exposed to 0.1 mg/mL mannose thiols (b) and boropolymer–cysteamine modified Au quartz exposed to 20 μL AuNPs (c). AuNPs size, 15 nm.

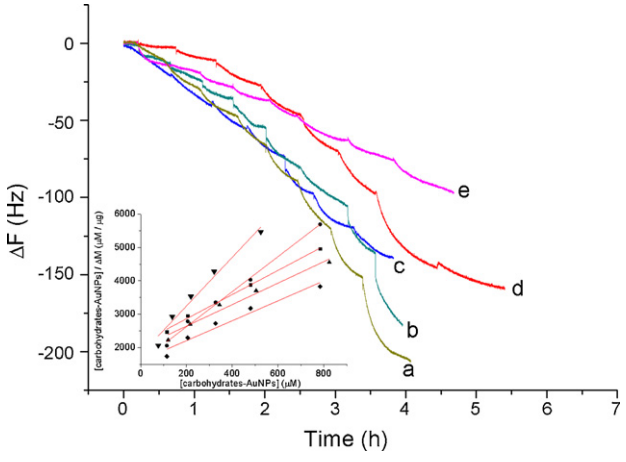

Fig. 4. Frequency change vs. time curves of boropolymer modified Au quartz electrodes exposed to mannose–AuNP (a), fucose–AuNP (b), galactose–AuNP (c), glucose–AuNP (d), and maltose–AuNP (e). 2.5, 7.5, 17.5, 37.5, 67.5, 107.5, 157.5 and 257.5 μg/mL carbohydrates–AuNPs were added in series. AuNPs size, 15 nm. Inset, $[carbohydrates–AuNPs]_0/\Delta M$ vs. $[carbohydrates–AuNPs]_0$ of carbohydrates–AuNPs binding with boropolymer. maltose–AuNP (▼), galactose–AuNP (●), glucose–AuNP (■), fucose–AuNP (▲), mannose–AuNP (♦).

constant and dissociation constant (K_d) for the binding between carbohydrates–AuNPs and boropolymer can be evaluated by Eq. (2) (Shen et al., 2005), where ΔM_{max} is the maximum binding amount, ΔM is the measured binding amount, and [carbohydrates–AuNPs]$_0$ is the original concentration of carbohydrates–AuNPs.

$$\text{boropolymer} + \text{carbohydrates–AuNPs} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{boropolymer–carbohycrates–AuNPs complex} \quad (1)$$

$$\frac{[\text{carbohydrates–AuNPs}_0]}{\Delta M} = \frac{[\text{carbohydrates–AuNPs}_0]}{\Delta M_{max}} + \frac{1}{\Delta M_{max} K_a} \quad (2)$$

The inset of Fig. 4 shows the plot of [carbohydrates-AuNPs]$_0/\Delta M$ versus [carbohydrates–AuNPs]$_0$ based on the Fig. 4 data. According to Eq. (2), the ratio of the slope to the intercept gives the association constant (K_a) as $1.69 \times 10^3\ M^{-1}$ for glucose, $3.33 \times 10^3\ M^{-1}$ for galactose, $1.61 \times 10^3\ M^{-1}$ for fucose, $4.06 \times 10^3\ M^{-1}$ for maltose and $1.85 \times 10^3\ M^{-1}$ for mannose. The electrode modified with the boropolymer showed a selective and enhanced response to carbohydrates. The K_a indicates that the interaction between the boropolymer and the carbohydrates were in the order of fucose < glucose < mannose < galactose. Since maltose is disaccharide, it is not appropriate to compare the affinity of this disaccharide with those of monosaccharides. However, we have confirmed that the small size of the carbohydrate did not play significant roles to generate the frequency change (Fig. 3), the association constant for maltose, which showed the highest value among the carbohydrates, was over 2 times higher than that observed for fucose. It has been reported that the order of the higher association constants of the boronic acid for the carbohydrate molecule is glucose ($110\ M^{-1}$) < mannose ($172\ M^{-1}$) < galactose ($276\ M^{-1}$) (James, 2007; Lorand and Edwards, 1959). The association constants for the carbohydrates in present study also exhibited a similar trend. The higher associate constant in our study confirmed the multivalent carbohydrate receptor and multiple carbohydrate moieties on AuNPs provide increased signal for the enhancement of carbohydrate detection. These results demonstrated that polyvalent interactions could be collectively much stronger than corresponding monovalent interactions. The different association constants can be explained from the structural point view of carbohydrates. It indicated that our synthesized boropolymer might be useful as selective carbohydrate-binding agents for carbohydrate purifications.

Different size and shape of AuNPs have different molar mass as well as different surface areas so they have different abilities to conjugate carbohydrates. In order to understand the size and shape effect of AuNPs on the carbohydrate binding ability to the boropolymers, three different diameters of AuNPs (15 nm, 30 nm, 50 nm) and a different shape of Au nanorod were used to conjugate with carbohydrates respectively. Fig. S2 shows the frequency responses of the boropolymer modified Au quartz electrodes when different sizes and shape of AuNPs conjugated with mannose were added. It can be seen that that the amplification ability of AuNPs was heavily dependent upon their size and shape. 30 nm AuNPs has a highest ability to amplify the signal than that of others. This may be explained that the smaller size of 15 nm AuNPs has smaller surface area and has less number of carbohydrates immobilized. On the other hand, the bigger size of 50 nm AuNPs even though has more surface area and higher molar mass but will have smaller diffusion coefficient as well as the steric effect due to its big size which could prevent the mannose approach to boropolymer. Meanwhile, 30 nm spheric AuNPs could more easily access the boropolymer layer compared with rod shape of Au nanorods. Thus 30 nm AuNPs was chosen in the further studies of boropolymer carbohydrate biosensors.

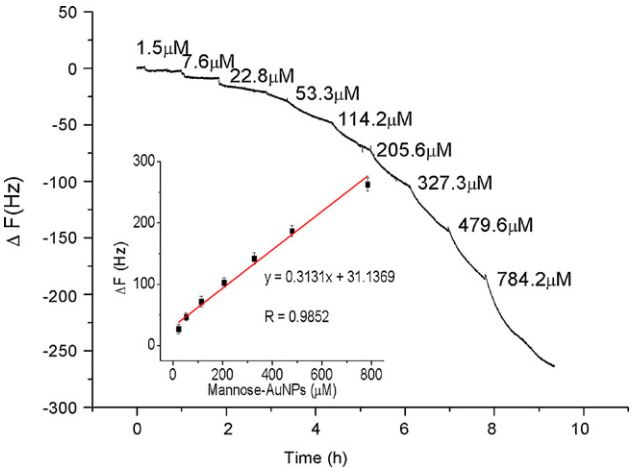

Fig. 5. Frequency change vs. time curve for mannose-AuNP (30 nm) binding with boropolymer modified quartz surface. Inset is the linear relation ship of the frequency vs. the concentration of mannose ($n = 3$).

Under optimized experimental conditions of AuNPs (spheric and 30 nm in size), quantification of mannose-conjugated AuNPs using the boropolymer sensor was carried out (Fig. 5). A good linear relationship in the mannose concentration ranging from 23 μM to 3.83 mM was obtained with a relative linear coefficient of 0.98. The experimental determined detection limit of this sensor was 1.5 μM, which is much lower than that of using phenylboronic acid monolayer as recognition element but without oriented immobilization and amplification as we show in this report (Takahashi et al., 2004). The multivalent carbohydrate receptor of boropolymer, the oriented immobilization of boropolymer via isourea bond formation, the stable and biologically active carbohydrate–AuNPs conjugates thus represent an innovative approach for the high sensitivity and low detection limit of carbohydrate detection for glycobiology research.

Carbohydrate–boronic acid complex formation is a pH-dependent reversible process. In the present study, we investigated whether the reversible interaction can be used to regenerate the boropolymer recognition element on the sensor surface. As shown in Fig. S3, the electron-transfer resistance R_{et} of the $Fe(CN)_6^{3-/4-}$ redox couple upon interaction with mannose–AuNP (Fig. S3,a) was found to decrease after regenerating the surface with 1 M acetic acid for 30 min (Fig. S3,b), implying that mannose–AuNP underwent a desorption process with boronic acid group. After reinjection of mannose–AuNP to the cell, the electron-transfer resistance R_{et} almost recovered to its initial value (Fig. S3,c), indicating little loss of binding affinity of boropolymer towards mannose on the sensor surface. Moreover, the process of regeneration using acetic acid can be repeated (Fig. S3,d). The reproducibility of carbohydrates binding to the boropolymer monolayer monitored during three subsequently repeated experiments with the same concentration of mannose–AuNP (10 μg/mL) was better than 84% via QCM detection methods (Fig. 6). The proposed carbohydrate sensor could be used for at least 10 regeneration cycles without any significant decrease of sensitivity. The stability of this sensor was also evaluated. Stored at 4 °C, this sensor could retain 81% of its initial sensitivity after 10 days. All of these results show that the boropolymer modified surface can be easily regenerated by dissociating the bond carbohydrates, previously captured by the immobilized boropolymer, using 1 M acetic acid. Furthermore, the exposure to 1 M acetic acid did not significantly affect the immobilized boropolymer, and did not reduce the ability of carbohydrate

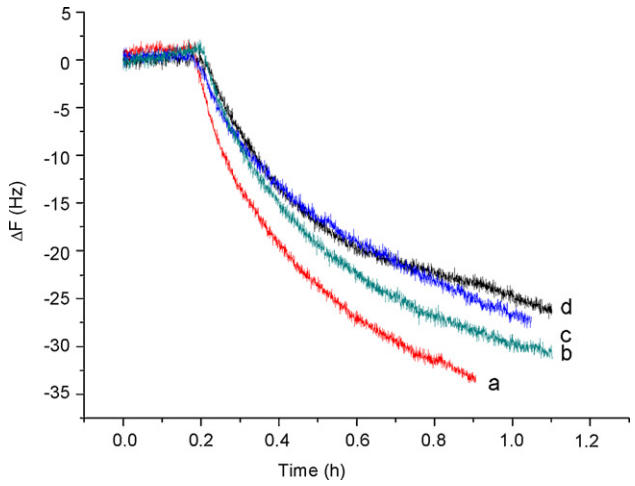

Fig. 6. Frequency change vs. time curves of boropolymer modified electrodes exposed to 10 μg/mL mannose–AuNP. (a)–(d), regeneration boropolymer surface from 0 to 3 times.

binding to boropolymer. This was attributed to the characteristic of boronic acid and the stabilized boropolymer structure. The reversibility and stability of the boropolymer based carbohydrate biosensor described in this work can greatly reduce the operating costs by allowing the electrode materials to be reused and the modified interface to be regenerated quickly and inexpensively.

3.4. Rigidity of the modified surface

Rigidity of a biofilm is important for quantitative analysis by QCM technique using Sauerbrey equation. By obtaining the damping resistance through fitting the Butterworth–Van Dyke equivalent circuit, we can verify the validity of Sauerbrey equation (i.e., $\Delta F = -C\Delta m$) and obtain information related to the dissipative properties if the modified layer shows viscoelastic behavior. As shown in Table S1, the damping resistance in all cases was $|\Delta R_q|/R_q$ (%) < 2. This proved that the attached biofilms behaved as a rigidly attached mass and the Sauerbrey equation is valid in our systems.

4. Conclusions

Multivalent interaction between a boronic acid-containing polymer and AuNP–carbohydrate conjugates was explored for a sensitive, selective and reusable biosensor for studying carbohydrate–receptor interaction and carbohydrate detection. The multivalent boronic acids on boropolymer increased its binding sites with the multivalent carbohydrates on the AuNPs and thus allow significant increase of the response signal. We studied the binding between five carbohydrate conjugated AuNPs with the boropolymer. In our results, the interaction between the boropolymer on the sensor chip and the monosaccharides was in the order of fucose < glucose < mannose < galactose. Disaccharide maltose showed the highest sensitivity among the carbohydrates. The boropolymer based carbohydrate sensor gave a linear response to mannose in the range from 23 μM to 3.83 mM and a lower detection limit of 1.5 μM. The reversible binding between boropolymer and carbohydrates allow the regeneration of the ligands by using 1 M acetic acid as the regeneration reagent. The greater progress achieved through this study will aid the effort to develop glycosensor and will enhance the potential for future discoveries in the fields of carbohydrate research and carbohydrate analysis. The study of multivalent interaction may provide new targets and new strategies for the design of pharmaceutical agents.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.08.025.

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