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Complex thiolated mannose/quinone film modified on EQCM/Au electrode for recognizing specific carbohydrate–proteins

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ABSTRACT

A complex thiolated mannose (TM)/quinone functionalised polythiophene (QFPT) thin film was modified on EQCM/Au electrode for recognition of specific carbohydrate–proteins. Different lectins such as those from *Sambucus nigra* (elder berry), *Arachis hypogaea* (peanut), *Ulex europaeus* (gorse, furze), *Triticum vulgaris* and Concanavalin A (ConA) was used for probes to evaluate bio-sensing performance of the TM/QFPT film. A specific response was observed for ConA from lectins when using the TM/QFPT film as sensing material and employing either elelctrochemical or the QCM method. No response was detected between thiolated mannose and other lectins. The linear relationship between current and ConA concentration is in the range of 0.5–17.5 nM by the elelctrochemical method and the linear relationship between frequency change and ConA concentration is in the range of 0.5–4.5 nM by the QCM method. This shows that the TM/QFPT-modified EQCM biosensor presents a paralleled determination by using electrochemical and the QCM method. The elelctrochemical method of the biosensor can be applicable in a large concentration range and its frequency change can be more precise.

1. Introduction

Carbohydrate–protein recognition plays an essential role in all living organisms, which governs a wide spectrum of pivotal biological and pathological events including cell–cell recognition, signal transduction, autoimmune estimation, tumormetastasis, *among others* by generating multivalent interactions with corresponding carbohydrate receptors on cells (Bertozzi and Kiessling, 2001; Seeberger, 2005; Raman et al., 2005). The sugar chains disturbed on the cellular membrane and wall surface can act as cellular malignancy, development, or differentiation as well as the real-time and accurate probing through the specific carbohydrate–protein interactions. Unfortunately, the method for detecting the interaction between natural carbohydrates and their receptors is much more difficult due to the amount and structural diversity of sugar. Glucose and galactose, for example, represent a pair of the simplest monosaccharide epimeric which are much different in their biological characteristics although the difference is only at the site of C4.With regards to recognizing carbohydrate–proteins, various indirect and direct methods have been used to examine the interactions between free and immobilized lectins to carbohydrates (Pfeufer et al., 1997; Hatakeyama et al., 1996).

Fluorescent biosensors for sugar and lectin affinities in chromatography have been developed (Hamachi et al., 2000; Yoshida et al., 1997). Ertl and Mikkelsen (2001) designed a lectin-based sensor arrays for qualitative discrimination of bacterial strains. They employed lectin-modified membranes to react with bacterial cell suspensions for 100 min and then fixed them at the surface of a Pt electrode to obtain electrochemical signals from respiratory cycle activity to identify bacterial strains. Lectins are extensively of concern since they are structurally diverse proteins from many sources (plants, viruses, microorganisms and animals) that selectively and reversible related with the mono- and oligosaccharide components of polysaccharide structures (Ertl and Mikkelsen, 2001). Min et al. (2010) reported a highly sensitive electrochemical lectin biosensor using carbohydrate-stabilized gold nanoparticles and silver-enhancement technique. A target lectin protein, Concanavalin A (Con A), was specifically bound to the self-assembled monolayer of thiolated mannose on a gold electrode. The mechanism of mannose group recognizes a specific lectin–ConA was shown by Z. Derewenda (Derewenda et al., 1989). Mannose-stabilized gold nanoparticles were added to form a sandwich-type complex with the Con A and were followed by silver-enhancement process to coat the mannose-stabilized gold nanoparticles with silver metal.

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In this study, a novel complex TM/QFPT film was modified on a gold electrode of quartz crystal monitor (QCM) to investigate whether the mannose moiety immobilized on the film can combine with lectins and whether the combination can be quantified. Herein we selected and synthesized the thiolated mannose based on the ideal features that glucose–quinone or galactose–quinone hybrids with a short and solid C-glycosidic linkage was resistant to acidic as well as enzymatic cleavage as reported by Praly et al. (2005) and Li et al. (2005). The thiolated mannose has two active groups (see Fig. S1(a and b)). One thiol group combines with quinone deposited on the electrode and the other mannose group recognizes a specific lectin–ConA (see Fig. S1(c)). A detailed estimation of carbohydrate–protein recognition and quantification was carried out through an EQCM method.

2. Experimental

2.1. Reagents

Concanavalin A (ConA), lectin from *Sambucus nigra* (elder berry), lectin from *Arachis hypogaea* (peanut), lectin from *Ulex europaeus* (gorse, furze), and lectin from *Triticum vulgaris*, and (4-(2-hydroxythyl)-1-piperazineethaneslfonic acid) (Hepes) were purchased from Sigma-Aldrich. Thiolated mannose and 3-((2, 5-dimethoxyphenyl)ethynyl) thiophene were obtained from Dr. Xiangqun Zeng (Oakland University, USA). The hydroquinone – functionalised thiophene (HQFT) was obtained by the electrochemical deprotection of the methoxy groups of 3-((2,5-dimethoxyphenyl) ethynyl) thiophene by holding the potential at 1.6 V/SCE for about 5 min until the current was constant (Stéphanie et al., 2008). All other reagents and material were analytical grade and solvents were purified by conventional procedure.

2.2. Assembly preparation of HQFT modified film on QCM

The 10 MHz crystals were mounted on the cell and removed possible surface contamination of Au electrode of QCM as described in Section 2.3. 1 ml 10.6 mM HQFT solution was added in the cell and kept overnight in 4 °C refrigerator to assemble HQFT film on QCM and washed with pH 7.4 Hepes buffers.

2.3. Apparatus and the electrochemical preparation of QFPT and TM films

The electrochemical electrosynthesis and analysis were performed in an EQCM system as shown in Fig. S2. The main compartment which contained the working electrodes, the polished 10 MHz quartz crystals with gold plate electrode area were purchased from the International Crystal Manufacturing Company, Inc (Oaklahom city, USA). To remove possible surface contamination, the Au electrode was carefully cleaned by pipetting one drop of fresh Piranha solution (H_2SO_4: H_2O_2, v/v 3:1) onto the surface and kept for 5 min, after which it was rinsed with copious Milli-Q ultrapure water. The treatment was repeated three times. The treated electrode was then scanned between 0 and 1.5 V versus SCE in 0.2 mol/L $HClO_4$ at 50 mV/s for a 10 cycles to obtain reproducible cyclic voltammograms (Yang et al., 2004).

The crystals were mounted on the cell by means of sealing nitrile o-rings with only one face in contact with solution. The active area of the gold electrodes was 0.21 cm^2. The working electrodes were connected to a CHI 660C electrochemical workstation (CH Instrument, Inc. U.S.A.) with a three-electrode system comprising a platinum wire as an auxiliary electrode, and Ag–AgCl as reference electrode. The preparation of the complex TM/QFPT film by the electrosynthesis processes of quinone-functionalised polythiophene (QFPT) and thiolated mannose films are depicted in Scheme S1. The QFPT film was prepared by oxidation of assembly prepared HQFT film on a QCM Au electrode through cycling the potential between 0 and 1.4 V at 20 mV/s in 2.2 mM of hydroquinone-functionalised thiophene (HQFT)+5 mM Hepes aqueous solution. After rinsed with 5 mM Hepes aqueous solution, a thiolated mannose–quinone composite film was then prepared by cycling the potential between −0.2 V and 0.8 V in 10.6 mM thiolated mannose+5 mM Hepes aqueous solution. Electrochemical impedance measurements were performed in the presence of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture as a redox probe in the frequency range between 0.1 Hz and 100 000 Hz at the formal potential of the system, E_o=0.17 V. The amplitude of the alternate voltage was 10 mV. A frequency change of a 10 MHz QCM was measured by a RQCM station through a 1 pF filter capacitor. All experiments were conducted at room temperature. All processes were monitored in situ by the EQCM system.

3. Results and discussion

3.1. The surface coverage of QFPT and TM film

A complex TM/QFPT film was modified on EQCM/Au electrode. The electrochemical deposition of QFPT conductive polymer film was performed in 2.18 mM HQFT and 5 mM Hepes buffer pH7.4 solution by successive scans in the positive potential 0–1.4 V as shown in Fig. 1.

The surface coverage of the QFPT film on EQCM can easily be counted from Fig. 2 through the formula $\Gamma_0=\Delta f\times 1.1/MA$ from Sauerbrey equation (Sauerbrey, 1959), where A is the area of QCM electrode and M is the molecular weight of HQFT. After 16 scans,

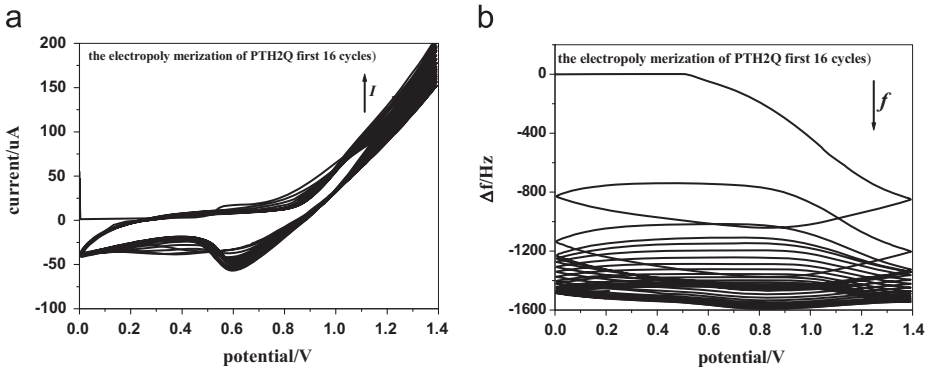

Fig. 1. Electrochemical polymerization process of cyclic voltammogram (A) and the simultaneously obtained EQCM frequency curve (B) for a gold electrode (f_0=10 MHz) in contact with 2.18 mM HQFT and 5 mM Hepes buffer pH 7.4 solution. Scan rate: 20 mV s^{-1}.

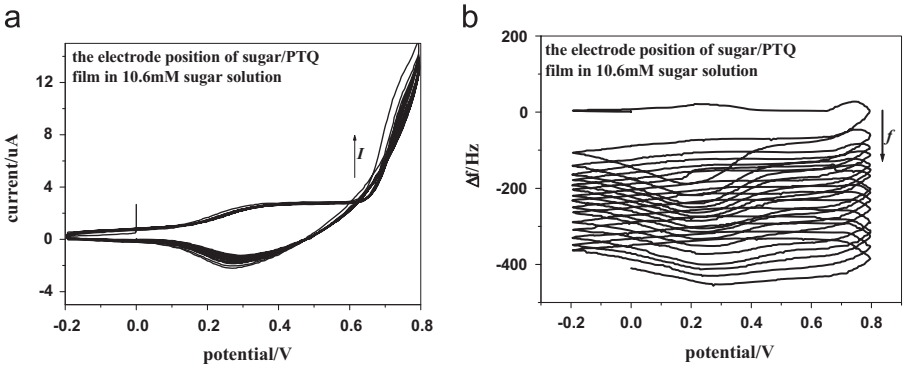

Fig. 2. Electrochemical polymerization process of cyclic voltammogram (A) and the simultaneously obtained EQCM frequency curve (B) for a QFPT modified gold electrode (f_0=10 MHz) in 10.6 mM thiolated mannose and 5 mM Hepes buffer pH 7.4 solution. Scan rate: 20 mV s^{-1}.

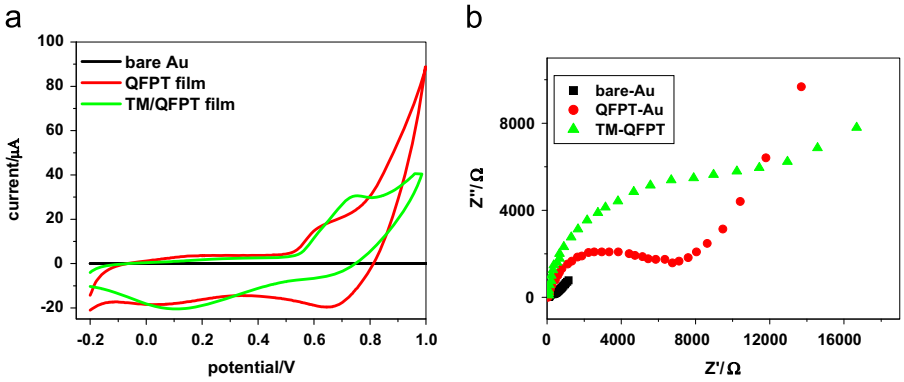

Fig.3. (a) Cyclic voltammograms of bare Au electrode (black); QFPT film (red); TM/QFPT composited film (green) in 5.0 mM Hepes pH 7.4, scan rate 100 mV/s. (b) Electrochemical impedance spectra of bare Au electrode (black); QFPT film (red); TM/QFPT composited film (green) in 0.1 M KCl containing 5 mM $Fe(CN)_6$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the corresponding frequency change measured in situ by a 10 MHz QCM with an area of 0.21 cm^2 was 1200 Hz. The surface coverage $\Gamma_0 = 3.6 \times 10^{-8}$ mol/cm^2 can be obtained for QFPT film.

The preparation of thiolated mannose film on QFPT film was performed in 10.6 mM thiolated mannose, 1 mM Ca^{2+}, 1 mM Mn^{2+} and 5 mM Hepes buffer 7.4 solutions by successive scans in the potential −0.2–0.8 V as displayed in Fig. 2.

The surface coverage of the TM film also can be easily counted from Fig. 2. The surface coverage $\Gamma_1 = 6.5 \times 10^{-9}$ mol/cm^2 can be obtained. The surface coverage of QFPT is about 6 times of that for thiolated mannose. This means that 6 mol QFPT will bind with one mole TM, as shown in Fig. S3. When potential window changes in the range from −0.4 to 0.8 V, the obtained surface coverage will be 8.0×10^{-9} mol/cm^2. This made it easier for the control, adjustment, and evaluation of the performance of the prepared films and biosensors.

3.2. The electrochemical properties of QFPT and TM film

In order to characterize the electrochemical properties of QFPT and TM/QFPT modified electrodes, we recorded their CVs in 5 mM pH 7.4 Hepes buffer (see Fig. 3). The current obviously decreases in comparing MT /QFPT electrode with QFPT electrode, showing that conductivity of TM/QFPT electrode reduces. The voltammogram of the QFPT-modified electrode clearly demonstrates a reversible peak near at 0.6 V/Ag–AgCl ($\Delta E_p = (0.68 - 0.62)/2 = 0.03$ V) corresponding to the BQ/BHQ couple immobilized at Au/QCM electrode. The formal potentials, $E_{f1} = 0.65$ V (for QFPT film), $E_{f2} = 0.74$ V (for TM/QFPT film), were calculated by taking the average of the anodic and cathodic peak potentials. The formal potentials were calculated to ascertain the large separation between the cathodic and anodic peaks for the TM/QFPT-modified electrode which suggests a slow transfer process due to modification of the electron density by sulfur addition (Finley, 1974).

The sulfur addition and substitution reaction to quinones has been thoroughly investigated (Seymour et al., 2002). According to Seymour et al., 1,4-Michael-type of thiol adds to quinines for the convent bound of 3-(2,4-dihydroxyphenyl)-2-(3-thienyl) and thiolated mannose. The scheme is presented in Fig. 4.

Electrochemical impedance (EIS) of electrodes at different modification steps was also studied. Fig. 3(b) shows the impedance features of them. Significant differences in the impedance spectra were observed during stepwise modification. The bare Au electrode exhibited an almost straight line (Fig. 3(b) black), which is characteristic of a diffusion limited electrochemical process. The appearance of the semicircular part of the spectrum (Fig. 4) of QFPT films on the electrode surface functioned as a barrier to the interfacial electron transfer. The diameter of the respective semicircular element corresponds to the electron-transfer resistance (R_{et}) at the electrode surface, which increased dramatically when QFPT and TM/QFPT films (Fig. 3b green) were deposited on the Au electrode indicating formation of a ferrocyanide transport-blocking layer on the electrode. These data were consistent with the results obtained from CV experiments and demonstrate that QFPT and TM/QFPT films are successfully immobilized on the Au electrode.

3.3. Current response of carbohydrate–lectin interactions at a TM/QFPT and TM modified EQCM biosensor

The voltammograms of the TM/QFPT-modified electrode with different concentrations of ConA (unit of C_{ConA}) were first detected,

shown in Fig. 5. Decrease of the current resulted from the binding of the specific molecular recognition domains of ConA to mannose (see Fig. 5(a)). The currents were plotted to ConA concentrations ranging from 0.5 nM to 30 nM. The linear relationship between peak current and ConA concentration, I_p (μA)=19.42–0.1039C_{ConA} (nM) with a fitting coefficient of R^2=0.99, represents the interaction of specific monosaccharide mannose to ConA ranging from 0.5 nM to 17.5 nM. The repeatability of 5 times measurement at each point are shown in error bar Fig. 5(b). In contrast, while the selected nonspecific lectins such as those from *Sambucus nigra* (elder berry), *Arachis hypogaea* (peanut), *Ulex europaeus* (gorse, furze), and *Triticum vulgaris* were assayed in the same concentration, very minor peak current changes were observed (see Fig. S4). For the TM-modified Au/QCM, the current response almost kept constant when lectins concentration changed from 0.5–30 nM.

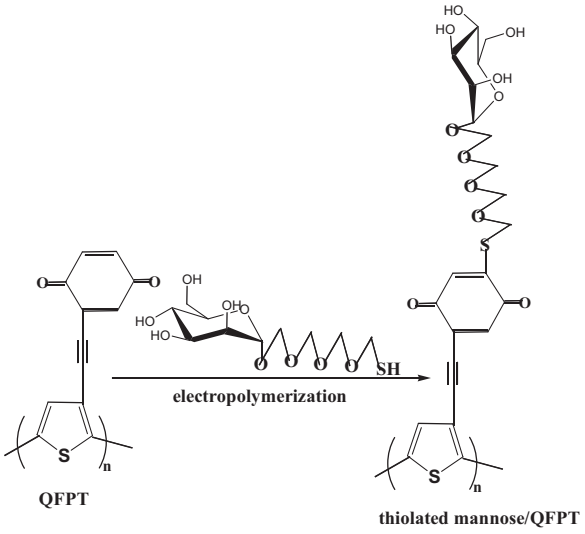

Fig.4. Schemes of covalent attachment between thiolated mannose and quinine via 1,4-Michael-type of thiol addition.

3.4. Frequency response of carbohydrate–lectin interaction at a TM/QFPT and TM modified QCM biosensor

The designed TM/QFPT QCM biosensor consists of quartz crystal and TM/QFPT-modified Au film. The method for preparation of biosensitive membrane by electrochemistry can be controlled, adjusted with short time. The experimental result of the TM/QFPT modified electrode in the absence or in the presence of different concentration of ConA solutions was shown in Fig. 6(a). After frequency stabilization in 1 ml pH 7.4 Hepes buffer containing 1 mM Ca^{2+} and 1 mM Mn^{2+} solution, The frequency decrease as 1 μl of 7.55 μM ConA solution was added to the cell which resulted from the binding of the specific molecular recognition domains of ConA to mannose. After the frequency remained stable for about 100 min, the difference in frequency was recorded as 75 Hz (as shown in Fig. 6(a)). Then another 1 μl of 7.55 mM ConA solution was again added to the cell, and again the frequency difference was recorded using the same method as before.

The decreased frequencies were plotted against ConA concentrations ranging from 0.5 nM to 4.5 nM. The linear relationship between frequency change and ConA concentrations, Δf(Hz)= 5.324–55.78 C_{ConA} (nM) (R^2=0.99), represents the interaction of specific monosaccharide mannose to ConA. The repeatability of 5 times measurement at each point are shown in error bar Fig. 6(b)

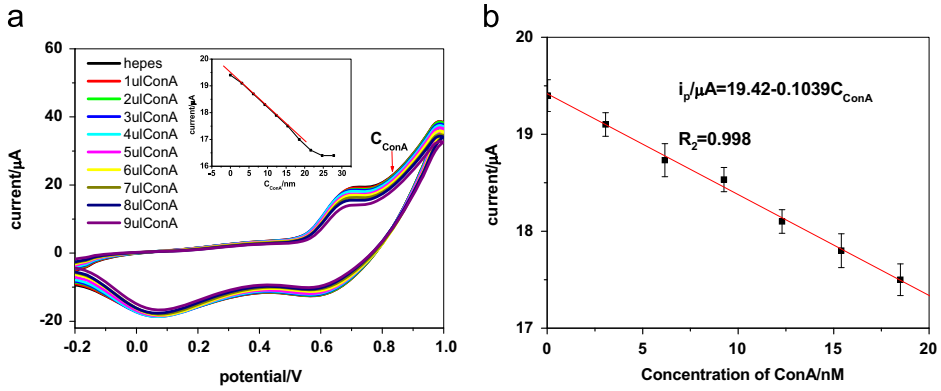

Fig. 5. (a) Voltammograms of TM/QFPT modified electrode in 5 mM Hepes, 1 mM Ca^{2+}, 1 mM Mn^{2+} with different concentration ConA solution. (b) The Error Bars of current with different concentration ConA (n=5).

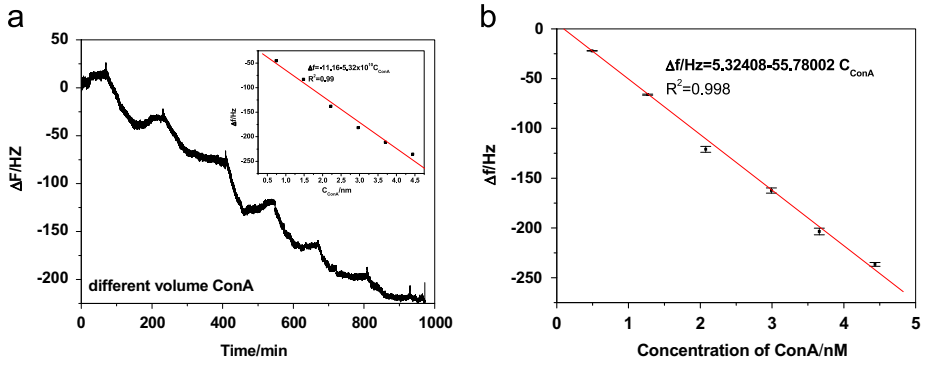

Fig. 6. (a) Frequency changes of TM/QFPT modified QCM biosensor when different concentration ConA are added to the cell containing 1 ml pH 7.4 Hepes buffer, 1 mM Ca^{2+}, and 1 mM Mn^{2+}. (b) The Error Bars of frequency with different concentration ConA (n=5).

with a coefficient of variation no more than 3%. In contrast, while the selected nonspecific lectins such as from *Sambucus nigra* (elder berry), *Arachis hypogaea* (peanut), *Ulex europaeus* (gorse, furze), and *Triticum vulgaris* were assayed under the same condition, very minor frequency changes occurred as shown in Fig. S5. For the TM-modified Au/QCM, the frequency response almost kept constant when ConA concentration changed from 0.5 to 4.5 nM. The mechanism of QFPT film in this experiment will be studied further. The stability of the TM/QFPT modified biosensor was measured in a period of 60 days, and the change percentage of current and frequency is less than 3%.

4. Conclusions

In summary, we have successfully fabricated a TM/QFPT-film-modified biosensor by electrochemical depositing TM/QFPT film on the surface of Au/QCM. The TM/QFPT-modified biosensor has more excellent sensitivity and selectivity to ConA than TM-modified biosensor. Most importantly, this TM/QFPT-modified EQCM biosensor presents a paralleled determination by using electrochemical and the QCM method. The linear range of ConA from 0.5 nM to 17.5 nM by the electrochemical method is superior to the report by Caixin et al. (2008). And the linear range of ConA from 0.5 nM to 4.5 nM by the QCM method is superior to the report by Mingchuan et al. (2007) (the linearity range from 0.1 μM to 0.5 μM, (Mingchuan et al., 2007)). The paralleled determination and more excellent sensitivity show a good applied prospect for the TM/QFPT film modified EQCM biosensor.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.11.018.

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