Microgravimetric lectin biosensor based on signal amplification using carbohydrate-stabilized gold nanoparticles†

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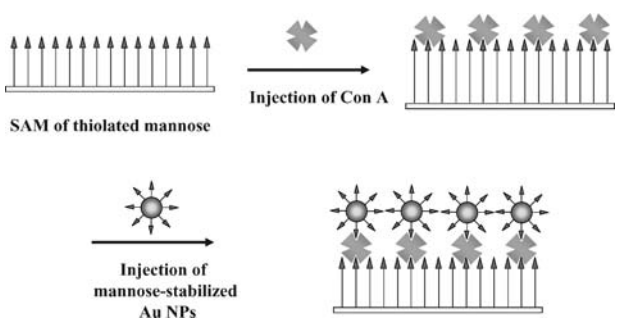
A highly sensitive microgravimetric lectin biosensor has been developed using carbohydrate-stabilized Au nanoparticles as a signal amplifier; mannose-stabilized Au nanoparticles formed a sandwich-type complex with the target Con A specifically bound to a mannose-modified Au QCM electrode to give an amplified frequency response.

In recent years, considerable attention has been focused on the exploration of the microgravimetric quartz crystal microbalance (QCM) as a highly sensitive biosensing transducer for the detection of biological analytes.[1] The interest in this area stems to a large extent from the versatility, simplicity and inherent sensitivity of the QCM transduction method. The QCM-based biosensor simply relies on the change in resonant frequency of the QCM that results from the mass change associated with biological processes on the surface of the quartz crystal. For example, direct association of an analyte to a selective receptor immobilized on the surface of a quartz crystal electrode results in a mass increase and thus leads to a decrease in the resonant frequency of the QCM. Therefore, a QCM-based biosensor can detect analytes without any label. Although the QCM can measure a small mass change with relatively high intrinsic sensitivity, approaches for improving the sensitivity of the QCM-based biosensor have been widely studied in order to apply the technique to the trace analysis of biological analytes. In particular, the use of gold nanoparticles (NPs) as a signal amplifier is noteworthy. For instance, the incorporation of biomolecule-functionalized Au NPs as a signal amplifier led to a two to three orders of magnitude sensitivity increase in QCM-based DNA hybridization assays[2–4] and protein binding assays.[5,6]

Lectins are carbohydrate-binding proteins and are found in most organisms from microorganisms to plants and animals. Lectin–carbohydrate interactions play key roles in a variety of important biological processes such as cell-surface recognition, cell–cell communications, and pathogen infection.[7] A variety of techniques have been developed for the investigation of these interactions including NMR, mass spectrometry, isothermal titration calorimetry and affinity chromatography.[8] Since the interactions are weak, the development of highly sensitive but simple detection methods for the carbohydrate–protein interactions is still one of the most important analytical challenges in the area of glycomics research. To date, a number of studies have been directed toward the use of QCM to study lectin–carbohydrate interactions. For example, the interactions of a specific carbohydrate and lectins have been studied to determine their affinity constants based on the thiol-tailored trisaccharide[9] as well as azido sugars[10] self-assembled monolayer (SAM) on a Au QCM electrode. In addition, the interaction of carbohydrate and lectin Con A has been further applied to the selective detection of *E. coli* without any label since the microorganisms contain lectin-binding pockets at their surface.[11]

As a highly sensitive microgravimetric lectin biosensor, we present herein a novel biosensor for the detection of a target lectin Con A as a model analyte. Con A from *Canavalia ensilformis* is a well-known tetrameric protein with four carbohydrate binding sites which specifically binds α-D-mannopyranosyl and α-D-glucopyranosyl groups.[12] Con A is similar to a thick square (12 nm × 12 nm) and each binding site of Con A is located at a corner of the thick square. Scheme 1 outlines the present biosensing system to detect lectin Con A. Once the interaction between Con A and mannoside SAM on a Au QCM electrode is completed, Au NPs capped with mannoside are added to form a sandwich-type complex through the binding of the mannose-stabilized Au NPs to the remaining opposite binding sites of Con A, which results in an increased frequency change of QCM. Therefore, the additional coupling of the high-mass Au NPs to the Con A effectively amplifies the mass increase. As a result, this method provides a substantial increase in the sensitivity of the QCM biosensing system.

A mannoside containing a thiol functional group at the terminal position has been synthesized in a multistep sequence from a mannosyl trichloroacetimidate and an alcohol (see ESI for synthesis and characterization†). The thiol-modified mannoside was self-assembled on a Au QCM electrode (AT-cut, 9 MHz, Seiko EG&G Co., geometrical area of 0.196 cm^2) by placing the electrode in 0.5 mM aqueous solution of thiolated mannose for



Scheme 1 Schematic illustration of the microgravimetric biosensing of lectin Con A based on mannose-stabilized Au NPs as a signal amplifier.

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12 h. The formation of mannose SAM on the Au electrode was verified by cyclic voltammetry. This experiment was carried out in a solution of 50 mM phosphate buffer at pH 7 containing 1.0 mM $K_3Fe(CN)_6$ using an EG&G 273A electrochemical system. The mannose SAM-modified Au electrode was used as the working electrode. A platinum wire and Ag/AgCl (3 M NaCl) were used as counter and reference electrodes. Cyclic voltammograms were obtained at a bare and the mannose SAM-modified Au electrode at a scan rate of 50 mV s^{-1} (see ESI for cyclic voltammograms of 1.0 mM $K_3Fe(CN)_6$ on a bare and the mannose SAM-modified Au QCM electrodes†). The formation of the mannose SAM on the Au electrode dramatically decreased the reversible redox peaks from 1.6×10^{-5} A to 1.0×10^{-7} A. In addition, double layer charging capacitance was measured at the bare and the mannose SAM-modified Au electrode in 50 mM phosphate buffer at pH 7.0. The double layer charging capacitance was significantly decreased from 28 μF cm^{-2} to 4.7 μF cm^{-2}. These results indicate that a highly compact mannose SAM has been formed on the electrode surface, which blocks the electrode surface for the redox reactions.

Fig. 1 shows a frequency response plot for the injection of 7.2 nM Con A into the mannose SAM-modified Au QCM electrode. The addition of Con A resulted in a frequency decrease because of the direct association of Con A onto the mannose SAM-modified Au QCM electrode.

In order to further confirm whether the mannose SAM maintains the specificity to the corresponding lectin, Con A, the resonant frequency shift of the mannose SAM-modified electrode was recorded while increasing the concentration of Con A added to the solution. The dependency of the frequency shift on the Con A concentration is plotted in Fig. 2(a). It follows the Langmuir-type adsorption isotherm,

$$\Delta f = \Delta f_{\max} \frac{K_a[\text{Con A}]}{1 + K_a[\text{Con A}]} \quad (1)$$

where Δf is the frequency change, $\Delta f_{\max}$ is the frequency shift on saturation, [Con A] is the concentration of the Con A solution and K_a is the association constant.[13] Fig. 2(b) presents a representative reciprocal plot of [Con A]/Δf *vs.* [Con A]. From the fitting of

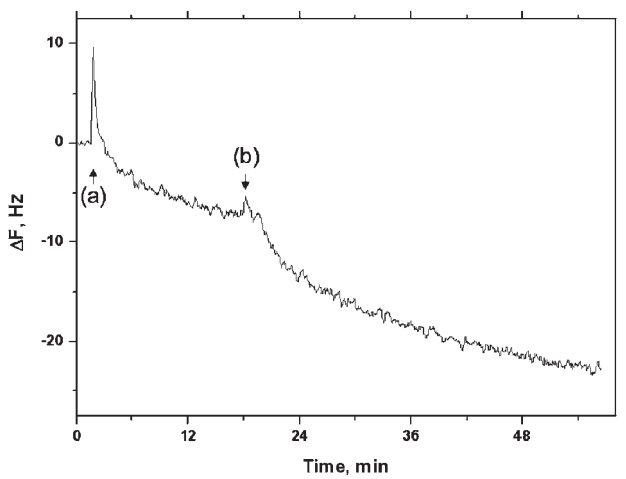

Fig. 1 Frequency response plot for the injection of 7.18 nM Con A into the mannose SAM-modified Au QCM electrode (a) before and (b) after the addition of mannose-stabilized Au NPs.

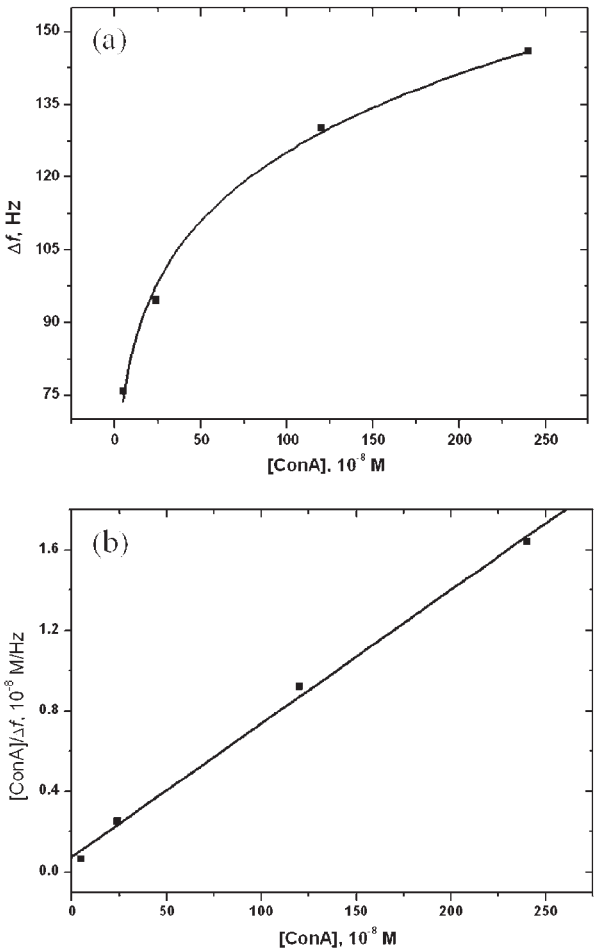

Fig. 2 Frequency change *vs.* concentration of Con A in 50 mM phosphate buffer at pH 7.0 with 1 mM Ca^{2+} and 1 mM Mn^{2+} (a) and its reciprocal plot of [Con A]/Δf *vs.* [Con A] (b).

eqn (1), the association constant was estimated to be *ca.* 2.24×10^7 M^{-1}. This value is slightly higher than the reported values in other literature (5.6×10^6 M^{-1} and 3.9×10^6 M^{-1} based on QCM[12] and SPR[10] measurements, respectively). The binding constant obtained in this study confirms that the mannose SAM was formed on the Au surface not only with a high density but also with specificity to its corresponding lectin Con A.

As shown in Scheme 1, in order to amplify the frequency change, the mannose-stabilized Au NPs were prepared by the displacement self-assembly of commercially available citrate-capped Au NPs (*ca.* 8 nm diameter, British Biocell International Co., 1 mL) solution with 1.0 mg of thiol-modified mannoside. The excess thiol-modified mannoside was then removed by filtration with Centricon YM-100 (Millipore Co.) in 50 mM pH 7.0 phosphate buffer. The final concentration of the Au NPs was determined to be 5.0×10^{12} particles mL^{-1} by UV–visible spectroscopy (UV-1650PC, Shimadzu Co.). TEM images of citrate-capped and mannose-stabilized Au NPs cast on a substrate of carbon grid type-A (300 mesh, TED PELLA, Inc.) are shown in Fig. 3(a) and (b), respectively. Mannose-stabilized Au NPs were monodisperse as were the citrate-capped Au NPs and their size was *ca.* 8 nm in diameter. The surface coverage of the Au NPs with mannose was calculated to be 196 pmol cm^{-2} according to

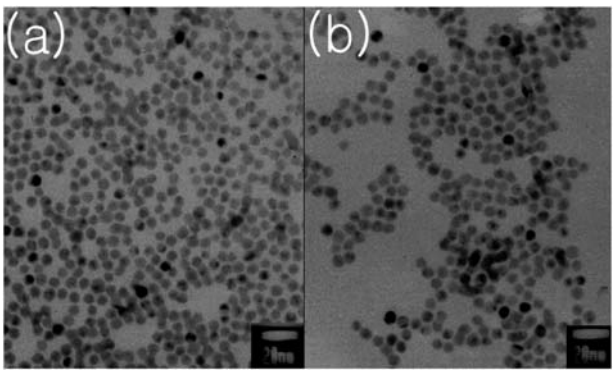

Fig. 3 TEM images of (a) citrate-capped Au nanoparticles and (b) mannose-stabilized Au nanoparticles.

the literature.[14] The mannose-stabilized Au NPs were stable in the phosphate buffer solution.

The prepared mannose-stabilized Au NPs were applied as a signal amplifier in the determination of Con A. As shown in Fig. 1(b), right after the interaction between Con A and mannose SAM on the Au QCM electrode was terminated, the mannose-stabilized Au NPs were added to form a sandwich-type complex through the binding of the mannose-stabilized Au NPs to the remaining opposite binding sites of Con A, which resulted in an increased frequency change of the QCM. As a control experiment, mannose-stabilized Au NPs were added to the mannose SAM-modified electrode in the absence of Con A. The frequency change was only 1.2 Hz, which indicates that mannose-stabilized Au NPs are specifically bound to the binding sites of Con A and non-specific adsorption of the mannose-modified Au QCM electrode has not occurred.

Since the additional coupling of the high-mass Au NPs to the Con A effectively amplifies the frequency change, the sensitivity of the QCM detection system has been substantially increased. Fig. 4 shows the calibration curves of Con A before (a) and after (b) the signal amplification by Au NPs. As shown in Fig. 4, the sensitivity (4.32 Hz/nM) obtained with the signal amplification using mannose-stabilized Au NPs was *ca.* 13-fold higher than that (0.34 Hz/nM) obtained with the simple detection method without the signal enhancement.

While the detection limit of 1.6×10^{-9} M (S/N = 3) for the target Con A has been estimated from the response of the QCM signal without the signal enhancement, the biosensing system based on signal enhancement with the mannose-stabilized Au NPs resulted in the improved detection limit of 1.3×10^{-10} M. The detection limit for the Con A obtained with the present system is much lower than the reported values. For example, QCM analysis of Con A based on an azido mannose SAM-modified electrode gave a reported detection limit of 9.0×10^{-8} M.[11] The higher association constant (2.24×10^{7} M^{-1}) obtained with the present mannose SAM than with an azido mannose SAM (5.6×10^{6} M^{-1}) might lead to a lower detection limit even in the unenhanced system. The colorimetric detection of the lectin Con A using mannose-stabilized Au NPs resulted in the detection limit of 1.9×10^{-7} M, which is three orders of magnitude higher than the present system.[15]

It is of interest to find out if the present lectin Con A biosensor has a nonspecific binding toward other proteins. When the IgG and cholera toxin were added to the mannose-modified Au QCM electrode, there was a negligible frequency change. For example, the frequency change was less than 5 Hz for the addition of 6.3 μM Con A, which is almost negligible in consideration of such a high concentration of Con A.

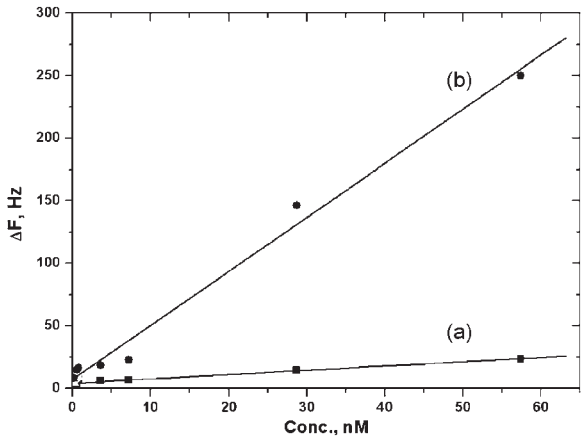

Fig. 4 Calibration curves of Con A before (a) and after (b) the signal amplification by mannose-stabilized Au NPs. Con A is dissolved in 50 mM phosphate buffer at pH 7.0.

In conclusion, we have presented a highly sensitive QCM lectin Con A biosensor based on mannose-stabilized Au NPs as a signal amplifier. Although the sensitivity with the present biosensing system is impressive, the sensitivity may be further enhanced by silver capping of mannose-stabilized Au NPs as in an immunoassay.[5] The present biosensing method should complement other methods such as ELISA in a favorable manner and provide a versatile tool for the analysis of lectin proteins containing several binding sites with various directions.

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