Analytica Chimica Acta 657 (2010) 204–209

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Construction of supported lipid membrane modified piezoelectric biosensor for sensitive assay of cholera toxin based on surface-agglutination of ganglioside-bearing liposomes

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ARTICLE INFO

Article history:
Received 13 July 2009
Received in revised form 15 October 2009
Accepted 18 October 2009
Available online 24 October 2009

Keywords:
Piezoelelctric biosensor
Surface-agglutination
Functionalized liposome
Supported lipid membrane
Renewable biosensor

ABSTRACT

A novel piezoelelctric biosensor has been developed for cholera toxin (CT) detection based on the analyte-mediated surface-agglutination of ganglioside (GM1)-functionalized liposomes. To achieve a CT-specific agglutination at the surface, the gold electrode is modified by a GM1-functionalized supported lipid membrane via spontaneous spread of the liposomes on a self-assembled monolayer of a long-chain alkanethiol. In the presence of CT, the GM1-incorporated liposomes in assay medium will rapidly specifically agglutinate at the electrode surface through the binding of CT to GM1 on the electrode surface and the liposome interface. This results in an enormous mass loading on the piezoelelctric crystal as well as a significant increase of density and viscosity at the interface, thereby generating a decrease in frequency of the piezoelelctric crystal. The combination of mass loading with interfacial change in the surface-agglutination reaction allows the developed piezoelelctric biosensor to show substantial signal amplification in response to the analyte CT. The detection limit can be achieved as low as 25 ng mL^{-1} CT. This is the first demonstration on CT detection based on specific surface-agglutination of GM1-modified liposomes. The supported lipid layer based sensing interface can be prepared readily and renewably, making the developed technique especially useful for simple, reusable and sensitive determination of proteins.

1. Introduction

Piezoelectric quartz crystals are very sensitive microgravimetric devices. The frequency change of the piezoelectric quartz crystal in gas phase arises from mass change of the adsorbates on the crystal surface, while in liquid phase it is generally attributed to both gravimetric and viscoelastic effects. Owing to its simplicity, low cost, high sensitivity and real-time output, the piezoelectric quartz crystal microbalance (QCM) has attracted considerable interest in biomedical analysis of various targets including nucleic acids [1–3], proteins [4–7] and small molecules [8,9].

QCM detection of proteins has been routinely performed using antibody-based immunoassay methodologies. In general, to achieve specific detection of proteins the antibody is immobilized on the QCM electrode surface, and the binding of target protein directly causes a change of mass loading on the crystal surface or mediates a downstream binding of secondary antibody with giant mass amplifier or enzyme label. Therefore, the immobilization protocol and the detection strategy both play crucial roles for the performance of QCM biosensors of protein targets.

Immobilization protocol should ideally not only impart a high loading of active recognition components for enhancing the sensitivity and dynamic range, but also allow ready and reproducible preparation and regeneration of the sensing interface. Immobilization of recognition components is traditionally implemented using covalent linking via polymer coating [10–12], surface self-assembled monolayer (SAM) [8,13], electrostatic assembled multi-layer [14–16] and sol–gel film [17,18]. To improve the surface loading of recognition components, there is an increasing interest in the utilization of nano-structured sensing interfaces including porous nanofilms [19,20] and nanoparticle-assemblies [21,22]. Alternatively, oriented immobilization procedures using protein A/G and antibody fragments are introduced for the purpose of optimizing the activity of immobilized antibodies [9,23]. Nevertheless, these immobilization methods usually require skillful operations to ensure reproducible preparation of the interfaces or harsh conditions to regenerate the surfaces in which the immobilized antibody might be partially inactivated. In this context, search for protocols by which the sensing interface can be readily prepared and renewed is still of great significance in the development of immunosensors.

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0003-2670/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.aca.2009.10.036

Piezoelectric sensors are responsible to protein analytes either via the gravimetric or the viscoelastic mode. Due to limited mass of the analyte protein, the frequency response of gravimetry-based piezoelectric sensor is often inadequate to generate a highly sensitive signal. Giant mass label [24–27] or enzymatic deposition [28,29] has been exploited in order to improve the dose-response sensitivity of piezoelectric sensors. These signal amplification techniques, though offering sensitivity enhancement, frequently involve multi-step reactions that might be time-consuming and thus impractical for rapid assay applications. Piezoelectric sensors based on viscoelastic detection are essentially physical transducers in response to liquid phase bio-recognition reactions [7,11,30]. Because of facilitated kinetics in homogenous interaction between antibodies and antigens in liquid phase, this technique features a relatively high analytical speed. However, since only interfacial viscoelastic effect is measured, viscoelasticity-based piezoelectric sensors are intrinsically limited in their detection sensitivity.

In this paper, a novel reusable piezoelectric biosensor with enormous signal amplification has been developed for detection of cholera toxin (CT) based on analyte-specific surface-agglutination of ganglioside (GM1)-functionalized liposomes on supported lipid membrane incorporated with ganglioside GM1. The analyte protein CT, a protein enterotoxin that is secreted by the bacterium *Vibrio cholerae* associated with a severe epidemic disease, comprises a pentamer of identical B units each specifically interacting with one ganglioside GM1 molecule. Then, the analyte protein not only binds to the biosensor surface, but also causes specific agglutination of the GM1-incorporated liposomes on the surface as well as in the assay solution. This produces an agglutinated network complex of liposomes and CT at the sensor surface, inducing substantial viscoelastic changes and enormous mass loading on the sensing interface. Compared with traditional piezoelectric biosensor, the proposed technique introduces a simple approach to combine the contribution of both gravimetric and viscoelastic effects, thereby enabling vast sensitive enhancement in protein determination. Furthermore, the supported lipid membrane modified interface is inherently biocompatible and resistant to non-specific adsorption of serum constituents, which mitigates the background responses and improves the selectivity of the developed sensor [31]. Additionally, the supported lipid membrane can be easily regenerated via brief wash with ethanol followed by spontaneous spread of the lipid membrane. This furnishes an ideal protocol for the immobilization of bio-recognition elements such as antibodies, aptamers and receptors for protein detection.

2. Experimental

2.1. Chemicals and solutions

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and monosialoganglioside (GM1) were purchased from Sigma Chemical Co. Phosphate buffer saline (PBS, pH 7.4) was prepared using 1/15 M Na_2HPO_4 and 1/15 M KH_2PO_4. Cholera toxin (CT) was kindly supplied by Shanghai United Cell Biotechnology Co., Ltd. (United Biotech). CT solution of varying concentrations were prepared in 1/15 M PBS (pH 7.4) and stored at 4 °C. BSA, IgG, HSA, IgE, thrombin, and lysozyme were obtained from Beijing Dingguo Biotechnology Company (Beijing, China).

2.2. Apparatus

The quartz crystal analyzer (QCA 922) was purchased from Serkoeg & G Co., Ltd. (Japan). Piezoelectric quartz crystals (9 MHz, gold electrodes) were supplied from Chenxing Radio Equipments (Beijing, China). One side of the crystal was sealed with an O-ring of silicone rubber. The detection was performed by setting the piezoelectric sensor in a laboratory-made reaction cell containing 300 μL sample solution. The experimental temperature was controlled at 25 °C. The electric wires of the crystal were extended to the quartz crystal analyzer to monitor the frequency responses. The surface morphology of assembled layers was investigated by AFM (SPI3800N Probe Station&SPA-400SPMUnit, Seiko, Japan)

2.3. Preparation of GM1-functionalized liposomes

Unilamellar liposomes were prepared according to a documented procedure [32] with slight modifications described as follows: Stock solutions of DPPC and GM1 were prepared in a chloroform/methanol mixture (6:1, v/v). The solution of DPPC (1 mg in total) and GM1 were mixed in the mole ratio of 40:1 (DPPC/GM1) in a 5-mL round-bottom flask. After the mixture was sonicated for 5 min, the organic solvent was removed by rotary evaporation under reduced pressure (0.09 MPa), leaving a thin lipid film on the inside wall of the flask. To the dried lipids, 2 mL of PBS (pH 7.4) was added. The lipids were hydrated in a water bath at 60 °C and rotated vigorously to form multilamellar vesicles (MLVs). The MLV suspension was sonicated using a probe-type sonicator to reduce the average size of the liposomes. The resulting liposome suspension was centrifuged at 3000 rpm (644 g) for 15 min to remove residual multilamellar vesicles and aggregated lipids. The preparation of GM1-free liposome was exactly performed using the aforementioned procedure except that the lipid solution only contained DPPC. The liposome solution was stored at 4 °C until further use. The diameter of the resulting liposome was measured to be 151 ± 15 nm using a dynamic light scattering system (Zeta Plus, Brookhaven Instruments Corp.). The average volume of a single liposome was calculated to be 1.8×10^{-12} μL. The amount of GM1 in the resulting liposomes was measured by the dimethyl methylene blue dye method [33], and 51% of the GM1 was found to be successfully incorporated in the GM1 liposome. Then it was estimated that 2200 ± 200 molecules of GM1 were on the outer surface of each liposome. Liposome concentration was determined to be 2.3×10^{12} liposomes mL^{-1} as explained elsewhere [34].

2.4. Fabrication of piezoelectric sensor modified with supported lipid membrane

The gold electrode of a piezoelectric sensor was treated with "piranha" solution, a mixture of 98% H_2SO_4 and 30% H_2O_2 in a volume ratio of 7:3, then washed thoroughly with a copious amount of doubly distilled water, and finally dried at room temperature. The bare electrode was immersed in a freshly made 2 mM octadecanethiol in ethanol overnight to form a hydrophobic SAM on the gold surface.

The hydrophobic surface of piezoelectric sensor was rinsed with ethanol thoroughly, and air-dried. Exposure of the modified sensor surface to an aqueous suspension of GM1-incorporated liposomes in the reaction cell resulted in spontaneous spread of supported planar lipid monolayer, which could be detected by the decrease of resonance frequency. The supported lipid membrane modified piezoelectric sensor was kept in PBS for further use. The piezoelectric sensor without surface-tether GM1 was prepared exactly using the aforementioned procedure except the use of GM1-free liposome in spontaneous spread of the membrane.

2.5. Measurement procedure

The piezoelectric sensor was immersed in the reaction cell containing 300 μL liposome suspension. After the resonance frequency became stabilized, 3 μL of sample solution with different CT concentrations was injected into the reaction cell. The frequency

responses were recorded as the reaction proceeded until equilibrium was reached. For all experiments, unless otherwise indicated, the recorded frequency responses were the mean (SD) frequency changes of triplicate measurements.

3. Results and discussion

3.1. Frequency response characteristics of piezoelectric sensor

The frequency change of a piezoelectric quartz crystal in liquid phase was determined by two factors, i.e. the gravimetric and the viscoelastic effects. To reveal that the surface-agglutination assay offered enormous signal amplification as compared with traditional gravimetry-based and viscoelasticity-based detection modes, the behaviors of the piezoelectric sensor with or without surface-tethered GM1 in assay media with or without GM1-modified liposomes were investigated.

Fig. 1 shows the real-time frequency response curve for this sensor in different cases. Curve a in Fig. 1 gives the response of the piezoelectric sensor with surface-tethered GM1 to CT protein in an assay medium with GM1-free liposome. In this case, the piezoelectric sensor with surface-tethered GM1 was set in the detection cell with 300 μL of PBS containing GM1-free liposome. After frequency stabilized, CT solution of 3 μL was added. From Fig. 1 one observes that the frequency decreased gradually and became stabilized after 5 min with a maximized frequency change of 155 (SD = 7) Hz. One also found that such a response curve was consistent with that obtained in the detection cell with 300 μL of PBS, indicating that there was no non-specific adsorption between CT and GM1-free liposome. To identify whether the frequency response in this case contained the contribution of viscoelastic effects of the bulk solution, the solution in the detection cell was replaced with PBS solution containing GM1-free liposome via a flow system. One sees from curve a in Fig. 1 that there is no significant frequency shift during this treatment, evidencing the bulk solution at the sensor surface has little contribution to the frequency response of the piezoelectric sensor. In other words, the frequency response in curve a of Fig. 1 was primarily contributed by the binding of CT to the surface. Considering that surface-tethered GM1 ligands were confined in a monolayer, the adsorbed CT would only form a sub-monolayer on the surface, which might only yield negligible viscoelastic response as compared to gravimetric contribution arising from surface adsorbates.

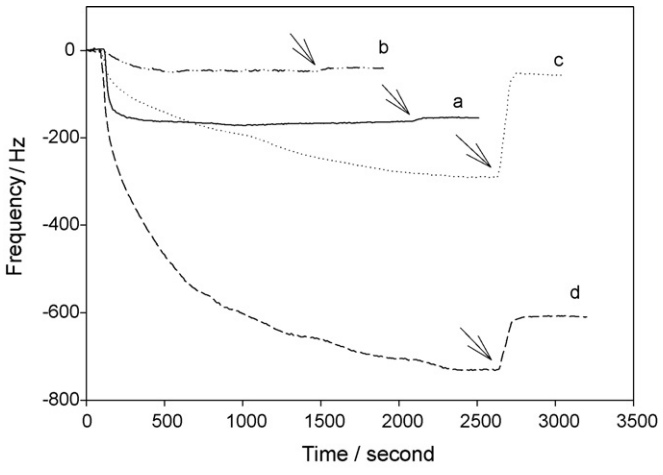

Fig. 1. The real-time frequency response to (a) 1 μg mL^{-1} CT using the piezoelectric sensor with surface-tethered GM1 in an assay media with GM1-free liposome, (b) 1 μg mL^{-1} CT using the piezoelectric sensor with a GM1-free supported lipid membrane in an assay media with GM1-free liposome, (c) 1 μg mL^{-1} CT using the piezoelectric sensor with a GM1-free supported lipid membrane in an assay media with GM1-functionalized liposomes and (d) 1 μg mL^{-1} CT using the piezoelectric sensor with surface-tethered GM1 in an assay media with GM1-functionalized liposomes. The arrows denote the substitution of assay medium.

Curve b in Fig. 1 gives the response of the piezoelectric sensor with GM1-free supported lipid membrane to CT protein in an assay medium with GM1-free liposomes. In this case, the sensor was placed in a detection cell with 300 μL PBS containing GM1-free liposomes. After frequency stabilized, 3 μL of CT was added in the solution. It is clear from curve b that the frequency change is relatively small, about 40 (SD = 3) Hz. This might be due to non-specific adsorption of the protein on the surface or the liposome. With the solution inside the cell replaced by PBS containing GM1-free liposomes, there was no remarkable change in the frequency, suggesting that non-specific adsorption primarily occurred on the surface, and the adsorption of protein on liposome was negligible.

Curve c in Fig. 1 gives the response of the piezoelectric sensor with GM1-free supported lipid membrane to CT protein in an assay medium with GM1-functionalized liposomes. In this case, the sensor was placed in a detection cell with 300 μL PBS containing GM1-functionalized liposomes. After frequency stabilized, 3 μL of CT was added in the solution. It is seen in curve c in Fig. 1 the frequency change becomes stable at about 300 (SD = 17) Hz after 40 min. With the solution inside the cell replaced by PBS containing GM1-functionalized liposomes, the frequency response was almost recovered to the value given in curve b. From these observations, one could conclude that the frequency shift in this case was primarily contributed by the agglutination of GM1-incorporated liposomes in the presence of CT. The formation of network complexes of CT and liposomes at the surface altered the interfacial viscoelasticity, thereby resulting in the frequency response of the sensor. Because the network complexes were only present in the bulk solution at the surface, the removal of the complexes in solution substitution recovered the frequency.

Curve d in Fig. 1 gives the response of the piezoelectric sensor with surface-tethered GM1 to CT protein in an assay medium with GM1-functionalized liposome. In this case, the GM1-modified sensor was immersed in 300 μL PBS with GM1-functionalized liposomes. On adding CT in the detection cell, one observes a large frequency decrease of 730 (SD = 38) Hz, as is depicted in curve d of Fig. 1. With the solution inside the cell replaced by PBS with GM1-functionalized liposomes, one observed a gradual increase of the frequency with a maximized frequency shift of 110 (SD = 10) Hz. Note that in this case where GM1 was present both on the sensor interface and the liposome surface, CT would not only bind to the surface, but also induce the formation of network complexes on the surface and in the solution, i.e., a surface-agglutination reaction. The network complexes of CT and liposome would form a multilayer structure on the surface and in the interfacial bulk solution, which not only increased the gravimetric loading on the sensor, but also altered substantially the viscoelasticity at the surface. That is, the surface-agglutination strategy introduced a simple approach to combine the contribution of both gravimetric and viscoelastic effects, thereby enabling vast sensitive enhancement, 730 Hz versus 155 Hz, in CT determination. Based on the results obtained via substitution of assay medium that removed the network complexes of CT and liposome in the bulk solution, the frequency response arising from viscoelastic effect in bulk solution in the surface-agglutination approach was estimated to be 110 Hz. This implies that the contribution of gravimetric and viscoelastic effects due to the adsorption of network complex on the surface was 620 Hz, which was also much greater than the gravimetric response obtained in curve a.

Moreover, atomic force microscope (AFM) photographs of the surface morphologies of QCM after spontaneous spreading of supported planar lipid monolayer and specific detecting of CT also verify above-mentioned results. As shown in Fig. 2, the two pho-

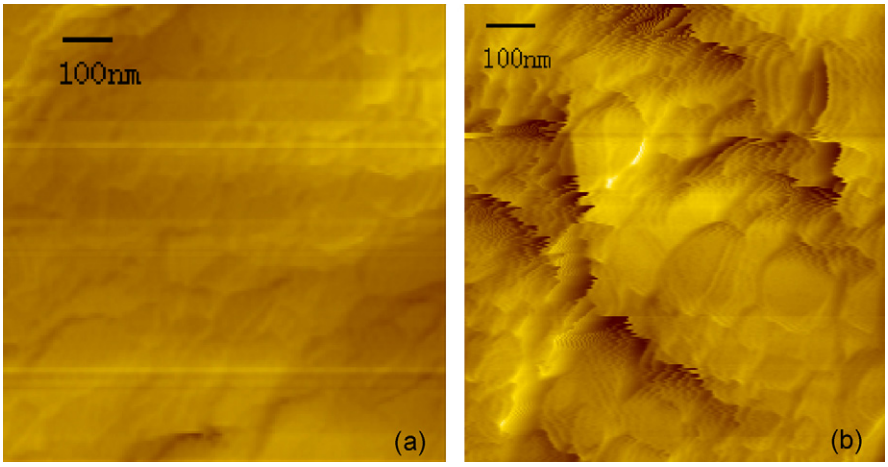

Fig. 2. AFM photographs of QCM surface morphology: (a) QCM modified with the GM1-incorporated supported lipid layer and (b) QCM with surface-aggregation of liposome–CT complex.

tographs of surface morphology are distinct markedly, which illuminates that the self-assembled supported planar lipid layer was even and compact and surface-agglutination of liposome-CT complex led to substantial change of surface morphology.

3.2. Concentration of GM1 and phospholipids in assay medium

It was reported that the surface density of GM1 in lipid membrane would influence the orientation of CT protein at the membrane [34]. To enable the analyte protein accessible by both the sensor surface and the liposome, the concentration of GM1 should be optimized in the lipid membrane. Under controlled conditions in preparing the liposome, the concentration of GM1 in the lipid membrane was determined by the amount of GM1 in the lipid solution. Fig. 3a depicts the frequency response of the biosensor as a function of varying GM1 fraction in the lipid solution. It can be seen from Fig. 3a that the maximal frequency response is obtained with the lipid solution with 40:1 in molar ratio for PC:GM1. Therefore, the molar fraction of GM1 was controlled to be 1/41 in the lipid solution throughout the experiments.

The sensitivity of agglutination assay was highly dependent upon the size of the network complex of CT and GM1-functionalized liposome, which was in turn determined by the concentration of phospholipids used for the preparation of liposome. Similar to the immunoagglutination assay, the network complex exhibited the maximized size only when the valence ratio of the reactive species coincides with the molar ratio of CT to GM1-functionalized liposome. Either excessive or scant liposomes in the assay media would induce shrinkage in the size of the network complex, resulting in a decreased frequency response. To optimize the signal gain of the surface-agglutination assay, effect of the concentration of phospholipids on the frequency response was investigated at a fixed concentration of CT. The results in Fig. 3b shows that the maximized frequency response is obtained when the concentration of phospholipids is around 0.5 mg lipids mL^{-1}, indicating that the network complex of CT and liposome reaches the biggest size at the phospholipids concentration of 0.5 mg lipids mL^{-1}. The phospholipids concentration of 0.5 mg lipids mL^{-1} was used throughout experiments.

3.3. Calibration curve

Fig. 4 depicts the frequency responses of the piezoelectric sensor to CT of varying concentrations. It was observed that the sensor gave increased frequency shift with increasing CT concentration, and the stabilized frequency shift (recorded 40 min after addition of CT) exhibited a linear correlation to the CT concentration in the range from 0.1 $\mu g\,mL^{-1}$ to 1 $\mu g\,mL^{-1}$, as shown in the inset of Fig. 4. The regression equation was $\Delta F/\mathrm{Hz} = 653.7C/(\mu g\,ml^{-1}) + 70.9$ with a correlation coefficient of 0.99. The detection limit (the concentration corresponding to a signal 3 SD above the mean for blank signals) was calculated as of 50 ng mL^{-1}.

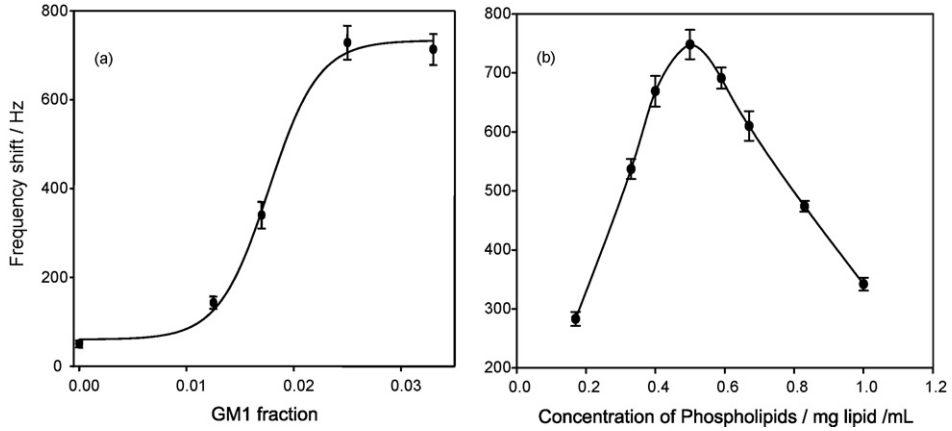

Fig. 3. (a) Frequency responses to 1 $\mu g\,mL^{-1}$ CT of the developed piezoelectric sensor in the assay media with varying GM1 fraction in phospholipids concentrations and (b) frequency responses to 1 $\mu g\,mL^{-1}$ CT of the developed piezoelectric sensor in the assay media with varying phospholipids concentrations.

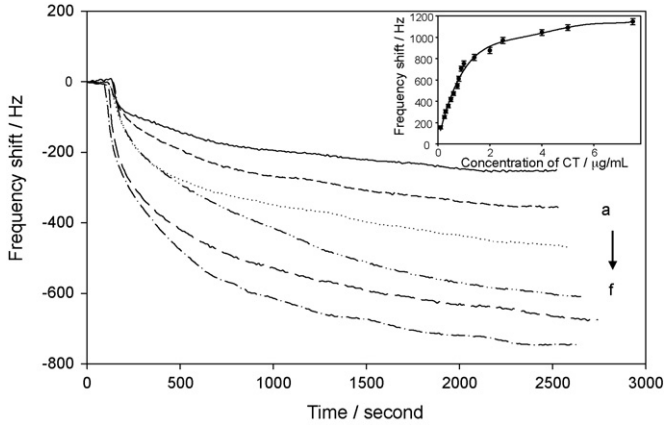

Fig. 4. The real-time frequency responses of the piezoelectric sensor to different concentrations of CT (a) 0.25 μg mL^{-1}, (b) 0.4 μg mL^{-1}, (c) 0.6 μg mL^{-1}, (d) 0.8 μg mL^{-1}, (e) 0.9 μg mL^{-1} and (f) 1 μg mL^{-1}. Inset: Calibration curve of the frequency responses versus the concentrations of CT.

Besides the use of stabilized response for the quantification of CT, the initial response rate, defined by the slope of the frequency change curve at the initial stage, could also be utilized for the detection of CT, which might be of particular significance in rapid assay practices. Fig. 5 gives the initial rate of frequency responses at the initial stage (30 s) after the addition of CT. One observed that the frequency responses almost exhibited a linear variation profile at the initial stage, and the slope (initial response rate) dynamically increased with increasing CT concentration. The inset plot revealed that the initial response rate had a linear correlation to the CT concentration in the range from 0.1 μg mL^{-1} to 5 μg mL^{-1}. The regression equations was $(\Delta F/\Delta t)/(\text{Hz s}^{-1}) = 1.4C/(\mu\text{g mL}^{-1}) + 2.1$ with a correlation coefficient of 0.99. The detection limit (the concentration corresponding to a signal 3 SD above the mean for blank signals) was calculated as 25 ng mL^{-1} for the kinetic approach. So, the developed biosensor might provide a useful approach for rapid clinical diagnosis, particularly for epidemic screening of CT.

3.4. Specificity and real sample analysis

Fig. 6 depicts the frequency responses of the developed biosensor to common coexisting materials. It was observed that the biosensor gave mean(SD) frequency responses of 55(6) Hz, 40(5) Hz,19(4) Hz, 60(7) Hz, 43(5) Hz, 39(4) Hz, 35(5) Hz, respectively, for BSA (50 μg mL^{-1}), HSA (50 μg mL^{-1}), human IgG (50 μg mL^{-1}), human IgE (50 μg mL^{-1}), lysozyme (50 μg mL^{-1}), thrombin (50 μg mL^{-1}) as well as whole serum negative for CT (10 fold dilution). These responses were all much less than that, 738(39) Hz, obtained with 1 μg mL^{-1} CT, indicating that the supported lipid membrane and the liposome were highly resistant to non-specific adsorption of common serum proteins. Then, the developed strategy was expected to show high selectivity for the detection of CT in serum specimens.

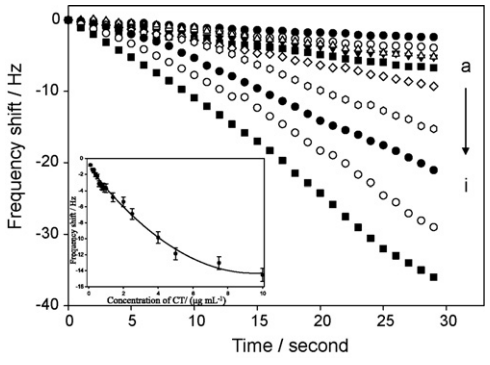

Fig. 5. The frequency responses of the sensor in 30 s after the addition of the different concentrations of CT (a) 0.1 μg mL^{-1}, (b) 0.25 μg mL^{-1}, (c) 0.3 μg mL^{-1}, (d) 0.4 μg mL^{-1}, (e) 0.6 μg mL^{-1}, (f) 1.4 μg mL^{-1}, (g) 2.5 μg mL^{-1}, (h) 4 μg mL^{-1} and (i) 5 μg mL^{-1}. Inset: Calibration curve of the initial response rates versus the concentrations of CT.

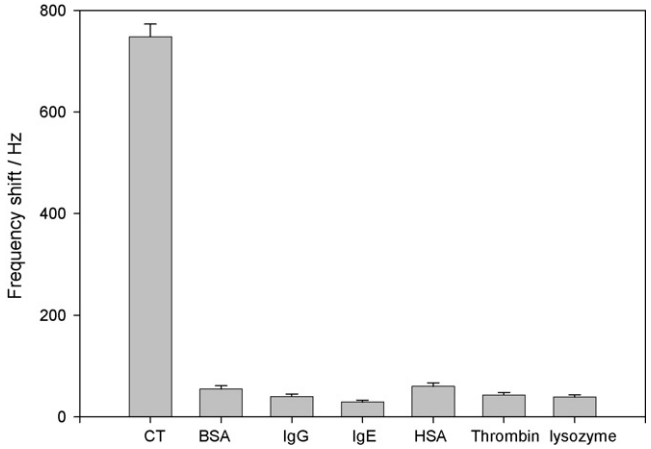

Fig. 6. The frequency responses of the piezoelectric sensor to different proteins: CT (1 μg mL^{-1}), BSA (50 μg mL^{-1}), hIgG (50 μg mL^{-1}), HSA (50 μg mL^{-1}), IgE (50 μg mL^{-1}), thrombin (50 μg mL^{-1}), lysozyme (50 μg mL^{-1}) as well as whole serum negative for CT (10 fold dilution).

3.5. Regeneration of piezoelectric sensor

The developed biosensor was prepared using the supported lipid membrane technology. This biologically compatible membrane not only alleviated of non-specific adsorption of serum proteins, but also allowed a ready regeneration of the sensor interface. Indeed, the external lipid membrane could be removed immediately via rinsing with ethanol, which renewed the octadecanethiol-assembled electrode surface. Then, the supported lipid membrane could be regenerated via immersing the electrode in a suspension of GM1-functionalized liposome for 30 min. Fig. 7 depicts typical frequency variation curve of the piezolelectric sensor in five regeneration cycles. It was seen that the piezoelectric crystal could response in very reproducible way to the spread of supported lipid membrane and the surface-agglutination of CT–liposome com-

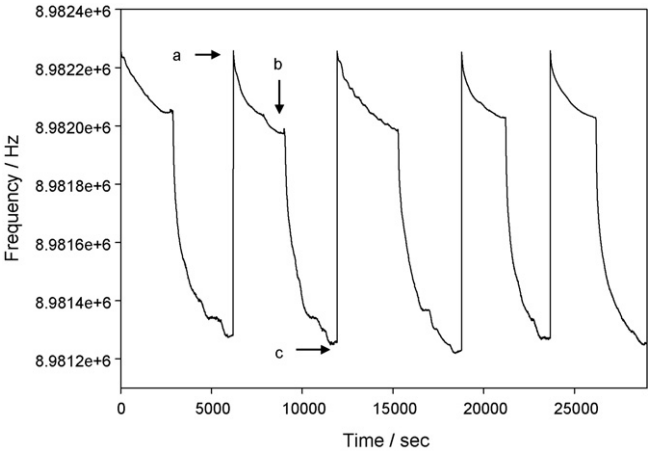

Fig. 7. The frequency variation curves of the piezolelectric sensor in five regeneration cycles. Arrow a denotes the formation of supported lipid membrane, arrow b represents the addition of 1 μg mL^{-1} CT and arrow c indicates the regeneration of the sensor surface.

plex. As a matter of fact, it was observed that the biosensor could be regenerated for more than 50 times with no significant loss of the sensitivity. The relative standard deviation for frequency measurements obtained with piezoelectric sensors in response to 1 μg mL^{-1} CT prepared via renewal in 50 runs was evaluated to be 4.1%. Furthermore, the octadecanethiol-assmbled electrode surface could also be prepared with high reproducibility. Actually, 20 biosensors were prepared via simple self-assembled monolayer of octadecanethiol-assmbled on different electrodes followed by the spread of the supported lipid membranes. One observed that the frequency shifts obtained with these 20 piezoelectric sensors in response to 1 μg mL^{-1} CT exhibited a relative standard deviation of 6.2%. In addition, the octadecanethiol-assmbled electrode surface could be stored for a long period in air (more than three months) and the resulting biosensor could be used throughout this period for CT detection without significant loss of sensitivity.

4. Conclusion

The present study proposed a novel strategy for the development of piezoelectric biosensor for CT based on surface-agglutination of GM1-functionalized liposomes on supported lipid membrane incorporated with GM1. The biosensor was prepared easily and reproducibly using the supported lipid membrane tethered with target-specific receptor GM1. This biocompatible modification enabled not only the alleviation of non-specific adsorption of serum proteins, but also a ready regeneration of the sensor interface. Also, a surface-agglutination assay method was developed for sensitive detection of CT using the piezoelectric biosensor. This method combined the viscoelastic and the gravimetric effects produced by the agglutinated network complex of liposomes and CT at the sensor surface, thereby creating an effective approach to signal amplification. The results demonstrated that the developed biosensor could allow sensitive, selective, rapid and reusable detection of CT with highly resistance to non-specific adsorption. It was expected that the developed strategy might furnish an ideal protocol in immobilization of bio-recognition elements such as antibodies, aptamers and receptors and sensitive detection of proteins in clinic diagnosis and medical researches.

Acknowledgment

This work was supported by “973” National Key Basic Research Program (2007CB310500) and NSF (20875027, U0632005) of China.

References

[1] F. Caruso, E. Rodda, D.F. Furlong, K. Niikura, Y. Okahata, Anal. Chem. 69 (1997) 2043–2049.
[2] X.C. Zhou, L.Q. Huang, S.F.Y. Li, Biosens. Bioelectron. 16 (2001) 85–95.
[3] M. Minunni, S. Tombelli, R. Scielzi, I. Mannelli, M. Mascini, C. Gaudiano, Anal. Chim. Acta 481 (2003) 55–64.
[4] S. Boujday, A. Bantegnie, E. Briand, P.G. Marnet, M. Salmain, C.M. Pradier, J. Phys. Chem. B 112 (2008) 6708–6715.
[5] H. Wang, H. Zeng, Z.M. Liu, Y.H. Yang, T. Deng, G.L. Shen, R.Q. Yu, Anal. Chem. 76 (2004) 2203–2209.
[6] B.L. Zuo, S.M. Li, Z. Guo, J.F. Zhang, C.Z. Chen, Anal. Chem. 76 (2004) 3536–3540.
[7] H. Chen, J.H. Jiang, Y.F. Li, T. Deng, G.L. Shen, R.Q. Yu, Biosens. Bioelectron. 22 (2007) 993–999.
[8] Y.S. Fung, Y.Y. Wong, Anal. Chem. 73 (2001) 5302–5309.
[9] L. Alfonta, I. Willner, D.J. Throckmorton, K. Singh Anup, Anal. Chem. 73 (2001) 5287–5295.
[10] V.V.R. Sai, S. Mahajan, A.Q. Contractor, S. Mukherji, Anal. Chem. 78 (2006) 8368–8373.
[11] H. Wang, C.X. Lei, J.S. Li, Z.Y. Wu, G.L. Shen, R.Q. Yu, Biosens. Bioelectron. 19 (2004) 701–709.
[12] C.J. Percival, S. Stanley, M. Galle, A. Braithwaite, M.I. Newton, G. McHale, W. Hayes, Anal. Chem. 73 (2001) 4225–4228.
[13] D.F. Tai, C.Y. Lin, T.Z. Wu, J.H. Huang, P.Y. Shu, Clin. Chem. 52 (2006) 1486–1491.
[14] T. Deng, H. Wang, J.S. Li, G.L. Shen, R.Q. Yu, Anal. Chim. Acta 532 (2005) 137–144.
[15] F. Caruso, H. Mohwald, J. Am. Chem. Soc. 121 (1999) 6039–6046.
[16] R. Pei, X.Q. Cui, X.R. Yang, E.K. Wang, Biomacromolecules 2 (2001) 463–468.
[17] B.Q. Wang, B. Li, Z.X. Wang, G.B. Xu, Q. Wang, S.J. Dong, Anal. Chem. 71 (1999) 1935–1939.
[18] D.C. Jiang, J. Tang, B.H. Liu, P.Y. Yang, J.L. Kong, Anal. Chem. 75 (2003) 4578–4584.
[19] S. Fujikawa, E. Muto, T. Kunitake, Langmuir 23 (2007) 4629–4633.
[20] E.W. Stein, P.S. Grant, H.G. Zhu, M.J. McShane, Anal. Chem. 79 (2007) 1339–1348.
[21] L. Han, D.R. Daniel, M.M. Maye, C.J. Zhong, Anal. Chem. 73 (2001) 4441–4449.
[22] M.J. Wang, L.Y. Wang, G. Wang, X.H. Ji, Y.B. Bai, T.J. Li, S.Y. Gong, J.H. Li, Biosens. Bioelectron. 19 (2004) 575–582.
[23] S.F. Chou, W.L. Hsu, J.M. Hwang, C.Y. Chen, Clin. Chem. 48 (2002) 913–918.
[24] F. Patolsky, A. Lichtenstein, I. Willner, J. Am. Chem. Soc. 122 (2000) 418–419.
[25] V. Pavlov, Y. Xiao, B. Shlyahovsky, I. Willner, J. Am. Chem. Soc. 126 (2004) 11768–11769.
[26] T. Liu, J.A. Tang, H.Q. Zhao, Y.P. Deng, L. Jiang, Langmuir 18 (2002) 5624–5626.
[27] F. Patolsky, A. Lichtenstein, I. Willner, J. Am. Chem. Soc. 123 (2001) 5194–5205.
[28] F. Patolsky, E. Katz, A. Bardea, I. Willner, Langmuir 15 (1999) 3703–3706.
[29] K.J. Feng, J.S. Li, J.H. Jiang, G.L. Shen, R.Q. Yu, Biosens. Bioelectron. 22 (2007) 1651–1657.
[30] H. Wang, Y. Zhang, B.N. Yan, L. Liu, S.P. Wang, G.L. Shen, R.Q. Yu, Clin. Chem. 52 (2006) 2065–2071.
[31] M. Stelzle, G. Weissmueller, E. Sackmann, J. Phys. Chem. 97 (1993) 2974–2981.
[32] A.K. Singh, P.K. Kilpatrick, R.G. Carbonell, Biotechnol. Prog. 11 (1995) 333–341.
[33] A.J. De Lucca, T.J. Jacks, K.A. Brogden, Mol. Cell. Biochem. 151 (1995) 141–148.
[34] A.K. Singh, P.K. Kilpatrick, R.G. Carbonell, Biotechnol. Prog. 12 (1996) 272–280.