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Construction of a novel macroporous imprinted biosensor based on quartz crystal microbalance for ribonuclease A detection

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ABSTRACT

A novel quartz crystal microbalance (QCM) biosensor with high selectivity and sensitivity has been developed for ribonuclease A determination. Macroporous protein imprinted films have been fabricated on the surface of QCM electrode using 2,2,3,4,4,4-hexafluorobutyl methacrylate (HFBMA) as the main matrix monomer, N-methacryloyl-histidine (MAH) as the functional monomer, and trimethylolpropane trimethacrylate (TRIM) as the cross-linker. The imprinted special surface area and the quantity of the imprinted sites were increased by the formation of macropores that were generated by employing calcium carbonate nanoparticles as the porogen. The selectivity factor was improved obviously for the fluoromonomer containing system, especially in dilute protein solution, which gets benefit from the reducing of the nonspecific adsorption of proteins. Furthermore, MAH can not only play the role as the functional monomer, but also improve the hydrophilicity of surface of the imprinted film, which makes for the adsorption of proteins. At last, the rigid skeleton structure made the films durable in the recycled tests.

1. Introduction

In the recent years, there has been a growing interest in molecular sensors demonstrating not only high specificity, sensitivity, and quick response, but also easy regeneration and use in analyte detection, medical diagnostics, stimulant control and drug delivery (Yano and Karube, 1999; Byren and Dianmond, 2006; Potyrailo, 2006; Sellergren and Allender, 2005). Although some progresses have been made towards improving the performances of sensors, it is still a challenge to find an efficient way to construct an extensively practicable sensor that simultaneously possesses all these desirable features rather than improving one feature at the expense of another.

With the development of biotechnology and modern electronics, quartz crystal microbalance (QCM) sensors, due to their less sample consumption and simple operation, have been widely used because of their high sensitivity, label-free, shorter analysis time and real-time analysis (Jonsson et al., 2008). For a rigid, uniform and thin-film layer, the oscillation frequency shift is directly proportional to the mass change on the surface of quartz crystal, which makes the QCM an increasingly popular platform for biosensor applications (Aizawa et al., 2004). In the recent years, the combination of QCM with molecular imprinting (MIP) technology for molecular recognition has attracted much attention (Cui et al., 2000; Lee and James, 2011).

Molecular imprinting is a promising technique for analyte detection. Although small molecule imprinting has been straightforward now (Wang et al., 2006; Pernites et al., 2011; Li et al., 2011), demands for recognition of macromolecules, such as DNA and proteins, are continually growing in recent years (Xia et al., 2005; Guo et al., 2004; Bossi et al., 2007; Cutivet et al., 2009; Zhang et al., 2011). But the major problem associated with the imprinting of proteins lies in their restricted mobility within highly cross-linked polymer networks and the poor efficiency in rebinding (Lu et al., 2012). Meanwhile, the flexible structure, easy denaturation and large size of proteins will hinder them from reaching and leaving binding sites. Thereupon, surface imprinting has been developed to overcome these problems (Tan et al., 2008).

During protein imprinting process, methacrylic acid (MAA) and acrylamide (AAM) are usually used as the functional monomers, because the "soft" character imparts the imprinted hydrogel matrices good conformation adaptability to the protein. However, disadvantages such as the structure instability and poor mechanical properties limit their practical application. So rigid organic polymer or organic/inorganic hybrid material was reconsidered by many researchers (Tan and Tong, 2007; Tatemichi et al., 2007). In our prior work, the rigid monomer methyl methacrylate (MMA) was also used as the main matrix (Zhou et al., 2011). The interactions between template protein and the

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MMA matrix are mainly based on the hydrophobic interaction, which is quite strong compared to other interactions such as hydrogen bond and ion interaction in aqueous environment. However the hydrophobic interaction does not have specificity, which would negatively interfere with the recognition properties (Bures et al., 2011). Thus a considerable reduction for the hydrophobic interaction between the MIP surface and the template is required, which was achieved through the introduction of hydrophilic components and this also would lead to a relatively higher selectivity of the material (Yang et al., 2011).

As we know, fluoropolymers have many unique characteristics such as high hydrophobicity, high thermal and mechanical stability, oil and water-repellency, and very interesting surface properties (Liu et al., 2012). So to reduce nonspecific adsorption and fabricate a rigid structure, 2,2,3,4,4,4-hexafluorobutyl methacrylate (HFBMA) was selected as the main matrix monomer. The fluorine-containing segments, due to their low surface free energy, would be prone to gather on the solid-air interface, forming an excellent protein barrier during application (Ernsting et al., 2007; Kwon et al., 2011; Zhang et al., 2012). Unlike acrylate based polymer as the monomer, the presence of fluorinated groups would lessen the nonspecific adsorption due to protein repulsion behavior. On the other hand, N-methacryloyl-histidine (MAH) was introduced as the functional monomer, which would enlarge the specific binding affinity to the target protein because of electrostatic interactions between MAH and proteins in neutral solutions. As far as we know, there are not related reports on protein imprinted film with the balance of reduced nonspecific adsorption and enhanced specific adsorption based on QCM sensor by now.

In this special system, imprinted films are fabricated in situ on the surface of QCM electrode. Once target molecules are adsorbed into the imprinted cavities, the oscillation frequency decreases. However the oscillation frequency shift usually derives from both mass change on the electrode and viscoelasticity variation at interface. But for a rigid system, the mass change is directly proportional to the frequency shifts and can be estimated more directly and efficiently. As a piezoelectric acoustic sensor, QCM has high sensitivity, but the efficient surface area of the electrode is so small that leads to a relatively low adsorption capacity and compromises the detection sensitivity. Consequently, increasing the specific surface area of sensing materials is a facile approach to further improve the sensitivity of QCM sensors (Zheng et al., 2008). For example, Ding et al. (2011) developed a polyethyleneimine (PEI) modified electrospun polystyrene (PS) (PEI-PS) nanoporous fibers coated QCM sensor to enhance formaldehyde sensing. In our previous work, calcium carbonate nanoparticles were used as the porogen in the preparation of imprinted film and performed excellent porous effect (Zhou et al., 2011). Thus, this method was still utilized to this new material system.

2. Experimental

2.1. Materials

2,2,3,4,4,4-hexafluorobutyl methacrylate (HFBMA, Xeogia Fluorine-Silicon Chemical, Harbin, China), butyl Methacrylate (BMA, Tianjin Chemical Reagent Institute, China) and trimethylolpropane trimethacrylate (TRIM, Tianjin Chemical Reagent Institute, China) was vacuum distilled prior to use. N-methacryloyl-histidine (MAH) was prepared as the reference (Say et al., 2002) and the ^{1}H NMR data are that δ_{ppm}: 5.36 (s, 1H), 5.45 (s, 1H), 6.97, 6.94 (d, 1H), 7.70, 7.68 (d, 1H) and the yield is about 53%. Darocur 1173 was purchased from Ciba Specialty Chemicals Co. Ltd (China). Ribonuclease A (pI=7.8, M_w=13,700) from bovine pancreas and Lysozyme (pI=11.2, M_w=14,300) from chicken egg white were purchased from Beijing Dingguo Biotech. Co. Ltd. (China). Phosphate buffer solution (PBS, pH=7.2, 0.05 M) was purchased from Lianxing Biotech. Co. Ltd. (China). Calcium carbonate nanoparticles (15–40 nm in diameter) were gained from Nanomaterials Technology Co. Ltd. (China).

2.2. QCM apparatus

The Quartz Crystal Microbalance (QCM200) from Stanford Research Systems, Inc. (USA) is composed of Crystal Holder, Oscillator Module, Frequency Counter and PC interface connection for signal output visualization. A 5 MHz AT-cut piezoelectric quartz wafer (2.54 cm in diameter) attached with two gold electrodes (0.40 cm^2 active oscillation area) on both sides was used as the transducer of the QCM biosensor of this study. The crystal was placed in the holder and positioned by two O-rings so that one side of the electrodes was exposed to the sample solution. The measurement chamber formed by the Teflon holder and flow cell was about 150 μL. And the crystal oscillation signal readout was recorded by QCM software installed on a host personal computer.

2.3. Preparation of ribonuclease A imprinted macroporous (porous-HFBMA-MAH-MIP) film

Prior to use, quartz wafer was first rinsed with deionized water and ethanol for three times respectively and dried under gentle nitrogen flow atmosphere. The freshly cleaned chip was dipped into allyl mercaptan solution overnight, the gold electrode was then washed with ethanol and deionized water to remove excess thiols and dried under nitrogen.

To get 4.0 mg/mL calcium carbonate nanoparticles suspension, 200.0 mg calcium carbonate nanoparticles were dispersed into 50.0 mL anhydrous ethanol through high-speed shearing at 5000 rpm for 5 min and ultrasonic vibration for 10 min. Then a clean round glass coverslip (12 mm in diameter and treated with trimethylchlorosilane solution beforehand) was dipped into the nanoparticles suspension, taken out vertically with a steady pace, placed flat and dried at ambient temperature. After 20 times of above operation, the coverslip was covered with a white layer of calcium carbonate nanoparticles. Dropping 50 μL of ribonuclease A phosphate buffer solution (25 mg/mL) onto the nanoparticles layer, the white layer became transparent, indicating the successful infiltration of protein solution. Then the coverslip was subjected to spin-coating at 600 rpm for 18 s and 5000 rpm for 30 s. Twice the dropping and spin-coating operation, then repeat the above procedure with MAH phosphate buffer solution (50 mg/mL). Afterwards, 2 μL of homogeneous mixture of HFBMA (160 μL), TRIM (80 μL) and 1173 (10 μL) was dropped onto the nanoparticles/template/MAH layer. Once again the layer became transparent, and a 5 MHz AT-cut quartz crystal electrode was covered on the top of the polymer precursors. Then the sandwich (glass coverslip/calcium carbonate nanoparticles layer/template protein/MAH/ polymer precursors/quartz crystal electrode) was exposed to UV light (365 nm, 1200 W, 50 cm high, every 30 s UV exposure following by 10 min cooling) to photopolymerize for 1 min under nitrogen atmosphere. After polymerization, the glass coverslip was peeled off and the sandwich (calcium carbonate nanoparticles layer/template protein/MAH/polymer precursors/quartz crystal electrode) was dipped into 2% hydrochloric acid solution for 0.5 h to fully dissolve the calcium carbonate nanoparticles. The obtained complex layer (protein imprinted polymer film/quartz crystal electrode) was washed with sodium chloride solution (3 M) for at least 10 h in order to remove the template completely. The obtained macroporous imprinted film was then