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Quartz crystal microbalance bioaffinity sensor for biotin based on mixed self-assembled monolayers and metastable molecular complex receptor

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Abstract

A quartz crystal microbalance (QCM) sensor was proposed for the detection of small molecule biotin based on the mixed self-assembled monolayer (SAM) of thiols on gold substrate and the bioaffinity difference between an analyte (biotin) and an analogue compound (HABA) in binding avidin. Avidin formed a metastable complex with 2-[(4-hydroxyphenyl)azo]benzoic acid (HABA) immobilized on the crystal surface. When the sensor contacts a sample solution containing biotin, the avidin was released from the sensor surface to form a more stable complex with biotin in solution. The frequency change recorded is proportional to the desorbed mass of avidin, and there is a clear mathematic relationship between the frequency change and the biotin concentration. The use of mixed SAMs allows the stable attachment of bioreceptor molecules on the QCM, and enhances the amount of the immobilized molecules on the QCM, as a longer "space arm" in the mixed SAMs makes this monolayer membrane more accessible to capture the immobilized molecules. The proposed bioaffinity sensor has nice response to biotin in the range of 0.017–1.67 μg/mL. The sensor could be regenerated under very mild conditions simply by reimmersion of the sensor into a biotin solution to desorb the surplus avidin.

Keywords: Quartz crystal microbalance; Bioaffinity sensor; Biotin; Mixed self-assembled monolayer; HABA; Metastable molecular complex

1. Introduction

Biotin is a water-soluble vitamin (Vitamin H), generally classified as a B-complex vitamin, playing an important biochemical role in living cells. Furthermore, biotin is a cofactor for necessary metabolic enzymes, and alterations in its content and the enzymes to which it is attached have been correlated with colorectal cancer and other diseases (Bramwell and Humm, 1992; Cherbonnel-Lasserre et al., 1997). Recent research indicates that biotin is broken down more rapidly during pregnancy and its nutritional status declines during the course of pregnancy. Although marginal biotin deficiency is relatively common in pregnancy (Mock et al., 2002), subclinical biotin deficiency has been shown to cause birth defects in several animal species (Mock, 1999). Thus, it is obvious that diagnosis of biotin deficiency as well as monitoring of biotin levels of patients receiving biotin treatment is very crucial. The detection of biotin is generally performed by use of some methods, such as spectrophotometry, colorimetry, polarography, thin layer chromatography, high-performance liquid chromatography and capillary zone electrophoresis (Green, 1970; Reio, 1970; Ahmed and Verma, 1979). These methods in common use are rather tedious or time-consuming as well as requiring sophisticated instrumentation. Searching some detection systems for biotin with low cost, high sensitivity and simplicity is of considerable interest for the biologic detection.

In recent decades, biosensors with various kinds of detection formats have received rapid development and wide applications, which provide a rapid and convenient alternative to conventional analytical methods for monitoring

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(bio)-chemical substances in various fields, such as medicine, environmental monitoring, fermentation and food processing. Of the various kinds of detection systems, the quartz crystal microbalance (QCM), which possesses some advantages including label- or radiation-free entities, low cost, high sensitivity and simplicity, has been the active subject of investigation. The binding of biomolecules on the QCM surface can be measured by this detection system, which is sensitive to the physical changes that occur on surface when a biomolecule binds. The QCM is an oscillating quartz crystal device whose resonance frequency changes with the change in the mass according to the Sauerbrey (1959) equation:

$$\Delta F = -2.3 \times 10^{-6} \frac{F^2 \Delta M}{A}$$

where ΔF is the change in frequency of the crystal, F the resonant frequency of the crystal, ΔM is the mass deposited on the electrode surface and A is the area of the coated crystal. It has been shown that, though the method is suitable for sensitive measurements in liquids as well as in air, it is rather difficult to measure the binding of small molecules (Bruckenstein and Shay, 1985; Ebersole and Ward, 1988; Ebato et al., 1994; Masson et al., 1995).

Since biotin is a small molecule (molecular weight: 244), it is unlikely that direct binding of biotin to the QCM sensor interface could result in a satisfactory detection limit and sensitivity. One would think to use some macromolecules, such as avidin (molecular weight: 68,000), containing four indentical binding sites to biotin. The binding constant is reported to be ca. $10^{15}\,M^{-1}$ (Florin et al., 1994; Anzai et al., 2000) which is one million times greater than that of the antibody-antigen pair and at the same time their binding is an irreversible process. Owing to the high affinity and stable nature of the resulting complex, the avidin–biotin system is currently widely used to label biomolecules with dyes, fluorophores, enzymes, etc. (Vreeke et al., 1995; Schriemer et al., 1998; Struthers et al., 1998). Moreover, avidin can also bind some compounds similar to biotin, such as desthiobiotin (Masson et al., 1995) and 2-[(4-hydroxyphenyl)azo]benzoic acid (HABA) (Anzai et al., 2000) to form metastable complexes. Thus, in this work, a new sensing principle for QCM based on different bioaffinity among the analyte and an analogue compound is brought forward (vide infra).

In the manufacture of QCM sensor, the techniques for immobilization of the biomaterials play a significant role. The immobilization process not only ensures the intimate contact of the biological entities with the transducer but also aids in the stabilization of the biological system, enhancing its operational and storage stability (Frederix et al., 2003). Conventional methods for biomaterials immobilization on the QCM sensor include the use of protein A (Minunni et al., 1996) or silanisation (Steegborn and Skladal, 1997). Other polymers have also been used, such as polyethyleneimine (Konig and Gratzel, 1993) and plasma-polymerized films (Nakanishi et al., 1996). Here, the biomaterials immobilization is based on the mixed self-assembled monolayers (SAMs) of thiols on gold substrate. One of the thiols in the mixed SAMs carries a functional group to attach the probe molecules. The other thiol compound used for the mixed SAMs construction is known to be used for limiting the non-specific adsorption of undesired biological entities (Wang et al., 1997; Chapman et al., 2000). Moreover, thiol compounds are famous for their stable bond to gold and for their reproducible behavior. On the other hand, the longer “space arm” in the mixed SAMs makes this monolayer membrane more accessible to capture the probe molecules.

Thus, we first formed the mixed SAMs of thiols on gold substrate used for the immobilization of ovalbumin (OA), which was used to immobilize HABA on the QCM sensor due to that the formed OA layer has more binding sites for HABA. Then, when the membrane-bound HABA was incubated in avidin solution, the metastable complexes of HABA and avidin were formed and tightly attached on the QCM. Since HABA shows lower affinity to avidin than the analyte (biotin) does, when the membrane-bound metastable complexes are exposed to the biotin sample solution, the avidin is displaced from the membrane-bound HABA by the biotin to form a stable biotin–avidin complex in solution. Thus, the frequency change should be proportional to the desorbed mass of avidin, and there is a clear mathematic relationship between the frequency change and the biotin concentration. Thus, a QCM sensor based on the different bioaffinity of the analyte comparing to an analogue compound in binding protein was proposed to use for the detection of small molecules like biotin. Moreover, it is noted that the proposed biosensor could be regenerated under very mild conditions simply by reimmersion of the sensor into a biotin solution to desorb the surplus avidin.

2. Experimental

2.1. Apparatus and reagents

The piezoelectric quartz crystals (AT-cut, 9 MHz) were purchased from Chenxing Radio Equipments (Beijing, China), AT-cut quartz wafer with vacuum-deposited gold electrodes with a diameter of 6 mm on both sides. One side of the crystals was sealed with an O-ring of silicone rubber covered by a plastic plate forming an air compartment isolated from the aqueous solution. To stabilize the oscillation frequency in the solution, QCM sealed on one side was mounted in a laboratory-made reaction cell containing 0.1 M phosphate-buffer solution (PBS, pH 7.0, 0.9% sodium chloride) under gently magnetic stirring. The frequency of the resonance crystal was recorded every 100 ms with data acquisition system connected to a computer system (QCA 922 Quartz Crystal Analyzer, SEIKO EG&G Co., Chiba, Japan). For alternating current (ac) impedance measurements, a Model VMP2 Multichannel Potentiostat with Ec-Lab V6.70 and ZsimpWin Version 2.00 software (EG&G Princeton Applied Research, Princeton, NJ, USA)