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A novel electrochemical impedance biosensor based on plasma-polymerized films for detection of biotin

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Abstract. An electrochemical impedance biosensor was proposed for the detection of small molecule biotin based on the plasma-polymerized films (PPF) 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 electrode 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 impedance spectra change recorded is proportional to the desorbed mass of avidin, and there is a clear mathematic relationship between the impedance change and the biotin concentration. The proposed electrochemical impedance bioaffinity sensor has nice response to biotin in the range of 4.8×10^{-9}–5.6×10^{-4} M. The sensor could be regenerated under very mild conditions simply by reimmersion of the sensor into a biotin solution to desorb the surplus avidin.

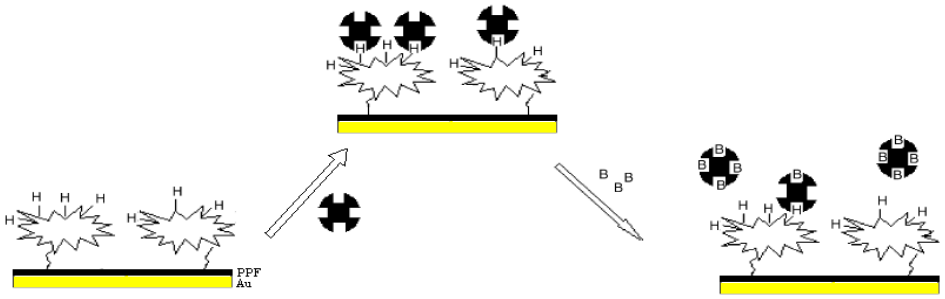
Introduction

Biotin is a vitamin of the B-complex, which plays an important biochemical role in every living cell. In the recent years, the interest in this vitamin has been rekindled, mainly due to its association with serious human disorders, such as the inherited syndrome multiple carboxylase deficiency, which can be successfully treated with biotin administration. The monitoring of biotin levels has some reports [1-3]. However, more or less the reported methods were not satisfactory. Therefore, development of a high sensitivity, unlabeled and low cost method for the direct detection of biotin has become necessary.

Due to the features of simplicity, portability and inexpensiveness, electrochemical detectors could be a useful tool in the biosensing assays [4,5]. As for the construction of an electrochemical biosensor based on the impedance transducer, the first and probably most important step is the immobilization of the recognition elements to provide the sites of analyte–receptor interaction. In order to circumvent the possible interference from electroactive species in the electrolyte solution and reduce the Faradic current, the layer containing the recognition sites should be electrically nonconducting and at the same time not too thick to guarantee a low detection limit.

Plasma-polymer deposition techniques, for the fabrication of the electrochemical impedance biosensor, offer a promising alternative to the conventional coating [6-11]. The plasma-polymerization films (PPFs) with high cross-linking possess mainly following advantages [12,13]: (i) the thickness of film is controllable, nanometer level film can easily be obtained; (ii) they have a strong adhesion to the substrate; (iii) they are homogenous and hole-free; (iv) they are mechanically and chemically stable; (v) many compounds can be used as precursors to deposit PPFs; (vi) PPFs can be deposited on various substrates, such as metals, glasses, Si plate, and organic materials. Such a polymer film seems very suitable for the used in preparation of biosensors.

In this paper, we described a sensitive electrochemical impedance biosensor based on n-butyl PPF for direct detection of D-biotin (VH), a very small molecule of 244.31. The sensing principle is shown in scheme 1. Due to the difference of affinity between biotin and 2-(4-Hydroxyphenylazo)benzoic acid (HABA) with avidin, when biotin was injected in running buffer, the exchange reaction between avidin-HABA complex and biotin would occur and the biotin-avidin complex was washed out into solution. So the interaction of avidin with the biotin leads to the decrease of the dielectric layer and induces an impedance decrease, which can be directly related to the amount of biotin to be quantified. For the detection of VH, alternative current (ac) impedance measurement method is applied.



Scheme 1. Schematic representation of the electrochemical impedance biosensor: , ovalbumin (OA); , avidin; H, HABA and B, biotin.

Experimental

Chemicals and apparatus. D-biotin, avidin, ovalbumin (OA), EDC and Sulfo-NHS were purchased from Sigma. 2-(4-Hydroxyphenylazo)benzoic acid (HABA) was obtained from ACROS. n-butyl (A.R.) was purchased from Changsha Chemical Reagents (Changsha, China). All other reagents and solvents were of analytical reagent grade and the doubly distilled water was used throughout. Phosphate buffer is a 8mM Na_2HPO_4–2mM NaH_2PO_4 solution (PBS) of pH 7.4. Hexacyanoferrate (III) or hexacyanoferrate(II) solutions used in electrochemical system were prepared using PBS of pH 7.4, which was 1mM in both hexacyanoferrate(II) and hexacyanoferrate(III). For alternating current (ac) impedance measurements, a Model VMP2 Multichannel Potentiostat with Ec-Lab V6.70 and ZsimpWin Version 2.0 software (EG&G Princeton Applied Research, Princeton, NJ, USA) was used.

Preparation of the sensor. The n-butyl PPFs were prepared as follows: the clean gold electrode was placed in a stainless reactor with a Microwave Plasma Chemical Vapor Deposition System (MPCDS) (Guowei Hightech limited, Hubei, China). n-butyl was used as a precursor, and pure hydrogen as a carrying gas. During the deposition process, the flow rate of carrying gas was kept at 50 ml min^{-1}, the pressure of the reactor chamber was 160 Pa, and the apparent rf power was 60 W. A covalent attachment of the OA to the surface of the PPF was obtained by glutaraldehyde (GA)[13]. For immobilization of HABA, the OA-modified electrode was incubated in a solution containing 5 mg HABA + 8 mg EDC. During coupling reaction the pH was controlled at 4.5. Then, the electrode was reduced with sodium borohydride under pH control at 7.0 with NaH_2P0_4. The HABA molecules not chemically but physically bound were washed out with 0.1 M carbonate buffer of pH 10 and then kept in storage in 1% ovalbumin solution at pH 7.0 to saturate the nonspecific binding sites. Finally, the HABA-bound electrode was incubated in avidin solution (0.1 M phosphate buffer, pH 7.0) to realize the formation of HABA/avidin metastable complex.

Measurement procedure. The sensor was first incubated in biotin solution (0.1 M phosphate buffer, pH 7.0) under stirring, and then was put into the cell containing 5.0 ml of Phosphate buffer (8mM Na_2HPO_4–2mM NaH_2PO_4 solution pH 7.4, 1 mM Hexacyanoferrate (III) and hexacyanoferrate(II), 0.1 M KCl). The formed gold electrode was as the working electrode, and the SCE as the reference.

Results and Discussion

Characterization of the PPF. To show the presence of amine groups in the PPF, IR reflection spectrum of the film was recorded. The IR spectra indicate that amine groups of different forms are present in the PPF. At the same time, Scanning electron micrograph of the PPF surfaces shows that the PPF surface is quite homogeneous, flat and nonporous (Fig.1). To study the stability of the PPF on the electrode surface, the electrochemical impedance spectroscopy (EIS) was measured after the electrode was immersed in pH 7.4 buffer solutions for different time. The results are shown in Fig. 2, it could be found that the EIS of the modified electrode was not changed obviously during a long time. This indicates that the insulting PPF was stable by the treatment in the buffer solution, which made the usage of the prepared impedance biosensor in buffer solution possible.

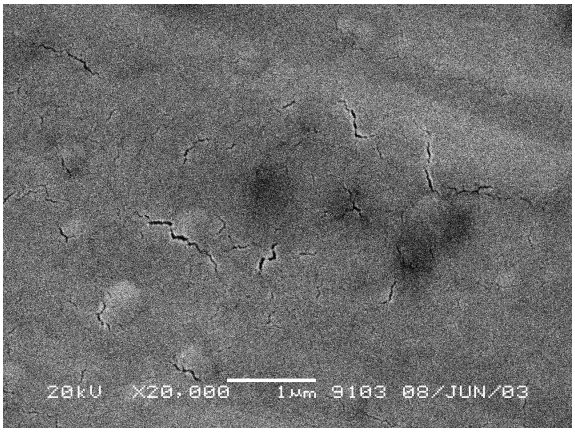


Fig. 1. Scanning electron micrographs of the PPF-coated electrode surface.

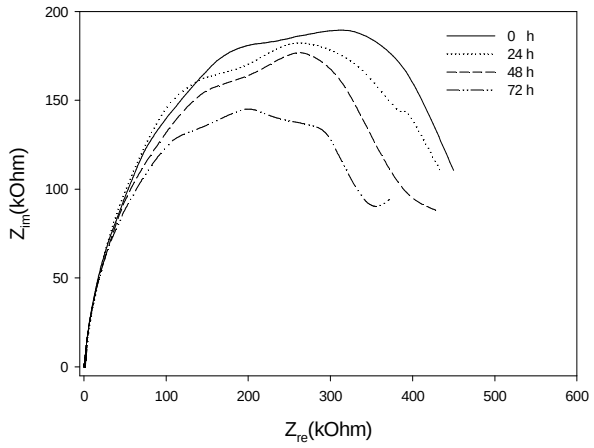


Fig. 2. Electrochemical impedance spectra of the PPF-coated electrode after treatment by using phosphate buffer for different time.

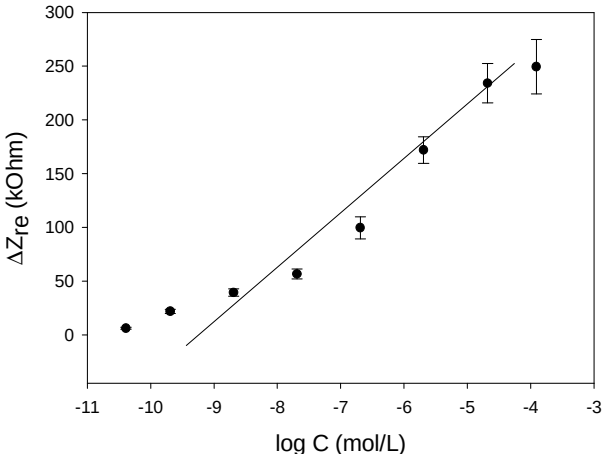


Fig. 3. Calibration curve of the bioaffinity assay with the proposed electrochemical impedance biosensor.

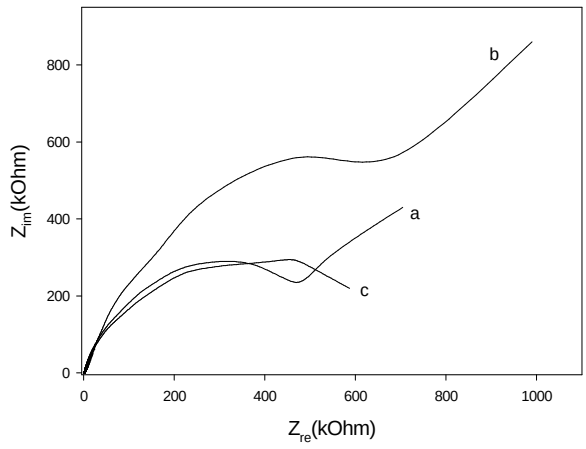


Fig. 4. Electrochemical impedance spectra during the regeneration process of the impedance biosensor.

Determination of biotin by using the impedance biosensor. After a set of affinity-sensing surfaces was prepared, the electrochemical impedance sensor was applied for the determination of the analyte. The results are shown in Fig. 3. The proposed bioaffinity sensor has nice response to biotin in the range of 4.8×10^{-9}–5.6×10^{-4} M. A linear equation ($y = 48.85 \log x + 446$) can be applied with a regression coefficient ($r = 0.987$).

Regeneration of the biosensor. In this work, after each detection, the used biosensor was reimmersed in a biotin solution to desorb the surplus avidin as well as restored the metastable molecular complex in the way mentioned above, and then the regenerated sensor is ready to be used in the next assay. Fig. 4 shows the change of the impedance spectra of the association/displacement reaction. When the HABA-immobilized membrane was incubated in avidin solution, the impedance spectra of interfacial resistance varies from curve a to curve b, however, when the prepared affinity-sensing surfaces was applied for the determination of biotin, due to that avidin was released from the surface to form a more stable complex with biotin in solution, the impedance spectra of interfacial resistance varies again from curve b to curve c (almost same as the cure a). Thus, the proposed electrochemical impedance biosensor can be regenerated via the repetitive association/displacement reaction cycles.

Conclusions

In summary, a novel sensing principle for electrochemical impedance biosensor has been explored to be used for the detection of small molecules biotin based on the plasma-polymerized film and the differences in the bioaffinity between an analyte and an analogue compound in binding protein. The feasibility of impedance measurements of analyte–receptor interaction based on the prepared polymer has been studied. Moreover, the proposed bioaffinity sensor can be regenerated under mild conditions without remarkable loss of activity. This sensor principle seems promising in further application for detection of small molecules with electrochemical impedance sensor.

Acknowledgements

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