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Impedimetric biotin—Immunosensor with excellent analytical performance for real sample analysis

Mai Mai Khoo[a], Khan Loon Ng[a], Yatimah Alias[a,b], Sook Mei Khor[a,b,*]

[a] Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[b] University Malaya Centre for Ionic Liquids (UMCiL), University of Malaya, 50603 Kuala Lumpur, Malaysia.

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ABSTRACT

Development of a simple electrochemical immunosensor, for the direct detection of biotin, is important for monitoring biotin content. A displacement assay was used in biotin detection, in which surface-bound antibodies were dissociated from the surface of immunosensor in the presence of free biotin. Pre-treatment of samples is not required for biotin detection using this electrochemical immunosensor. Using this electrochemical immunosensor, the recoveries of biotin in the two infant formulas A and B were 93.9% and 91.3%, respectively, of their biotin concentrations as stated on their packaging. The direct detection of biotin, with this electrochemical immunosensor, in supplements X, Y, and Z, showed recoveries as high as 92.9%, 106.5%, and 100.0%, respectively. The accuracy of our electrochemical immunosensor was validated with high pressure liquid chromatography (HPLC). The surface of immunosensor had a strong anti-fouling property and high specificity for actual applications in complex matrices. Additionally, the developed immunosensor shows good stability, reproducibility, and intra- and inter-day precision. This electrochemical immunosensor can directly detect biotin in infant formulas, biotin-containing supplements, and serum.

1. Introduction

Biotin is vitamin H or vitamin B_7, which is a primary water-soluble coenzyme in certain carboxylase-mediated metabolisms [1]. Biotin is found in the human serum, plasma and urine [2,3]. Human biotin is essential for cell growth [4], body metabolism [5], healthy skin, hair and nail strength [5] as well as for balanced blood sugar levels [6,7]. Adequate biotin consumption is vital for normal fetal development because even a marginal biotin deficiency during pregnancy can cause teratogenesis in humans [1,3,8]. Therefore, sufficient consumption of biotin is important for human health. The prescribed daily biotin intake for adults and pregnant women is 30 μg, whereas the amount of biotin required for infants (0–5 months) is 5 μg per day [3].

Numerous methods are used to detect biotin, such as microbiological assays, liquid chromatography (LC) with fluorescence detection, high performance liquid chromatography (HPLC) with various detection methods [tandem mass spectrometer (MS/MS), corona-charged aerosol detection (C-CAD), fluorescence detection, or horseradish peroxidase (HRP)-avidin assay], and electrochemical immunoassays with monoclonal antibodies [5,8–10]. Apart from traditional microbiological assays, such as those using *Lactobacillus plantarum*, a new microbiological VitaFast test kit is also available. The VitaFast test kit has several advantages over traditional microbiological assays, such as reduced time of sample preparation and improved reliability, productivity, and accuracy. However, compared with other methods, it is still time-consuming because incubation requires 44–48 h [4]. Chromatographic techniques are highly accurate and sensitive but are limited by high cost, portability of the instrument, the necessity to pre-treat samples, and the need for skilled operators [5,8,10]. Recently, the detection of biotin by electrochemical immunosensors has gained prominence because of their simplicity, low cost, portability, and short analysis time [11]. An electrochemical magneto biosensor which based on a competitive assay, used for biotin determination, has been reported by Kergaravat et al. [12]. However, the electrochemical magneto biosensor has some limitations such as a narrow dynamic range.

In this study, an electrochemical immunosensor with monoclonal antibodies was used to quantitatively detect small organic molecules, such as biotin, in real samples via a displacement assay. The surface of immunosensor was developed as follows: a clean glassy carbon (GC) plate was passivated with aryl diazonium salts, then attached with gold nanoparticles (AuNPs), and fabricated with 1,4-phenyl diamine and sulfo-NHS-biotin (which is a surface-bound epitope), was complexed with a monoclonal anti-biotin IgG antibody. The immunoassay is based

* Corresponding author at: Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
E-mail address: naomikhor@um.edu.my (S.M. Khor).

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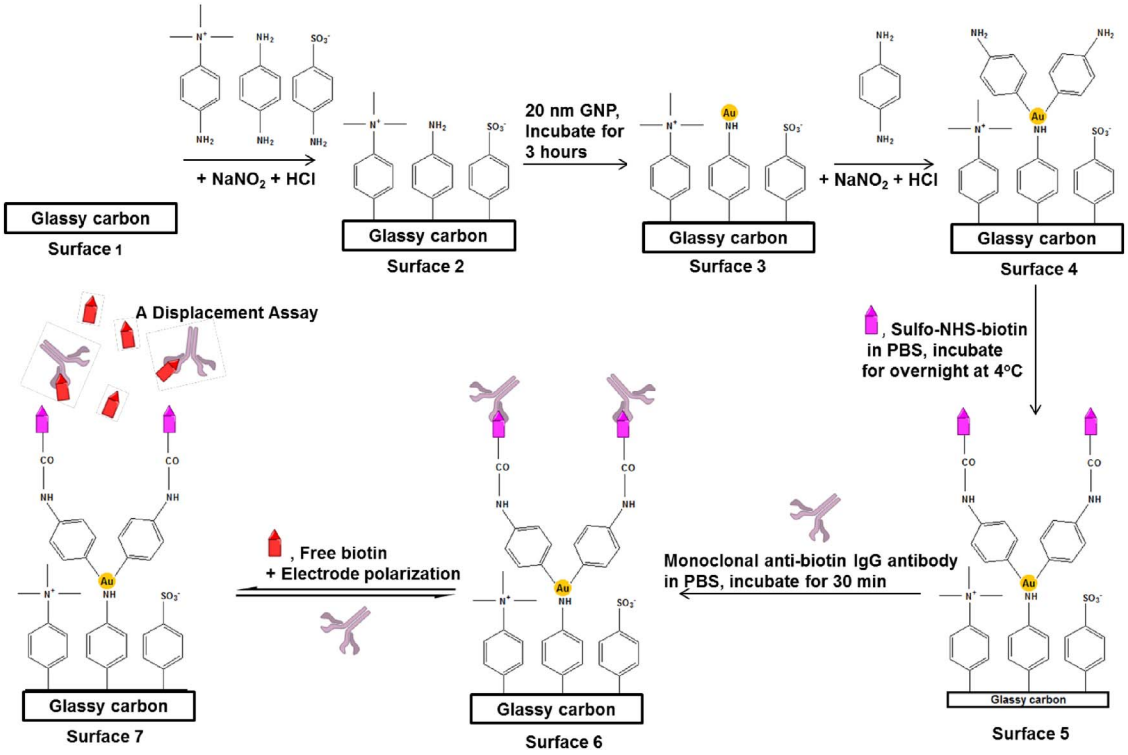

Scheme 1. Schematic representation of fabrication of the surface of an electrochemical immunosensor. Note that the AuNPs is not drawn to scale and is many orders of magnitude larger than the molecules, as drawn.

on the dissociation of the surface-bound antibodies in the presence of free biotin. This dissociation is caused by high binding affinity between the monoclonal anti-biotin IgG antibody and the free biotin present in the sample. Using monoclonal anti-biotin IgG antibodies on the surface of immunosensor can greatly enhance the specificity, selectivity, and sensitivity of the immunosensor towards biotin [13]. The displacement of surface-bound antibodies from the functionalized surface of immunosensor, in the presence of free biotin, was confirmed using frequency response analyzer (FRA) impedance measurements in potassium ferricyanide ($K_3Fe(CN)_6$) solution.

The main challenge of this study was the detection of biotin in real samples such as infant formulas, biotin-containing supplements, and serum. Various types of interference, such as additional nutrients in infant formulas and supplements, as well as proteins in serum, are present in real samples. These present a high probability of non-specific protein-surface absorption, in which a target analyte is hindered from reaching the surface of immunosensor because of interference in the samples. This can affect the sensitivity and selectivity of the electrochemical immunosensor. Therefore, anti-fouling molecules, such as phenyl-SO_3^- (Ph-SO_3^-) and phenyl-$N^+(CH_3)_3$ (Ph-NMe_3^+), with a negative or positive charge, respectively, were used to prevent the non-specific protein-surface absorption in this study. Previously Nadiya et al. developed an electrochemical immunosensor, fabricated using Ph-SO_3^-/Ph-NMe_3^+, which was successfully used for the direct detection of dengue virus non-structural protein 1 (NS1) in dengue virus-infected human serum [14]. To enhance the sensitivity of immunosensor in the detection of biotin in complex matrices, electrode polarization of − 800 mV for 10 min was performed to enhance the dissociation of the bound antibody from the surface-bound epitope in the presence of free biotin via a displacement assay. This improves the selectivity and sensitivity of the electrochemical immunosensor and enables the quantitative detection of biotin in real samples. Therefore, this approach can be used in place of current techniques for the detection of biotin in industries and dispensaries.

2. Materials and methods

2.1. Chemicals, reagents and apparatus

Dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), potassium chloride (KCl), 1,4-phenyl diamine, sodium nitrite ($NaNO_2$), sulfanilic acid, sodium chloride (NaCl), AuNPs (20 nm), monoclonal anti-biotin IgG antibody from goat, cytochrome C (cyt-C), ascorbic acid, uric acid, guanine, and biotin were purchased from Sigma-Aldrich, USA. Potassium ferricyanide ($K_3Fe(CN)_6$), and (4-aminophenyl) trimethylammonium iodide hydrochloride were purchased from Acros, USA. Hydrochloric acid, 99.7% ethanol, sodium hydroxide (NaOH), citric acid, and sucrose were purchased from R & M, UK. Di-sodium hydrogen phosphate dehydrate ($Na_2HPO_4.2H_2O$) and acetonitrile were purchased from Merck, Germany. Sulfo-NHS-biotin was purchased from Thermo Scientific, USA. Purified nitrogen gas was purchased from Linde, Malaysia. Human serum was purchased from Life Technologies Corporation, USA. Bovine serum albumin (BSA) was purchased from Amresco, USA. Micropolish alumina powder (1.0 μm, 0.3 μm, and 0.05 μm) was purchased from Buehler, USA. All reagents were used as received, and all aqueous solutions were prepared using Milli-Q™ water with resistivity of 18.2 MΩ·cm at 25 °C (Millipore, Sydney, Australia). Phosphate buffered saline (PBS) was composed of 0.137 M NaCl and 0.1 M $NaH_2PO_4{\cdot}2H_2O$, and adjusted to pH 7.4. Phosphate buffer solution was prepared using 0.05 M KCl and 0.05 M K_2HPO_4/KH_2PO_4, and adjusted to pH 7.0 with NaOH or HCl solution [13].

2.2. Instrumentation

All electrochemical experiments were conducted using Autolab PGSTAT204 (Metrohm, the Netherlands). GC electrodes (BASi, USA) were used as working electrodes. All potentials were set against an Ag/AgCl reference electrode (3 M KCl); a platinum (Pt) wire was used as the counter electrode. AuNPs-modified surfaces were morphologically