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

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A B S T R A C T
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, 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 B7, 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...