Sensors and Actuators B 257 (2018) 672–677

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Research Paper

# Functional magnetic nanoparticles–assisted electrochemical biosensor for eosinophil cationic protein in cell culture

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ARTICLE INFO

*Article history:*
Received 2 May 2017
Received in revised form 15 October 2017
Accepted 7 November 2017
Available online 7 November 2017

*Keywords:*
Functional magnetic nanoparticles
Eosinophil cationic protein
Biomarker of asthma
Heparin
Electrochemical biosensor

ABSTRACT

A low-cost electrochemical biosensor, assisted by a new type of functional magnetic nanoparticles (NPs), has been developed to detect eosinophil cationic protein (ECP), a known biomarker of asthma, in cell culture. The heparin-modified magnetic NPs were mixed with a sample solution containing ECP. After ECP had been captured by the NPs, a magnetic field was applied behind a graphite-based screen-printed electrode. The functional magnetic NPs were attracted to the electrode, raising the ECP concentration near its surface. Because of the use of the functional magnetic NPs, the difference in the signal was amplified when applying larger sample volumes for detection, thereby enhancing the sensitivity of the biosensor. This approach provided a linear range for the analysis of the logarithm of the ECP concentration from 1 to 1000 nM, with a coefficient of determination 0.992; the limit of detection was 0.30 nM. The fabricated biosensor displayed good recovery in a cell culture medium incubated with the Beas–2 B cell line. The ability to detect the concentration of ECP in a cell culture at any time point should be useful for explaining contradictory findings regarding the relationship between the initial ECP concentration and the cell line.


## 1. Introduction

Eosinophil cationic protein (ECP) is a biomarker for asthma and some other airway diseases [1–3]. It is produced from eosinophils when inflammation or parasitic infection occurs. Most ECP exists in tissue and body fluids, including serum and sputum [3,4]. The ECP concentration in serum can reflect the morbidity of asthma and be used as an indicator for drug doses (e.g., $\beta$-agonists). The common method used for detecting ECP in serum is Immuno CAP. The presence of highly basic residues (e.g., arginine) in the ECP structure results in its having a high isoelectric point (pI) of 10.8 [5–8]. Therefore, ECP bears a large net positive charge at neutral pH and can interact with negatively charged molecules on cell membranes. ECP has been reported [9] to bind specifically with heparin and heparan sulfate, one of the major glycosaminoglycans found on cell membranes. The resulting aggregation leads to ECP cytotoxicity during asthma attacks [10]. Considering this specific binding mechanism, heparin was used in this study as a replacement for heparan sulfate (they have similar structures and specific binding affinities) for the investigations of ECP.

Magnetic nanoparticles (NPs) find applications in various fields [11–15]. Such NPs can readily be positioned in specific areas under the influence of a magnetic field. Iron oxide has been the most common material for fabricating magnetic NPs because of its simple synthesis at low cost. Nevertheless, most magnetic iron oxide NPs suffer from ready oxidation, non-specific adsorption, and cytotoxicity. Coating of their surfaces can overcome these issues [16]. Accordingly, the resulting materials can behave as functional magnetic NPs. In recent years, functional magnetic NPs have been used, for example, in sample pre-concentration and isolation and as drug delivery carriers and MRI monitoring reagents [16–18].

Electrochemical sensors are among the most sensitive in analytical chemistry, providing relatively fast detection, ease of operation, high sensitivity, and low cost compared to methods using spectrometry. In most cases, an electrochemical sensor requires its surface to be modified to ensure recognition ability. Several kinds of molecules, including antibodies, aptamers, and enzymes, have been applied in probe/target strategies [19–25]. Various signal amplification strategies having also been developed [26–29] to further enhance the sensitivity of fabricated electrochemical sensors.

In most of ECP-related cell culture experiments, only the initial ECP concentration and the estimated number of cells have been revealed as experimental information [30–32]. Nevertheless, even at the same ECP concentration, the volume of the cell culture medium used for incubation and the total amount of ECP

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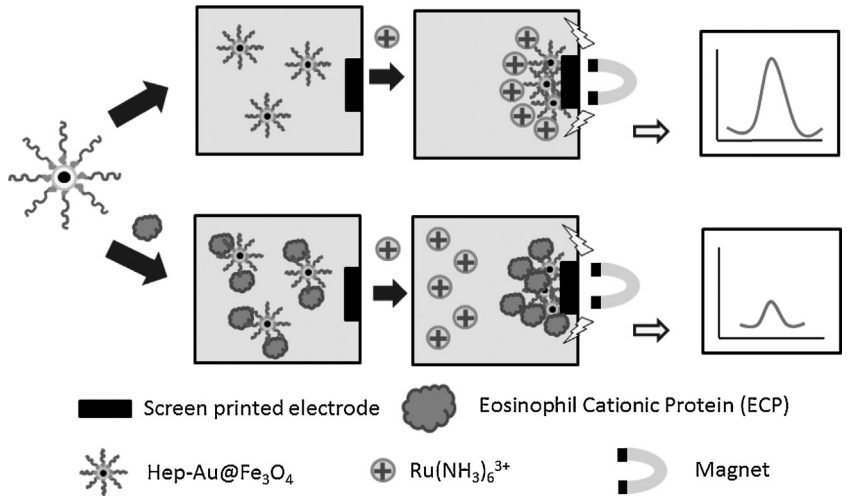
https://doi.org/10.1016/j.snb.2017.11.033
0925-4005/




**Scheme 1.** Cartoon representation of the electrochemical ECP biosensor.

added might be different in experiments performed in different labs. Moreover, the added ECP must bind with heparan sulfate at the cell membrane and then penetrate into the cell to trigger the activity. In chemical or biological kinetic experiments, most discussions of the cell line have been the activity at a target time point usually estimated based on the initial ECP concentration. This approach of estimating the level of activity from the initial ECP concentration may result in misleading conclusions—for example, inconsistencies in the relationship between the initial ECP concentration and the activity in studies of time-dependent cell cultures. Furthermore, knowing the exact amount of ECP that binds and penetrates into cells during incubation can help us understand the effects of ECP on cells. Because inexpensive procedures have been unavailable for real-time monitoring of ECP, the development of cheap, fast, and accurate detection methods for time-dependent cell culture experiments would be welcome.

In this study, an electrochemical biosensor based on new functional magnetic NPs has been developed for measurement of the concentration of ECP in cell cultures at any point in time. This biosensor was constructed using heparin-modified gold-coated magnetic NPs as a probe displaying specific affinity for ECP. Several aspects of the ECP biosensor have been examined, including the production of the heparin-modified gold-coated magnetic NPs; the ECP sample volume required to achieve adequate sensitivity; and the quantitation and recovery.

Scheme 1 presents the strategy employed for the detection of ECP in a cell culture using the biosensor. Cysteamine was used as a linker, covalently bound to the terminus of heparin to avoid significantly changes to heparin's physical or chemical properties. Cysteamine-tagged heparin was then immobilized, through thiol–gold self-assembly, onto the surfaces of gold-coated magnetic NPs. The heparin-modified Au@$Fe_3O_4$ NPs were added into a sample solution containing ECP. The ECP in the sample was adsorbed onto the dispersed heparin-modified Au@$Fe_3O_4$ NPs, which aggregated onto the surface of a screen-printed electrode (SPE) after an external magnetic field had been applied behind it. The SPE was rinsed with DI water and then placed into 10 mM KCl containing 100 μM $Ru(NH_3)_6^{3+}$ (RuHex). In the absence of ECP, the heparin-modified Au@$Fe_3O_4$ NPs, which carried net negative charge, attracted RuHex, leading to a significant signal for RuHex. When ECP was present in the sample solution, ECP bound to the heparin units of the heparin-modified Au@$Fe_3O_4$ NPs. Because ECP carries a net positive charge, it repelled RuHex, which also carries positive charge, leading to a decrease in the intensity of the RuHex signal. The different in the currents for the sample solutions in the absence and presence of ECP could be used to estimate the concentration of ECP in the sample. A higher concentration of ECP would cover more of the surface of the heparin-modified Au@$Fe_3O_4$ NPs, thereby decreasing the signal of RuHex. Because of the pre-concentration effect of the heparin-modified Au@$Fe_3O_4$ NPs, most of the ECP molecules in the sample solution were indirectly attracted to the SPE surface, thereby amplifying the sensitivity of the assay.

## 2. Materials and methods

### 2.1. Chemicals and reagents

All chemicals were of reagent grade or higher. All stock and working solutions were prepared using Milli-Q de-ionized (DI) water (Millipore, Bedford, MA, USA) having a measured resistance of 18.2 MΩ·cm. Sodium chloride was obtained from Fluka (St. Louis, MO, USA). Potassium chloride, hydrochloric acid, acetic acid, and sodium cyanoborohydride were purchased from J. T. Baker (Phillipsburg, NJ, USA). Sodium dihydrogen phosphate anhydrous was obtained from Showa (Tokyo, Japan). Disodium hydrogen phosphate anhydrous was purchased from Scharlau (Sentmenat, German). Hydrogen tetrachloroaurate trihydrate, tris(2-carboxyethyl)phosphine hydrochloride, hexaammineruthenium chloride, 2-mercaptoethylamine hydrochloride (cysteamine), heparin sodium salt, isopropanol, and RPMI 1640 with 10% fetal bovine serum (FBS) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Potassium ferricyanide was purchased from Riedel–de Haën (Seelze, Germany). ECP was kindly provided by the laboratory of Prof. Margaret Dah-Tsyr Chang (National Tsing Hua Univ., Hsinchu, Taiwan). Disposable graphite-based SPEs were obtained from Zensor (Taichung, Taiwan).

### 2.2. Apparatus

Square-wave voltammetry (SWV), cyclic voltammetry (CV), and amperometry were performed using a CHI 8021 B electrochemical analyzer (CH Instruments, Austin, TX, USA). Electrochemical impedance spectroscopy (EIS) was performed using a CHI 619E electrochemical analyzer (CH Instruments, Austin, TX, USA). Chronocoulometry (CC) was performed using a CHI 8121 B electrochemical analyzer (CH Instruments, Austin, TX, USA). A three-electrode system was used, with Ag/AgCl as the reference electrode, a platinum wire as the auxiliary electrode, and an SPE-based electrode as the working electrode. Field emission scanning