Novel ultrasensitive immunosensor based on magnetic particles for direct detection of transferrin in blood

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ABSTRACT

A novel immunosensor for transferrin (Tf) detection in blood samples has been developed. The stable layer of carbon-encapsulated magnetic nanoparticles containing carboxylic groups anchored with the monoclonal antibody ensured good electric contact for direct electron exchange between transferrin and the electrode surface. The interactions of the immobilized antibody with transferrin (at various concentration) were monitored via various responses, i.e.: voltammetric (reduction current of Tf), gravimetric (amplitude of frequency oscillations) and impedance (charge transfer resistance). For the determination of transferrin in blood samples the best procedure is the gravimetric one that gave the highest sensitivity. The obtained results show excellent linear response in the Tf concentration range between 5×10^{-7} and 5×10^{-2} g dL^{-1} ($5 \times 10^{-3} \div 500$ μg mL^{-1}) with the detection limit of ca. 12.0 ± 1.8, 15.0 ± 2.4 and 24.0 ± 5.2 ng dL^{-1} for gravimetric, impedance and voltammetric analysis, respectively. The functionality of the electrochemical immunosensor has been also demonstrated during the analysis of the Tf level in rat blood samples.

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1. Introduction

Transferrin (Tf) belongs to the transferrin family of evolutionarily related iron-binding proteins [1]. In contrast to heme-containing iron-binding proteins such as hemoglobin and myoglobin, transferrin is classified as a non-heme iron-binding protein, which facilitates the iron uptake in different cells and tissues [2,3]. In humans, transferrin is mainly synthesized in the liver and secreted into the blood and plays a key role as an iron-transporter molecule from the liver [4], intestine and reticuloendothelial cells to tissues requiring this essential metal in different physiological processes [5]. In normal human serum, the concentration of transferrin is between 0.20 and 0.38 g dL^{-1} and is usually about one-third saturated with iron, thus providing an efficient buffering capacity in case of a large body of increase in blood iron levels [6]. One molecule of transferrin can bind two ferric irons [7]. The studies evidence that transferrin is also involved in the immune defenses mainly via its ability to bind to iron ions required by some microorganisms [8].

Transferrin is a well-established biomarker in different human pathologies and the human recombined transferrin is clinically applied in many diseases as thalassemia, ischemia-reperfusion injury, bacterial infections and diabetes. Therefore, it is suggested that a direct assay of this glycoprotein in the blood and cells could have a potential diagnostic value in the measurement of erythron activity and the body iron deposits [9]. For example, the level of transferrin in blood serum is linked to iron-overload states which is accompanied in such diseases as hereditary hemochromatosis, hypotransferrinemia, atransferrinemia, transfusion siderosis and ferroportin-associated disorders [10]. The clinical value of serum transferrin and transferrin receptor measurements was evidenced in anemic persons reflecting iron deficiencies in such pathologies [11]. In other words, the assay of serum transferrin is a valuable diagnostic parameter in iron-associated diseases in humans including some pre-neoplastic and neoplastic states, therefore, a much more sensitive and precise analytical tools such as immunosensors are really needed to be developed and determine the discrete levels of transferrin in both normal and cancer cells. Please note that some immunosensor-based assays are of especially interest in modern nanomedicine because of their potential application as specific and direct non-invasive detection tools and their simplicity and accuracy compared with standard biochemical or molecular techniques.

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In recent years, a great deal of attention has been especially paid to develop so-called target electrochemical immunosensors composed of nanoparticles that were applied to immobilize specific polyclonal and/or monoclonal antibodies against selected antigens including transferrin [12–14]. Compared with the capacitive immunoassay used to data, the nanomaterial-based immunosensors presented a lower detection limit and a wider linear response range. However, the major challenge of the modern design of immunosensors is still an open debate to properly combine the specific antibody on the surface of the working electrode to get the proper electrochemical signal addressing to discrete antigen levels in comprehensive biological matrices such as human cells, serum and some intracellular and extracellular fluids. Because the key component of any immunosensors is the antibody-recognition layer, the nanoparticle surface, on which this antibody-recognition layer is deposited, is also of crucial importance in order to obtain a highly sensitive and selective diagnostic electrochemical platform. In other recent studies, a highly sensitive impedance immunosensor based on gold nanoparticles and multiwall carbon nanotube-ionic liquid electrode was developed and decorated with monoclonal antibodies (Herceptin) recognizing the tyrosine kinase receptor [15]. Interestingly, a new electrochemical immunosensor was recently constructed to detect some amyloid beta (Aβ) (1–40) peptides as biomarkers of Alzheimer's disease [16]. To date, a number of excellent review papers have been recently published on how to construct and modify different nanoparticles with further applications in the development of electrochemical immunosensors [17–19]. The carbon-encapsulated iron nanoparticles (Fe@C Nps) have been shown to be a well-designed nanoplatform in modern electrochemical analysis and have been successfully used in our bioassays of hemoglobin and ceruloplasmin in human and animal blood samples [20,21].

In this study, we have developed, to the best of our knowledge, for the first time, a novel type of electrochemical immunosensor based on Fe@C Nps decorated with monoclonal antibodies recognizing human transferrin glycoprotein. We proved that the control of magnetic properties of Tf and the application of magnetic nanoparticles (Fe@C-COOH Nps) as the electrode modifier and the use of external magnetic field lead to the direct electron transfer between the Tf protein attached to the sensing layer via protein-antibody interaction. Additionally we know that the cooperation of magnetic field with ferromagnetic nanoparticles enabled to control the protein conformation and in consequence its electroactivity [22]. The carbon-encapsulated magnetic nanoparticles were attached to the gold surface via covalent bonding with the cysteamine monolayer. The results of this study present a clear evidence that such modification allows the direct electron exchange between the protein and the electrode surface without participation of any mediator. To characterize the proposed Tf immunosensor various techniques were applied: voltammetry, electrochemical quartz crystal microbalance (EQCM), surface plasmon resonance spectroscopy (SPR) and electrochemical impedance spectroscopy (EIS).

2. Experimental section

2.1. Reagents and materials

All chemicals were of the highest available purity. Human transferrin (Tf), cysteamine (CSH), polyethylene glycol sorbitan monolaurate (Tween-20), KH_2PO_4, K_2HPO_4, K_2SO_4, N-hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich and used as received. Monoclonal anti-human transferrin-IgG1 (Ab) was purchased from ThermoFisher. All experiments and solutions were done in 0.02 M phosphate buffer (PB; pH 7.40) obtained by mixing of KH_2PO_4, K_2HPO_4 with a subsequent addition of 0.15 M K_2SO_4 and Tween-20 (0.2% v/v). To prepare all solutions the ultrapure water (Hydrolab, conductivity 0.056 μS cm^{-1}) was used. The ferromagnetic electrode modifier, carbon-encapsulated iron nanoparticles functionalized with carboxylic acid groups (Fe@C-COOH Nps; mean diameter 65 nm), was synthesized according to the procedure described and characterized in elsewhere [23,24].

2.2. Electrochemical measurements

Voltammetry, electrochemical impedance spectroscopy (EIS) and electrochemical quartz crystal microbalance (EQCM) measurements were performed using an Autolab (Eco Chemie B.V., Utrecht, Netherlands), model PGSTAT 12 potentiostat equipped with an ECD amplifier module (noise-filter RC time settings: 0 s for scan rates >10 mV/s and 0.1 s for scan rates <10 mV/s). All electrochemical experiments were done in the three-electrode system in which: the 6-MHz Au/TiO$_2$ quartz crystal resonators ($A_{Au-EQCM}$ = 0.495 cm^2), gold wire and double junction Ag/AgCl/3 M KCl/0.1 M KNO$_3$ (abbreviation: Ag/AgCl) electrode were used as working-, counter- and reference electrode, respectively. A large bar neodymium magnet (Fe$_{14}$Nd$_2$B; 40 × 40 × 15 mm) placed under the cell was applied as a source of an external magnetic field. The presence of the magnet did not influence the work of the quartz crystal microbalance.

Before the modification, the gold crystals were electrochemically cleaned by voltammetric cycling: first, between 0 V and 1.8 V (with a 10-s scan stop at 1.8 V) in 0.1 M NaOH and with the scan rate of 100 mV/s, and then between −0.3 and 1.5 V (vs Ag/AgCl/3 M KCl/0.1 M KNO$_3$) in 0.1 M H$_2$SO$_4$ solution until a stable voltammogram, typical for a clean gold electrode, was observed. Finally, the electrode was activated by cycling in 0.1 M HClO$_4$ in the potential range −0.65 ÷ 0.95 V with the relatively high scan rate of 1.2 V s^{-1}. All solutions before and during measurements were degassed with pure argon to remove oxygen.

2.3. Surface plasmon resonance measurements

Surface plasmon resonance (SPR) measurements were carried out at 23 ± 2 °C on a Autolab Springle (Metrohm Autolab B.V., Netherlands) system on glass slides covered with 40 nm of gold
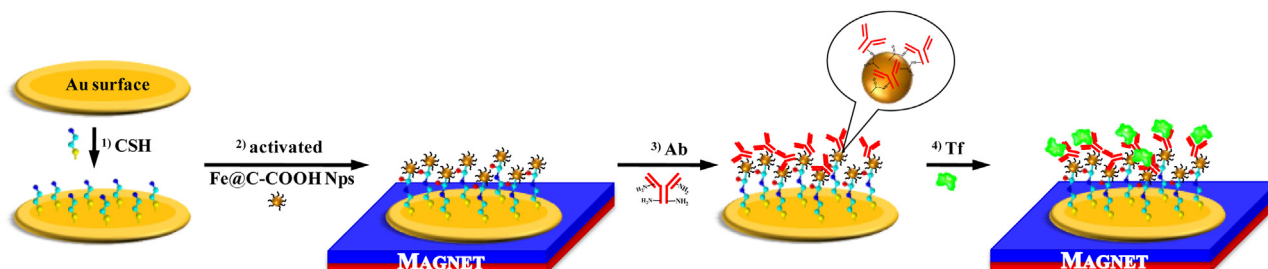
Scheme 1. Scheme of the construction of Tf immunosensor.