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ScienceDirect
Analytical Biochemistry 356 (2006) 208–214
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

# Application of electrochemical impedance spectroscopy for monitoring allergen–antibody reactions using gold nanoparticle-based biomolecular immobilization method

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Received 29 March 2006
Available online 5 June 2006

**Abstract**

Gold nanoparticles were used to enhance the immobilization amount and retain the immunoactivity of recombinant dust mite allergen Der f2 immobilized on a glassy carbon electrode (GCE). The interaction between allergen and antibody was studied by electrochemical impedance spectroscopy (EIS). Self-assembled Au colloid layer (ø = 16 nm) deposited on (3-mercaptopropyl)trimethoxysilane (MPTS)-modified GCE offered a basis to control the immobilization of allergen Der f2. The impedance measurements were based on the charge transfer kinetics of the $[Fe(CN)_6]^{3-/4-}$ redox pair, compared with bare GCE, the immobilization of allergen Der f2 and the allergen–antibody interaction that occurred on the electrode surface altered the interfacial electron transfer resistance and thereby slowed down the charge transfer kinetics by reducing the active area of the electrode or by preventing the redox species in electrolyte solution from approaching the electrode. The interactions of allergen with various concentrations of monoclonal antibody were also monitored through the change of impedance response. The results showed that the electron transfer resistance increased with increasing concentrations of monoclonal antibody.


*Keywords:* Gold nanoparticles; Recombinant allergen; Antibody; Electron transfer resistance ($R_{CT}$); Electrochemical impedance spectroscopy (EIS)

Epidemiological studies have revealed that 10 to 40% of the populations from industrialized countries suffer from allergy diseases, including asthma, allergic rhinitis, and atopic dermatitis [1]. And type I allergic reaction, an immune disorder initiated by inhalant allergens, represents a health problem for approximately 30% of adults and for up to 40% of children in Western countries [2]. This allergic response results in the release of biological mediators (as histamine and leukotrienes) due to the crosslinking of cell-bound immunoglobulin E (IgE)[1] antibodies by allergen. Therefore, during recent years, the allergen-specific IgE antibodies have received significant attention both in industry and in academia. They serve as probes in allergen identification and characterization based on the allergen-specific IgE interaction [3,4]. Besides the IgE antibodies, there are also the productions of immunoglobulin G (IgG) antibodies during specific allergen immunotherapy or immunizing animals for monoclonal antibodies. These IgG antibodies are of high affinity to allergen and may act as blocking antibodies interfering with the allergen–IgE interaction [5–7]. But the role of IgG antibodies in allergic reactions has remained controversial [8,9].

Immunoassay is based on a specific interaction between an antigen and a complementary antibody. With the extension of the application field of electrochemical analysis, there has been a growing interest in the

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[1] *Abbreviations used:* IgE, immunoglobulin E; IgG, immunoglobulin G; SPR, surface plasmon resonance; SERS, surface enhanced Raman scattering; AFP, α-1-fetoprotein; EIS, electrochemical impedance spectroscopy; GCE, glassy carbon electrode; MPTS, (3-mercaptopropyl)trimethoxysilane; PBS, phosphate buffer solution; TEM, transmission electron microscopy.

0003-2697/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2006.05.021

development of electrochemical immunosensors for biochemical analysis, clinical diagnosis, and environmental monitoring [10–12]. The crucial aspect of construction of an immunosensor is the deposition of antigen or antibody molecules in high amounts with the retention of their immunoactivity. For these reasons, many different immobilization procedures, such as physiosorption, covalent attachment, and polymer entrapment, have been investigated [13–15]. However, most of the current methods for the immobilization of biological molecules still have some problems, such as low loading capacity and partial loss of bioactivity, resulting in depressed detection sensitivity [16].

Gold nanoparticles have been used in nonenzyme immunoassay and label technologies for their friendly biocompatibility with antibody, antigen, enzyme, and DNA [17–20]. The utility of gold nanoparticles as an intermediator to immobilize antibody or antigen without the loss of its activity has been recognized since the early 1980s [21]. The monolayers of colloidal gold particles have been successfully coated on quartz or glass slides with the aim of serving as bases for biosensing devices such as surface plasmon resonance (SPR) and surface enhanced Raman scattering (SERS) [22,23]. Tang and coworkers [24] successfully constructed a novel potentiometric immunosensor for the detection of hepatitis B surface antigen by immobilizing hepatitis B antibody on a platinum disk electrode based on gold nanoparticles and other immobilization matrices. They proved that this gold nanoparticle-based biomolecular immobilization method allowed for antibodies immobilized with a higher loading amount and better retained immunoactivity compared with the traditional glutaraldehyde crosslinking procedure. The fabrication of amperometric immunosensor for rapid determination of α-1-fetoprotein (AFP) in human serum was achieved recently by Zhuo and coworkers [25], who also used gold nanoparticles layer for the immobilization of AFP antibody. Wang and coworkers [26] used the same method to enhance antibody immobilization through colloid–Au layer self-assembled on 4-aminothiophenol-modified gold electrode.

The conventional methods applied in determination of allergen–antibody interaction were immunoadsorbent techniques that may lack sensitivity due to the background effects and the partial loss activity of the immobilized proteins [27–29]. Recently, the preparation of allergen microarrays [30–32] for screening of allergen-specific IgE were reported based on the detection of the intensity of fluorescence or chemiluminescence. In the current article, a simple route for the immobilization of recombinant dust mite allergen Der f2 is provided by using gold nanoparticles as immobilization intermediators. The immobilization of allergen Der f2 and the interaction of allergen with murine monoclonal antibodies are monitored by electrochemical impedance spectroscopy (EIS).

## Materials and methods

### *Reagents and materials*

Recombinant dust mite allergen Der f2 and murine monoclonal antibody were kindly provided by Zhigang Liu (Shenzhen University, China). (3-Mercaptopropyl)trimethoxysilane (MPTS, 95%) was purchased from Fluka, and trisodium citrate and $HAuCl_4 \cdot 3H_2O$ were purchased from Aldrich. $K_4[Fe(CN)_6]$, $K_3[Fe(CN)_6]$, phosphate buffer solution (PBS, pH 7.4), and other chemicals used were of analytical grade. All commercially obtained chemicals were used as received without further purification. All aqueous solutions were made using double distilled water, which was further purified to 18.2 MΩ with a 0.22-μm Millipore syringe filter.

### *Apparatus*

Cyclic voltammetry and electrochemical impedance measurements were performed at room temperature on an Autolab PG30 electrochemical analyzer (Eco Chemie, The Netherlands). A three-electrode setup was employed with an Ag/AgCl (saturated KCl) reference electrode, a pt flag as the counter electrode, and a modified glassy carbon electrode (GCE) as the working electrode. The impedance spectra were recorded within the frequency range of $10^{-1}$ to $10^5$ Hz. The amplitude of the applied sine wave potential in each case was 5 mV, whereas the direct current (dc) potential was limited at the formal potential of the redox pair $[Fe(CN)_6]^{3-/4-}$ (0.23 V vs. Ag/AgCl). The electrolyte solution was 2.5 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ in 20 mM PBS (pH 7.4) containing 0.1 M KCl. Prior to each experiment, the electrolyte solution was bubbled with high-purity nitrogen for 20 min to remove dissolved oxygen.

### *Preparation of gold nanoparticles*

Gold nanoparticles used in this study were prepared by citrate reduction of $HAuCl_4$ in aqueous solution. All glasswares used were thoroughly cleaned in aqua regia (HCl–$HNO_3$, 3:1), rinsed in triply distilled water, and oven-dried prior to use. Then 25 ml of 0.01% $HAuCl_4$ aqueous solution was brought to boiling, and 0.45 ml of 1% sodium citrate solution was quickly added under stirring. The solution turned to red in 1 min and was kept boiling for 10 min before the heater was removed. The solution of prepared Au colloid suspension was stored in a brown glass bottle at 4 °C before use.

### *Stepwise modification of GCE*

GCE was mechanically polished with 1.0, 0.3, and 0.05 μm α-$Al_2O_3$ powder successively and was washed ultrasonically in distilled water. At first, the cleaned electrode was applied to 1.5 V for 3 min in 0.1 M NaOH, rinsed with water, and dried under a high-purity nitrogen