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Magnetic beads-based electrochemical immunosensor for monitoring allergenic food proteins

Michaela Čadková [a], Radovan Metelka [b], Lucie Holubová [a], Daniel Horák [c], Veronika Dvořáková [a], Zuzana Bílková [a], Lucie Korecká [a,*]

[a] Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, 532 10 Pardubice, Czech Republic
[b] Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, 532 10 Pardubice, Czech Republic
[c] Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, 162 06 Prague, Czech Republic

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ABSTRACT

Screen-printed platinum electrodes as transducer and magnetic beads as solid phase were combined to develop a particle-based electrochemical immunosensor for monitoring the serious food allergen ovalbumin. The standard arrangement of enzyme-linked immunosorbent assay became the basis for designing the immunosensor. A sandwich-type immunocomplex was formed between magnetic particles functionalized with specific anti-ovalbumin immunoglobulin G and captured ovalbumin molecules, and secondary anti-ovalbumin antibodies conjugated with the enzyme horseradish peroxidase were subsequently added as label tag. The electrochemical signal proportional to the enzymatic reaction of horseradish peroxidase during the reduction of hydrogen peroxide with thionine as electron mediator was measured by linear sweep voltammetry. The newly established method of ovalbumin detection exhibits high sensitivity suitable for quantification in the range of 11 to 222 nM and a detection limit of 5 nM. Magnetic beads-based assay format using external magnets for rapid and simple separation has been proven to be an excellent basis for electrochemical detection and quantification of food allergens in highly complex sample matrices.

Adverse food reaction, including food intolerance or allergy, is a broad term referring to any abnormal clinical response associated with ingestion of a food [1] and results in life-threatening reactions [2]. One of the most serious allergic reactions to foods is egg hypersensitivity in children caused by the major allergens ovalbumin (OVA)[1] and ovomucoid [1,3–9].

Ovalbumin, a water-soluble phosphoglycoprotein with molecular mass of approximately 45 kDa [7,8,10], is often present in various foods as an emulsifying and foaming agent [11]. A rapid and sensitive ovalbumin detection method, therefore, would be of great value. Methods commonly used for ovalbumin determination consist of laborious, time-consuming, and often expensive procedures [8,11,12] such as radioimmunoelectrophoresis [10,13,14], immunoblotting [15], competitive immunoassay [16], Western blot [17], and enzyme-linked immunosorbent assay (ELISA) [18–20]. An element common to all of the aforementioned methods is the use of specific antibodies for selective capture of the target antigen. Such traditional methods also are instrumentally challenging and must be performed by analytical experts in fully equipped laboratories. Combinations of immunochemical and electrochemical methods are becoming more attractive today, mainly for their wide accessibility, ease of implementation, and ability to perform measurements even outside of laboratories.

The great advantage of electrochemical methods is the possibility they offer to use screen-printed sensors, characterized by factors such as low-cost fabrication, low sample consumption, and possible surface modification [21,22]. The electrode surface used as a solid phase for incubation of target antigen and transduction of the electrochemical signal has certain restrictions consisting in a limited number of accessible recognizing biomolecules, its influence on the kinetics of the antibody–antigen reaction, and the possibility of electrode defects caused by washing steps [23]. All of

* Corresponding author. Fax: +420 466 037 068.
E-mail address: lucie.korecka@upce.cz (L. Korecká).

[1] *Abbreviations used:* OVA, ovalbumin; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; sulfo-NHS, *N*-hydroxysulfosuccinimide sodium salt; BSA, bovine serum albumin; OPD, *o*-phenylenediamine; Mes, 2-morpholinoethane-1-sulfonic acid; HRP, horseradish peroxidase; anti-OVA^{HRP}, anti-ovalbumin IgG antibodies labeled with horseradish peroxidase; p(GMA-MOEAA)-NH_2, poly[glycidylmethacrylate-(methacryloyloxy)ethoxy]acetic acid)-NH_2; HA, hyaluronic acid; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; LSV, linear sweep voltammetry.

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these drawbacks could be overcome by the use of magnetic beads [21,23–25] having large specific surface areas and functional groups for the attachment of various ligands [26]. Magnetic particles with all of these advantages already have been successfully coupled with electrochemical sensors [21,23–25].

To date, there have been only a few published articles devoted to electrochemical detection of the allergen ovalbumin. These studies used different analytical approaches enabling demonstration of the presence or even quantification of the desired allergen in biological samples such as by using a specific electrode modification with concanavalin A in combination with an aminoferrocene mediator [27], a covalently bound ovalbumin antibody with 4-carboxyphenyl film [28], peptides labeled with the electroactive compound daunomycin [29], pulsed electrochemical detection on a rotating gold electrode after high-performance liquid chromatography separation [30], and a reagentless immunosensor based on a multifunctional conjugated quinone-type copolymer [31]. Kuramitz and coworkers [32] used hydrodynamic voltammetry with a rotating disc electrode for detecting ovalbumin captured by magnetic particles modified by anti-ovalbumin immunoglobulin G (IgG) molecules.

We present here a selective and sensitive magnetic beads-based electrochemical immunosensor for rapid assay of ovalbumin using screen-printed platinum electrodes. The novelty of the current work lies in its combining the advantages of magnetic separation, selectivity of specific antibodies, and sensitivity of electrochemical detection. All of this is performed without the need for any additional preanalytical steps. To achieve sufficient shuttling of electrons between the substrate and redox center of the enzyme [33], an electron mediator could be used. We selected thionine for this purpose. Moreover, this approach avoids prolonged preparation steps (e.g., direct grafting of the electrodes with ovalbumin [31]) or other various modifications [27,29].

Materials and methods

Chemicals

Albumin from chicken egg (OVA), Oligo-HA4, thionine acetate, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), *N*-hydroxysulfosuccinimide sodium salt (sulfo-NHS), hydrogen peroxide, bovine serum albumin (BSA), *o*-phenylenediamine (OPD), 2-morpholinoethane-1-sulfonic acid (Mes), and Tween 20 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Horseradish peroxidase (HRP, 800 IU/mg) was provided by Fluka (Buchs, Switzerland). Affinity purified rabbit monoclonal anti-OVA IgG antibodies and secondary rabbit polyclonal anti-OVA IgG antibodies labeled with HRP (anti-OVA^{HRP}) were obtained from Patricell (Nottingham, UK). Precision Plus Protein unstained standard 10 to 250 kDa was a product of Bio-Rad (Hercules, CA, USA). Sera-Mag Double Speed magnetic carboxylate-modified microparticles (0.771 μm in diameter) were supplied by Thermo Fisher Scientific (Indianapolis, IN, USA), and poly[glycidylmethacrylate-(methacryloyloxy)ethoxy]acetic acid)-NH_2 (p(GMA-MOEAA)-NH_2) magnetic particles (4.5 μm in diameter) were kindly provided by the Institute of Macromolecular Chemistry (Academy of Sciences of the Czech Republic, Prague, Czech Republic) [34,35]. All other chemicals were supplied by Sigma–Aldrich or Penta (Chrudim, Czech Republic) and were of reagent grade.

Apparatus

All electrochemical measurements were performed with a PalmSens compact electrochemical sensor interface (PalmSens, Utrecht, Netherlands) connected to screen-printed three-electrode sensors (BST-120) composed of working and auxiliary electrodes made of platinum and Ag/AgCl pseudo-reference electrode (Bio Sensor Technology, Berlin, Germany).

Immobilization of anti-OVA antibodies on carboxylate-modified magnetic microparticles

The two kinds of magnetic particles—p(GMA-MOEAA)-NH_2 coated with hyaluronic acid (HA) [36] and commercially available carboxylate-modified Sera-Mag magnetic particles—were exploited for covalent coupling of specific antibodies using a slightly modified two-step carbodiimide method and with EDC as zero-length cross-linker and sulfo-NHS according to Hermanson [37]. Here, 1 mg of magnetic particles was washed five times with 50 mM Mes buffer (pH 5.0). EDC (2 mg) and sulfo-NHS (2.2 mg) were then dissolved in 500 μl of Mes buffer, and the reaction mixture was stirred for 30 min at room temperature. Particles were then washed two times with 0.5 M Mes buffer with the addition of 100 μg of affinity-purified monoclonal anti-OVA antibodies (IgG) dissolved in 1 ml of 50 mM Mes buffer in the final step. This was incubated overnight at 4 °C (with gentle mixing) and then washed three times with Mes buffer, followed by four times with 0.1 M phosphate buffer (pH 7.3). Immobilization efficiency was estimated using Tris/glycine sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).

Immobilization of HRP on Sera-Mag microparticles

Similarly, the modified common carbodiimide method described by Hermanson [37] was used for covalent immobilization of HRP onto carboxylate-modified Sera-Mag magnetic particles. Here, 1 mg of magnetic particles was washed five times with 0.1 M phosphate buffer (pH 7.3) and activated by 7.5 mg of EDC and 1.25 mg of sulfo-NHS dissolved in 1 ml of phosphate buffer for 10 min at room temperature with gentle mixing. After removing the supernatant, 1.5 mg of HRP in 1 ml of phosphate buffer was added. After overnight incubation at 4 °C with gentle mixing, the magnetic particles were washed with phosphate buffer five times. The activity of immobilized HRP was determined as published previously [38] with slight modification. Here, 50 μg of magnetic beads with immobilized HRP was incubated with substrate solution (5 mg OPD with 5 μl of 30% hydrogen peroxide in 10 ml of 0.1 M phosphate buffer, pH 6.2) protected from light using gentle mixing for 10 min at 37 °C. This was followed by spectrophotometric measurement of 100 μl of supernatant in a microwell plate at 492 nm.

Sandwich-type immunocomplex formation

Immunocomplex formation was performed as the mixture of OVA and Sera-Mag with immobilized monoclonal anti-OVA antibodies in molar ratio 2:1 (OVA/anti-OVA) within 0.1 M phosphate buffer (pH 7.0) was incubated for 1 h at room temperature under gentle mixing. It was then washed three times with phosphate buffer. Afterward, secondary anti-OVA^{HRP} IgG in 0.1 M carbonate buffer (pH 9.49) (1:10,000, v/v) with 0.1% BSA and 0.05% Tween 20 was added. Incubation followed for 1 h at 37 °C [39]. In parallel, the blank sample eliminating the negative effect of nonspecific sorption of secondary anti-OVA^{HRP} IgG was prepared by the same protocol without antigen addition.

Electrochemical measurement

Linear sweep voltammetry (LSV) was selected as the most suitable voltammetric technique. It was used for all electrochemical recordings of current decrease during hydrogen peroxide oxidation