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Voltammetric immunosensor for the diagnosis of celiac disease based on the quantification of anti-gliadin antibodies

Marta M.P.S. Neves[a,b,c], María Begoña González-García[c], Alice Santos-Silva[a,d], Agustín Costa-García[c,*]

[a] Faculdade de Farmácia da Universidade do Porto, Rua Aníbal Cunha 164, 4099-030 Porto, Portugal
[b] REQUIMTE, Instituto Superior de Engenharia do Porto, Rua Dr. António Bernardino de Almeida 431, 4200-072 Porto, Portugal
[c] Departamento de Química Física y Analítica, Universidad de Oviedo, C/. Julian Clavería 8, 33006 Oviedo, Spain
[d] Instituto de Biologia Molecular e Celular da Universidade do Porto, Rua do campo Alegre 823, 4150-180 Porto, Portugal

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ABSTRACT

Antibodies against gliadin are used to detect celiac disease (CD) in patients. An electrochemical immunosensor for the voltammetric detection of human anti-gliadin antibodies (AGA) IgA and AGA IgG in real serum samples is proposed. The transducer surface consists of screen-printed carbon electrodes modified with a carbon nanotube/gold nanoparticle hybrid system, which provides a very useful surface for the amplification of the immunological interactions. The immunosensing strategy is based on the immobilization of gliadin, the antigen for the autoantibodies of interest, onto the nanostructured surface. The antigen–antibody interaction is recorded using alkaline phosphatase labeled anti-human antibodies and a mixture of 3-indoxyl phosphate with silver ions (3-IP/Ag^+) was used as the substrate. The analytical signal is based on the anodic redissolution of the enzymatically generated silver by cyclic voltammetry. The electrochemical behavior of this immunosensor was carefully evaluated assessing aspects as sensitivity, non-specific binding and matrix effects, and repeatability and reproducibility. The results were supported with a commercial ELISA test.

1. Introduction

Celiac disease (CD) is an autoimmune enteropathy characterized by an inappropriate T-cell mediated immune response to the ingestion of gluten in genetically susceptible individuals [1]. Gluten is a complex mixture of wheat storage proteins that can be insoluble in alcohol, the glutenins, or soluble in alcohol, the gliadins. The alcohol-soluble fractions have analogous toxic proteins that are present in barley, the hordeins, in rye, the secalins, and in oats, the avenins [2]. It is generally accepted that gliadins are the major triggering factors in CD [3]. The mechanism underlying CD pathogeneses can be explained by the ingestion of these alcohol-soluble proteins. The deamidation of these gluten peptides is mediated by tissue transglutaminase (tTG) creating epitopes with increased immunostimulatory potential. The deamidated epitopes are then presented, in association with the human leucocyte antigens DQ2 and DQ8 of antigen-presenting cells, to CD4+ T cells [4]. The humoral autoimmune response leads to an abnormal intestinal mucosa characterized by villous atrophy and crypt hyperplasia [4] resulting in malabsorption related problems. Common serological changes include the appearance of antibodies against gliadin, tTG and endomysium, which are specific indicators of the disease.

In spite of efforts toward the development of new therapeutic strategies [5] the recovery of the intestinal mucosal still requires the total elimination of gluten proteins from the patient's diet. Therefore, an early and accurate diagnosis of CD is extremely important to control the gastrointestinal damage and to ensure the patient's quality of life.

Small bowel biopsy has been the gold standard diagnostic test for CD during the last 30 years. However, the different clinical presentations of this condition can complicate the diagnosis and, as a result, delay the treatment [6]. Recent guidelines from the European Society of Paediatric Gastroenterology and Nutrition [7] and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition [8] have suggested serological tests as the front-line test to clear clinical suspicion of celiac disease in patients presenting characteristic symptoms or in those who belong to a risk group. In the case of patients that have the symptoms but present negative serological tests, IgA class antibodies deficiency should be

Abbreviations: CD, celiac disease; EIs, electrochemical immunosensors; AGA, human anti-gliadin antibodies; Anti-H-IgA-AP, anti-human IgA antibodies conjugated with alkaline phosphatase; Anti-H-IgG-AP, anti-human IgG antibodies conjugated with alkaline phosphatase; SPCEs, screen-printed carbon electrodes; MWCNTs, carboxylated multiwalled carbon nanotubes; NPAus, gold nanoparticles.

* Corresponding author. Tel.: +34 985103488; fax: +34 985103125.
E-mail address: costa@uniovi.es (A. Costa-García).

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considered. The determination of the IgG class of antibodies against gliadin, tTG and endomysium has been suggested as an alternative [9].

The advances in the efficacy of serological antibody testing potentiate the possibility of accurate screening programs in the community, serving as a first-line method to clarify clinically suspicious cases in an underdiagnosed stage and also to manage the follow-up of this multifactorial disease [10]. Currently, enzyme-linked immunosorbent assay (ELISA) is the most used methodology for clinical serological CD diagnosis; nonetheless the development of more rapid, sensitive, and cost-effective strategies that allow point-of-care analysis are required.

Electrochemical immunosensors (EIs) are specific, fast, portable and low cost devices which offer exciting opportunities for numerous decentralized clinical applications, ranging from "alternative-site" screening to home self-testing [11]. The nature of the electrochemical sensor's surface and the strategies for the antibody or antigen immobilization have been one of the major issues in immunosensing development. The transduction of the biorecognition events requires innovative approaches that couple different sensing platforms. The use of hybrid structures formed by combining carbon and metal nanomaterials has demonstrated to improve the electrocatalytic efficiency of many electrochemical processes allowing a synergic enhancement of each of the material properties [12–14].

In the recent years, the electrochemical biosensors field have witnessed a growing interest in the development of analytical devices to celiac disease diagnosis. The employment of EIs for the detection of specific antibodies for CD was first reported by Balkenhohl and Lisdat [15,16]. These authors developed an impedimetric immunosensor for the detection of antibodies directed against tTG using screen-printed gold electrodes modified with a polyelectrolyte layer of poly(sodium 4-styrenesulfonic acid) [15]. The same authors also developed a similar immunosensor for the determination of anti-gliadin antibodies (AGA), using 3-mercaptopropionic acid to modify the screen-printed gold electrode surface [16]. The amount of these autoantibodies present in positive and negative serum samples was evaluated. The high charge transfer resistance values obtained by impedance spectroscopic measurements were related to the content of antibodies; however only a qualitative analysis was performed. Moreover, an acceptable sensitivity was achieved but the results suggest a lower precision, when compared with ELISA assays. On the other hand, Pividori et al. proposed an amperometric immunosensor based on the physical adsorption of tTG from guinea pig liver onto graphite–epoxy composite (GEC) electrodes [17]. For 10 positive and 10 negative processed serum samples a sensitivity of 70% and a specificity of 100% were achieved, compared with the commercial ELISAs, but this method was also based on a yes/no binary qualitative system and no direct correlation between the analytical signal and antibody concentration was achieved. Pereira et al. [18] have proposed a microfluidic immunosensor coupled with electrochemical detection for AGA IgG quantification. The methodology consists of a 30-min assay using a pore glass bed coated with the antigen gliadin on a bare gold electrode for the detection of the labeled anti-human IgG. The results obtained in this work were comparable with those obtained by commercial ELISA kits but the work lacks the detection of IgA-class antibodies and immunoglobulin A (IgA) isotype is considered to be the most specific [19]. An electrochemical supramolecular platform based on cyclodextrin-modified gold surface electrodes to detect antigliadin antibodies in real serum samples was also proposed [20]. In this work an amperometric detection of AGA IgA and AGA IgG in samples of CD patients in 3 stages of the disease was achieved and the results showed a good correlation with an ELISA assay. Two more EIs that provide a qualitative and semi-quantitative estimation of the antibody content in real sera were reported. One was developed for the detection of human anti-tTG antibodies [21] and the other for the detection of human AGA antibodies [22]. In the construction of both sensors, the antigen (i.e. tTG or gliadin) was covalently attached onto self-assembled monolayers of a carboxylic-terminated bipodal alkanethiol group which was immobilized on gold electrodes. The immunosensors performance was corroborated with a commercial ELISA kit and the results were in good agreement with those obtained with the conventional methodology. However, in almost every case, surface renewable electrodes and conventional electrochemical cells are used and this has serious disadvantages has memory effects and necessary cleaning processes. In spite of the good analytical results, the employed electrochemical devices do not allow to carry out a continuous analytical performance. Moreover, for the decentralization of the diagnostic toward a point-of-care analysis, the research was to be directed toward more simple, miniaturized and portable devices.

In our group, we already developed a disposable electrochemical immunosensor for the detection of IgA and IgG type anti-tTG autoantibodies in real patient's samples [23] using a screen-printed carbon electrode modified with a carbon nanotube (CNT)/gold nanoparticle hybrid system as transducer surface. That nanostructured electrodic surface was developed in a previous work [24] in order to be applied as a transducer surface in several biosensor devices. Due to the growing need to perform rapid "in situ" analyses, disposable screen-printed electrodes have been successfully employed in the development of several biosensors devices in many fields [25]. Furthermore, hybrid CNT-nanoparticle materials are interesting because the combination of the physical and chemical properties of the individual components leads to a robust and efficient transducer amplification platform [12].

In this work we propose the same single-use hybrid transducer platform [24] for the quantification of AGA IgA and AGA IgG in human serum. The antigen and secondary labeled antibodies' concentrations were optimized and non-specific binding was also studied. The evaluation of real patient's samples was performed with the developed immunosensor and the results were compared with commercial ELISA kits.

2. Materials and methods

2.1. Instrumentation

Voltammetric experiments were performed with an Autolab PGSTAT 12 (Eco Chemie, The Netherlands) potentiostat/galvanostat interfaced to an AMD K-6 266 MHz computer system and controlled by Autolab GPES 4.8 (software version for Windows 98). All the measurements were carried out at room temperature. Disposable screen-printed carbon electrodes (SPCEs) were purchased from DropSens (Spain). These electrodes incorporate a conventional three-electrode cell configuration, printed on ceramic substrates (3.4 × 1.0 cm). Both the working- (disk-shaped 4 mm diameter) and the counter electrode are made of carbon inks, whereas the pseudo-reference electrode and the electric contacts are made of silver. A ring-shaped layer printed around the working electrode constitutes the reservoir (50 μL) of the electrochemical cell. The SPCEs were easily connected to the potentiostat through a specific DropSens connector (Spain).

2.2. Reagents and solutions

Tris(hydroxymethyl)aminomethane (Tris), magnesium nitrate, bovine serum albumin fraction V (BSA), β-casein from bovine milk