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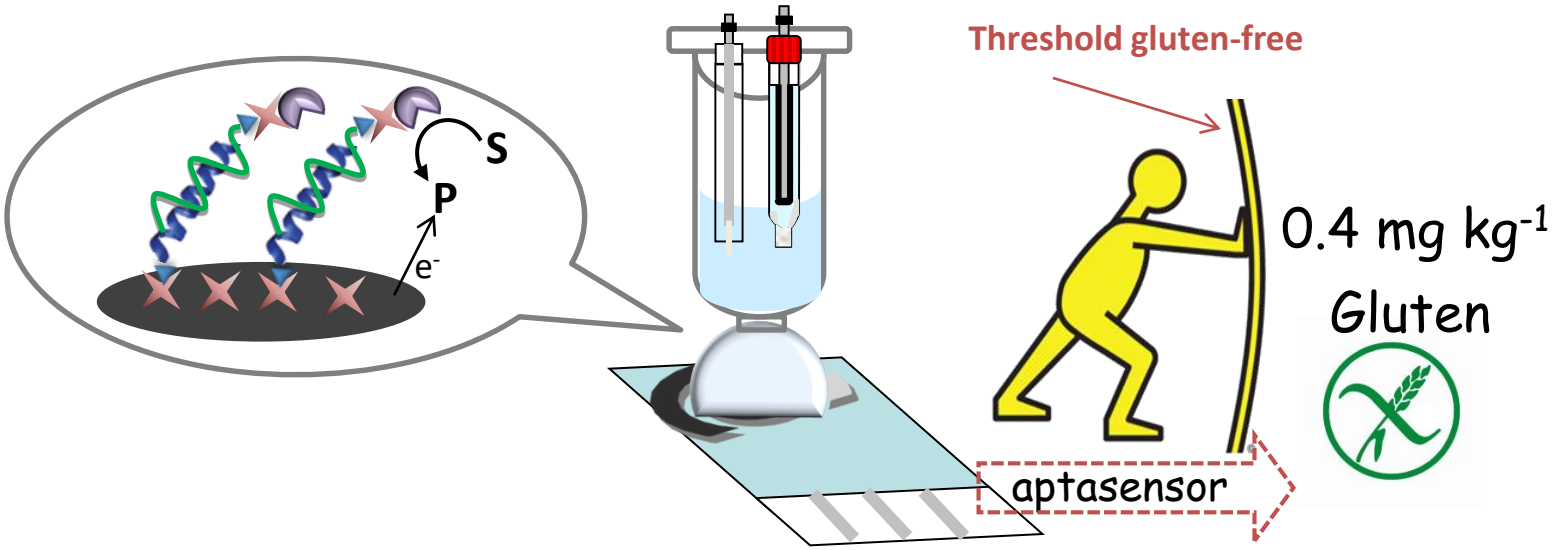
Abstract: Reliable detection of decreasing amounts of gluten in food is the only way of ensuring the safety of all coeliac patients. Results obtained with the method of choice, immunochemical assays, are not entirely comparable and many of them are sandwich assays that cannot recognize hydrolyzed proteins. In this work, we propose a competitive electrochemical sensor based on a recently described aptamer targeting the gliadin immunodominant peptide 33-mer that triggers the coeliac disease. The sensing layer is built on the surface of a screen-printed carbon electrode (SPCE) by adsorption of streptavidin and subsequent peptide immobilization. A competition between the peptide and gluten proteins from samples for a defined concentration of biotinylated Gli 4 aptamer is established. The aptamer bound to the peptide on the surface is finally measured after enzyme labelling and chronoamperometric detection of an enzymatically obtained electrochemically active product. This method is able to detect as low as 0.113 µg L-1 of gliadin, which corresponds to 380 µg kg-1 of gluten in food, taking all dilutions and conversion factors into consideration, with a reproducibility lower than 11%. The aptasensor was applied to food samples with gluten contents above and below the legislated threshold for gluten-free labelling in the EU, obtaining good agreement with the official R5 immunochemical method.

Graphical Abstract (for review)



Highlights

- An electrochemical aptamer-based sensor on peptide-modified carbon screen-printed electrodes for gluten determination
- Sensitivity is improved compared with reference enzyme-linked immunosorbent assays
- The sensor was successfully applied to detect gluten in various food samples

Disposable electrochemical aptasensor for gluten determination in food.

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Abstract

Reliable detection of decreasing amounts of gluten in food is the only way of ensuring the safety of all coeliac patients. Results obtained with the method of choice, immunochemical assays, are not entirely comparable and many of them are sandwich assays that cannot recognize hydrolyzed proteins. In this work, we propose a competitive electrochemical sensor based on a recently described aptamer targeting the gliadin immunodominant peptide 33-mer that triggers the coeliac disease. The sensing layer is built on the surface of a screen-printed carbon electrode (SPCE) by adsorption of streptavidin and subsequent peptide immobilization. A competition between the peptide and gluten proteins from samples for a defined concentration of biotinylated Gli 4 aptamer is established. The aptamer bound to the peptide on the surface is finally measured after enzyme labelling and chronoamperometric detection of an enzymatically obtained electrochemically active product. This method is able to detect as low as 0.113 μg L^{-1} of gliadin, which corresponds to 380 μg kg^{-1} of gluten in food, taking all dilutions and conversion factors into consideration, with a reproducibility lower than 11%. The aptasensor was applied to food samples with gluten contents above and below the legislated threshold for gluten-free labelling in the EU, obtaining good agreement with the official R5 immunochemical method.

Keywords: aptamer; gliadin; gluten; 33-mer peptide; electrochemical sensor

1. INTRODUCTION

Coeliac disease patients face the challenge to interpret a non-harmonized labelling in food across the world to avoid the offending compound that triggers their chronic autoimmune illness: gluten. This is in fact a mixture of storage proteins in wheat, barley, rye and possibly oat. The meaning of "gluten-free" label can vary from undetectable in Australia and New Zealand to 20 mg kg^{-1} in the European Union [1]. The latter was set since the advent of immunochemical methods based on R5 antibody [2]. Unfortunately, the established level is still harmful for specially sensitized individuals. A proposal to decrease this value to 3 mg kg^{-1} has been claimed [3] but the lack of more sensitive analytical methods along with the absence of a clinically proved maximum tolerated intake precludes its decrease so far.

Currently, there are two main groups of methods for gluten determination: those targeting directly the allergenic proteins or their peptide fragments, and those directed to identify DNA sequences specific of the carrier cereals. However, only a few of them have seen the transition to point of care by integration into chemical sensors.

Electrochemical genosensors have recently emerged as a novel low-cost strategy to detect gluten in food samples using a gene encoding the immunodominant peptide 33-mer as a target [4]. In combination with a structured capture probe the sensor was able to discriminate wheat from other gluten-containing cereals [5] unlike real-time PCR methods [6]. Though good correlation between immunochemical and genosensor results has been reported for a set of samples, DNA-based methods are indirect methods because they do not measure the offending compounds. They are excellent for identification and complementary to ELISA assays in samples where proteins are lacking or are highly degraded.

Proteomic-based methods are powerful tools for identification of immunotoxic peptides from different cereal sources but only the combination of HPLC with electrospray ionization (ESI) and tandem mass spectrometry detection (LC-MS/MS) allow quantitation at low mg kg^{-1} level at expense of cumbersome and time-consuming extraction and digestion steps to avoid matrix effects [7]. The final peptide content, however, depends on the composition of the gluten sample and the yield of enzymatic digestion. It also requires a conversion into gluten units, a non-trivial challenge currently unavailable, to serve as surveillance method about the compliance with legislation. Therefore, this methodology is restricted to large research facilities and regarded as complementary to immunochemical assays in case of controversy [1].

The development of chemical sensors targeting the allergen requires a specific receptor, recognizing the proteins with high affinity. In the last decade, new antibodies have been raised against different epitopes present in prolamins, especially in gliadin, the wheat prolamin. These receptors are mainly used in the development of immunoasays. There are 36 different commercial ELISA kits in several formats: sandwich, competitive, lateral flow devices and dipsticks [1]. However, results obtained with them are not fully comparable even in simple samples. Not a single kit performs well in all matrices. The differences increase in challenging samples containing heated or hydrolyzed gluten or interference ingredients such salt, sugar or spices [8]. This indicates that there is a long way to reliably measure gluten in food samples using immunochemical approaches. Only two electrochemical immunosensors have been reported and used in real samples: a sandwich immunosensor that needs multiple steps to covalently attach the capture antibody to a self-assembled monolayer in addition to the binding and labeling steps and a 10-min enzymatic reaction before measurement [9]; and a competitive immunosensor

with a limit of detection above 100 mg kg^{-1} due to incompatibility with extraction reagents [10].

At the research level, antibodies raised against deamidated gluten have been recently reported. This type of gluten is more toxic for coeliac patients and is profusely used in industry for several purposes in meat products, baked items and other food products. However, current antibodies fail to detect them, which compromise their safety. A lateral flow device with a limit of detection of 2 mg kg^{-1} in food can detect both native and deamidated gluten [11]. Superparamagnetic microsphere-based suspension array platforms are emerging as systems for multiplex analysis by flow cytometry. These beads contain different proportions of two dyes and each set is linked to a different antibody, which allows the simultaneously detection of up to 100 analytes. Quantification is based on a third reporter fluorophore. With these systems three allergens (casein, soy and gluten) were detected in a single assay in prepared mixtures [12]. Other platform under evaluation for commercialization can detect simultaneously 15 allergens including gluten [13].

Another possibility is to employ a glutamine-binding protein, which is able to bind glutamine residues in glutamine-rich peptides. This protein is successfully used to detect gluten-digested peptides with higher affinity than the intact gluten by fluorescence on a protein microarray. However, this strategy also relies on peptide detection similarly to MS proteomics [14].

Recently aptamers have emerged as novel receptors for gluten determination. Aptamers are short DNA or RNA oligonucleotides that can recognize a great variety of ligands with high specificity and selectivity. They are less prone to thermal denaturation and can be easily modified to include marker or anchoring functionalities. Aptamers were evolved against the immunodominant peptide known as 33-mer [15], the main peptide

triggering the coeliac disease due to its resistance to digestion. Limits of detection as low as 0.5 mg kg^{-1} of gluten have been reported in competitive electrochemical aptamagnetoassays [16]. In spite of the easiness of handling and amenability to washing steps, magnetic microbeads are not fully accepted in routine agri-food labs yet. In response to industrial demands, we have developed an electrochemical competitive aptasensor. This approach showed a slightly improved detectability that rivals the most sensitive immunochemical assays and was successfully validated against a commercial ELISA assay in unknown food samples.

2. MATERIALS AND METHODS

Reagents

5'-biotin tagged DNA aptamer (Gli 4) 5'-CCA GTC TCC CGT TTA CCG CGC CTA CAC ATG TCT GAA TGC C-3' was obtained from Integrated DNA Technologies, IDT, Leuven, Belgium. Biotinylated 33-mer peptide was obtained from Biomedal, (Sevilla, Spain) with the following sequence: LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPFHHHHHH-lysine-biotin.

Gliadin standard solutions were prepared using the gliadin standard provided by the Working Group on Prolamin Analysis (PWG) and acquired to R-Biopharm. Streptavidin-peroxidase conjugate (Str-HRP_2) was obtained from Thermo Fisher (Madrid, Spain). Reagents for buffer preparation Tris:HCl pH 7.4, phosphate buffer saline (PBS, pH 7.4), NaCl, bovine serum albumin (BSA), $MgCl_2$, tween® 20 and ethylendiaminetetraacetic acid (EDTA),were DNAse and RNAse free (for molecular biology) and purchased from Sigma-Aldrich (Madrid, Spain). Streptavidin, biotin and 3,3',5,5'-tetramethylbenzidine (TMB) liquid substrate system for ELISA were also acquired to Sigma. All solutions were prepared with MilliQ purified water.

Instrumentation

Electrochemical measurements were carried out with a μAutoLab type II potentiostat (Eco Chemie, The Netherland) using a three-electrode system. Screen-printed carbon electrodes from Dropsens (Oviedo, Spain) were used as working electrodes. External Ag|AgCl| KCl (sat) reference electrode and Pt counter electrode were used instead of the ones printed in the cell that were disassembled by covering with non-surfactant enamel. Both electrodes were inserted in a syringe filled with saturated KCl and stopped with filter paper as a liquid junction and placed just over the drop on the working electrode.

Procedures

Conditioning and preparation of the sensing layer on SPCE. Electrochemical screen-printed cells were washed with ethanol and MilliQ water and dried with a N_2 stream. Then the pseudoreference and counter electrodes were covered with non-surfactant enamel to precisely define the electroactive area avoiding non-specific adsorption.

All steps were performed in 0.1 M PBS. A 10 μL drop of 1 mg mL^{-1} streptavidin was placed onto the SPCE, incubated overnight at 4 °C and then washed with PBS with and without 0.001% tween® 20 (Figure 1A, step 1). The electrode surface was blocked with 40 μL of 1 % BSA for 30 min at room temperature and then washed as previously indicated (Figure 1A, step 2). The biotinylated 33-mer peptide at 0.2 μM concentration was bound to the streptavidin layer for 1 h and then a washing step was also carried out (Figure 1A, step 3). The unoccupied streptavidin binding sites were blocked with an excess of biotin (2 μM) for 30 min (Figure 1A, step 4).

Binding curve: 10 μL of increasing concentrations of Gli-4 aptamer prepared in binding buffer (50 mM Tris-HCl pH 7.4, 250 mM NaCl and 5 mM $MgCl_2$) were incubated onto different modified electrode surfaces for 30 min at room temperature. Several washing steps with binding buffer with and without 0.001% tween® 20 to remove the weakly

bound aptamers were carried out. The labelling step was performed by incubating 2.5 µg mL^{-1} Strep-HRP_2 in binding buffer containing tween® 20 for 30 min at room temperature. After several washing steps, 40 µL of TMB were added and the enzymatic reaction proceeded for 30 s. Then the current intensity due to the reduction of the enzymatically oxidized substrate was measured chronoamperometrically at 0.2 V for 60 s.

Competitive assay: Increasing concentrations of PWG gliadin standard or extracted samples prepared in binding buffer were incubated with 250 nM biotinylated Gli 4 aptamer in a total volume of 250 µL for 30 min at 25 °C under continuous shaking in a Thermomixer (Eppendorf Ibérica, Spain). 10 µL of the solution were deposited onto the sensing phase, where the competitive step takes place for 30 min at room temperature. The electrode was washed with binding buffer containing 0.001 % tween® 20. The labelling and detection steps were carried out as indicated above (Figure 1B).

Samples treatment: For the extraction of gluten from food samples 0.8 g of homogenized food sample were placed in a 15 mL Falcon tube. Prolamins were extracted twice with 4 mL of 60 % ethanol solution. The mixture was incubated at room temperature for 30 min under continuous shaking in a rocking mixer (Selecta, Spain) and then centrifuged for 45 min at 2500 rpm. Supernatants obtained in both extractions were finally combined. 6 µL of the extract was diluted up to 1 mL with binding buffer and this final solution was subjected to the same competitive assay protocol than the PWG standards.

3. RESULTS AND DISCUSSION

Commercial kits for gluten detection are usually based on ELISA sandwich assays. However, they cannot recognize hydrolyzed or degraded fragments so their usefulness is

somehow limited. Recently, on the bases of an exhaustive comparison study, the preference of developing competitive assay formats has been claimed because their superiority performance in most samples that can contain both whole or hydrolyzed gluten [8]. In line with this recommendation, we have developed a competitive assay using an aptamer previously raised against the immunodominant peptide 33-mer. In order not to modify the recognition ability of the aptamer, a rational design of the assay was performed. Immobilization of the aptamer was found to be more deleterious in terms of affinity than the immobilization of the 33-mer peptide [17], so the latter was anchored through streptavidin-biotin interaction to the electrode surface. Among the aptamers raised against 33-mer [16], Gli 4 was selected because of its highest affinity and good performance in competitive magnetoassays [18]. Likewise, biotin was used as a tag for subsequent enzymatic labelling. This molecule was reported to influence the actual affinity between the aptamer and the peptide but in a minor extent in comparison with other markers [17].

Competitive assays require a fixed amount of aptamer that can bind both the analyte in solution or immobilized, so the concentration of Gli 4 was first selected. To this purpose a binding curve with increasing concentrations of aptamer ranging from 10 nM to 1 μM was constructed, measuring the current intensity at each concentration on non-activated working electrodes modified with streptavidin and blocked with 1 mg mL^{-1} BSA before immobilization of 33-mer. No analyte was present in solution. As can be seen in Figure 2, the current intensity increased with the concentration of the aptamer indicating that aptamer can be fixed onto the 33-mer modified-electrode surface due to affinity binding. Data were fitted to a logistic model (eq. 1) using Origin software

$$y = \frac{B-A}{1+\left(\frac{x}{EC_{50}}\right)^{p}} + A \quad \text{(eq. 1)}$$

where A is the response at infinite concentration of aptamer in µA, B is the response to zero concentration of aptamer also in µA, EC_{50} is the concentration leading to 50% of the maximum signal in nM and p is the Hill slope representing the slope at the inflection point of the increasing sigmoidal curve. The best fitting values of the four parameters are the following:

$$I\ (\mu A) = 28.1(\pm 1.6) + [2.3(\pm 0.8) - 28.1(\pm 1.6)] / [1 + ([Gli\ 4]\ (nM) / 250(\pm 0.3)]^{1.6(\pm 0.3)}$$

The regression coefficient was 0.995. It is accepted that a maximum sensitivity is usually achieved using a limited reagent concentration providing about 50% binding of the reagent at saturating concentration of analyte [19], so EC_{50} value, that is, 250 nM was used in further experiments. This value is in excellent agreement with that obtained on magnetic microbeads [18]. The reproducibility of the measurements ranged from 6 to 32%. In order to improve it, electrochemical activation of the carbon electrode surface was tested in sulfuric acid at 1.3 V for 120 s. This type of activation creates an oxidized surface that improves the adsorption of proteins such streptavidin giving rise to higher analytical signals and better reproducibility [20]. However, this pretreatment led to high blank currents probably due to unspecific adsorption of the enzyme conjugate on an exceedingly oxidized surface. The concentration of BSA was also very high in comparison with the above mentioned protocol. Therefore, electrode surfaces with several levels of oxidation were prepared using a lower amount of BSA namely 1 %. In Figure 3A, the current intensity for 500 nM Gli 4 was compared with the blank experiment in the absence of aptamer and plotted in the form of signal to blank ratio (S/B). The best ratio was obtained when no pretreatment was carried out. However, low reproducibility was noted. For this reason the less oxidized surface (60 s at 1.3 V) was selected for further experiments varying the time of the BSA incubation. The S/B ratio (Figure 3B) was much higher when the incubation time was shorter than 60 min and the

reproducibility was mostly unaffected. A close inspection to the data shows that the largest contribution to the irreproducibility is associated to the current measured in the presence of Gli 4, which is almost constant at this aptamer concentration. On the contrary, the standard deviation of the blank signals is quite different, namely 0.4, 0.3 and 2.2 μA for 15, 30 and 60 min respectively. Therefore, a time of 30 min was selected for the following experiments because of the largest S/B ratio and lower standard deviation of the blank signal.

3.1. Competitive assay

The design of the assay is based on the competition between the PWG gliadin standard or gluten from the samples and the 33-mer peptide immobilized on the electrode surface for a fixed amount of Gli 4 aptamer in solution. As a result, the higher the PWG or gluten concentration the lower the amount of aptamer that binds to the modified electrode. The bound aptamer is measured through an enzymatic labelling and further detection of the enzymatic product by chronoamperometry. A direct relationship between the amount of aptamer bound to the surface and the current measured is expected. Preliminary experiments with increasing concentrations of PWG gliadin showed very high currents irrespective of the concentration assayed. Since this behavior was not observed on non-activated surfaces, it was attributed to a large unspecific adsorption of the prolamin on the activated surface that induces adsorption of the enzyme conjugate. Consequently, the electrochemical pretreatment of the surface was finally discarded. On non-activated surfaces, an inverse dependence between the current intensity and the concentration of PWG gliadin from 1 to 100 μg L^{-1} was obtained as anticipated (Figure 4). The decreasing calibration plot was fitted to a four-parameter logistic equation (eq 1). In this case, A and B correspond to the infinite and zero concentration of analyte, respectively, and A was

fixed at zero because it is a signal-off assay. The values of the parameters that best fit the experimental data are the following: $I(\mu A)= 9.4(\pm0.3) / [1+([PWG](\mu g\ L^{-1}) / 76(\pm14)]^{0.45(\pm0.06)}$

with a regression coefficient of 0.992. In this case, the concentration of PWG required to inhibit the binding of the labelled aptamer (IC_{50}) is 76 ± 14 μg L^{-1}. Considering that the amount of immobilized peptide will be much lower than IC_{50}, this value is a reasonable approximation to the dissociation constant for the PWG-aptamer complex (Gulukota et al. 1997). In this way, a K_d value of 1.5 ± 0.3 nM was estimated for the aptamer-PWG interaction under the experimental conditions used. This points out to a stronger interaction than that obtained using magnetic particles as support for the sensing phase.

The limit of detection was estimated as the concentration that gives the 95 % of the maximum signal resulting to be 0.113 μg L^{-1} of PWG gliadin. This value was lower than the one reported with the electrochemical magnetoassay [18] and corresponds to 0.226 μg L^{-1} of gluten considering that an average of 50% of gluten is constituted by gliadin [21]. This is the ratio used by convention though it can vary depending on the gluten source [22]. In a typical analysis of gluten a 1:500 dilution is carried out meaning that the limit of detection is equal to 0.113 mg kg^{-1} of gluten in real samples. Most sensitive immunological methods reach 0.125 mg kg^{-1} in a direct assay using R5 antibody or 0.250-0.3 mg kg^{-1} in sandwich formats with different monoclonal or polyclonal antibodies [8]. Competitive assays are usually less sensitive. 0.72 and 0.88 mg kg^{-1} were claimed for R5 [23] and G12 [24] antibodies, respectively. Recently, a competitive electrochemical immunosensor was proposed. It uses gold nanoparticles-SPCE and alkaline phosphatase as an enzyme. The limit of detection was 8 μg L^{-1} gliadin, more than an order of magnitude larger than the one provided with this aptasensor [10]. As mentioned above, the aptamer-based electrochemical magnetoassay has a reported limit of detection of 0.5

mg kg^{-1}. Therefore, this aptasensor is the most sensitive method for the detection of gluten in food reported so far. The relative standard deviation ranges from 2% at 1 µg L^{-1} and 11 % at 75 µg L^{-1} of PWG, which is remarkable for this type of assays.

3.2. Application to food samples

Extraction of gluten from food is an unsolved issue that has experienced a significant advance in the last years with the development of several extraction solutions that helps the extraction of heat treated gluten. There is a variety of protocols, all of them finally relying on alcohol:water mixtures. Some of them recommend the previous removal of albumins and globulins using 0.5 M NaCl [25, 26]. In our experience this step increases the irreproducibility of the extraction because some food can absorb water during this separation and then become dehydrated when extracted in alcoholic mixtures. For this reason, the extraction was directly carried out twice with 60% ethanolic solution and the supernatants were combined. The high ethanol content of the extracts precludes the affinity interaction with the receptor so a large dilution (1:166) was performed with the binding buffer. The influence of ethanol on the aptasensor response was evaluated measuring the current intensity for zero concentration of PWG (maximum signal). Values of 9.9(±0.9) and 10.0(±0.9) µA were obtained in the absence and in the presence of 1.2% ethanol, respectively, a concentration larger than the one actually present in the assay. This indicates that this sensor tolerates an ethanol concentration as high as 1.2% without affecting its performance.

Therefore, a calibration plot without ethanol was used to evaluate the gluten content in three food samples with unknown gluten content and tested by two external laboratories accredited to be competent in the use the official method based on R5 antibody. In Table 1, the results obtained with the aptasensor herein reported and the two external analyses

are provided. Both methods report concordant results. Two samples contain gluten above the threshold set up by European Regulations, so they are not safe for coeliac disease patients and cannot be labelled as gluten-free. Fixamyl sample, a thickener for light sauces, has a low amount of gluten, below the threshold with all approaches, so it is a gluten-free product.

CONCLUSIONS

A simple method for detection of gluten in food samples was reported. Preparation of the disposable sensing phase is based on adsorption of streptavidin on carbon surfaces as a mean of immobilizing the biotinylated 33-mer peptide. The competitive electrochemical assay was successfully applied to three food samples and the results were in good agreement with those obtained with the official method. The aptasensor show neither false positive nor false negative results. Taking into account the dilution performed in real samples, the aptasensor approach is able to detect as low as 380 µg kg^{-1} of gluten. This low value would allow the reliable determination of the claimed 3 mg kg^{-1} to protect most sensitive coeliac disease patients. It can be considered as a consistent step forward to reduce the threshold for labeling food as "gluten-free" in countries where the 20 mg kg^{-1} value is in force.

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TABLE 1

Sample	Aptasensor	R5 Lab I	R5 Lab II
Fixamyl	1.8	7	13.3
Rolled oats	91	77	> 80
Fit Snack	61	77	> 80

* in mg kg^{-1} of gluten

CAPTIONS FOR THE FIGURES

Figure 1: Scheme representing the sensor construction (A) and the three steps involved in the measurement (B).

Figure 2: Binding curve for biotinylated Gli 4 aptamer to a SPCE modified with biotinylated 33-mer through streptavidin-biotin interaction. No electrode pretreatment was carried out. The detection was performed by chronoamperometry at 0.2 V after labeling with Str-HRP_2 and addition of TMB+H_2O_2 as a substrate for 30 s.

Figure 3: A) Effect of electrode pretreatment and B) incubation time of BSA on the signal to blank ratio in the absence of PWG gliadin. Signal was obtained using 500 nM of biotinylated Gli 4 aptamer. Other parameters were as indicated in Figure 2.

Figure 4 Competitive assay curve obtained using 33-mer modified SPCE in the presence of 250 nM biotinylated Gli 4 aptamer and 1 to 100 µg L^{-1} PWG gliadin as a standard. Other parameters as indicated in Figure 2.

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Figure 1

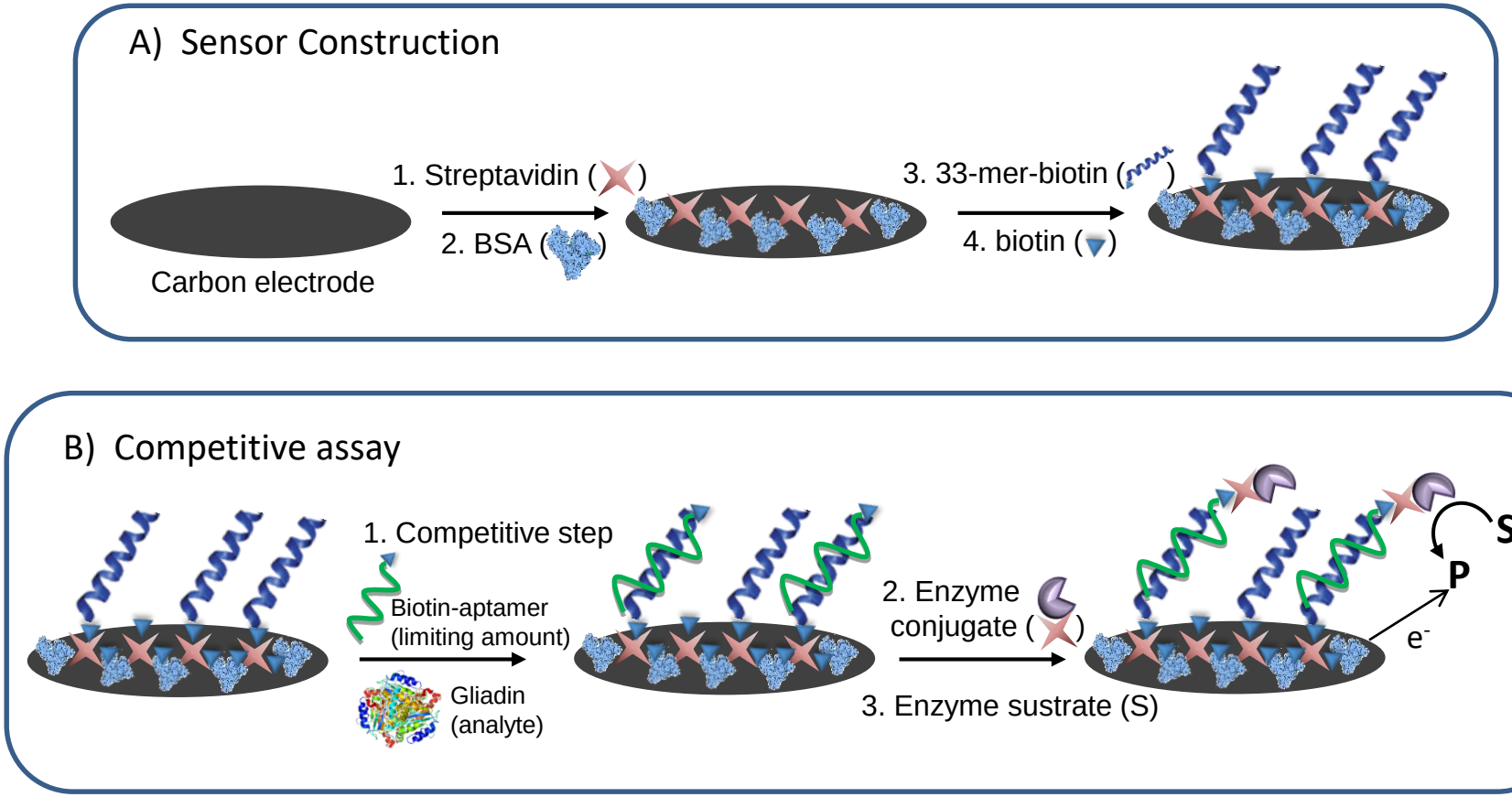

A) Sensor Construction
Carbon electrode
1. Streptavidin ( )
2. BSA ( )
3. 33-mer-biotin ( )
4. biotin ( )
B) Competitive assay
1. Competitive step
Biotin-aptamer
(limiting amount)
Gliadin
(analyte)
2. Enzyme
conjugate ( )
3. Enzyme sustrate (S)
S
P
e-

Figure 2

FIGURE 2

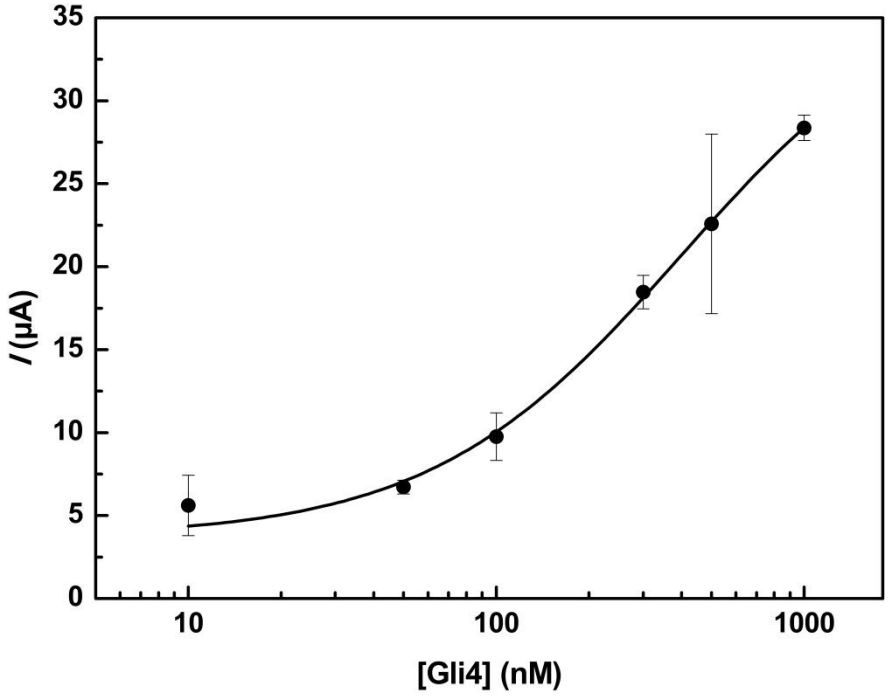
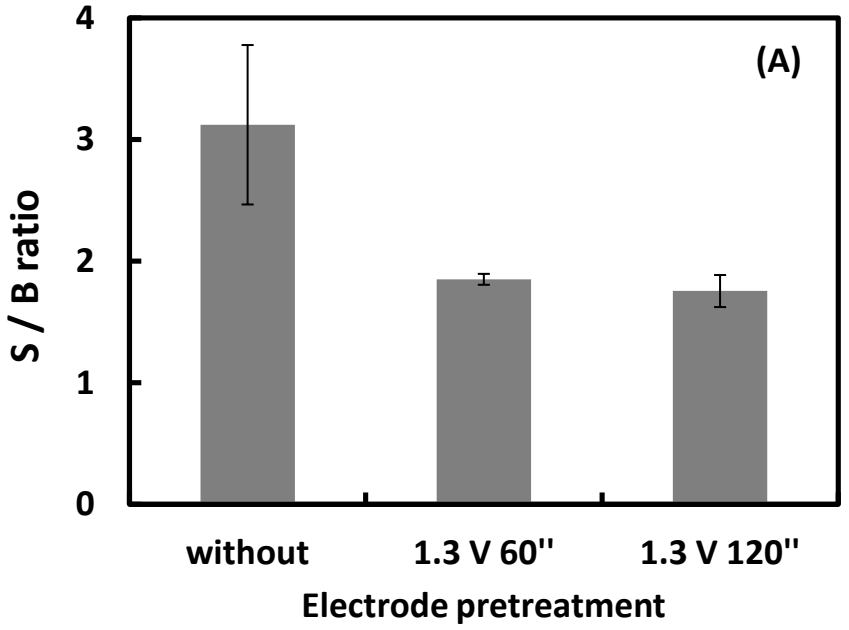


Figure 3

FIGURE 3



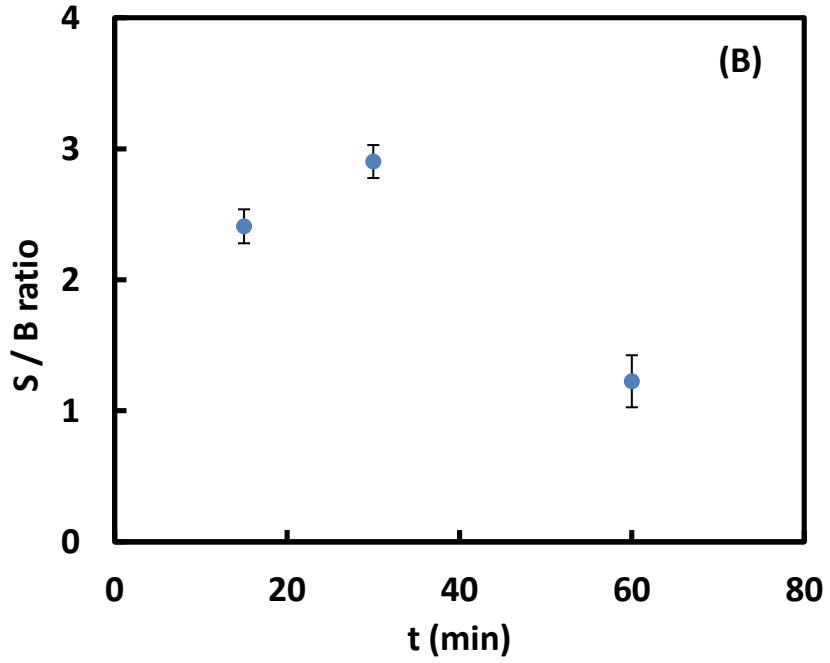
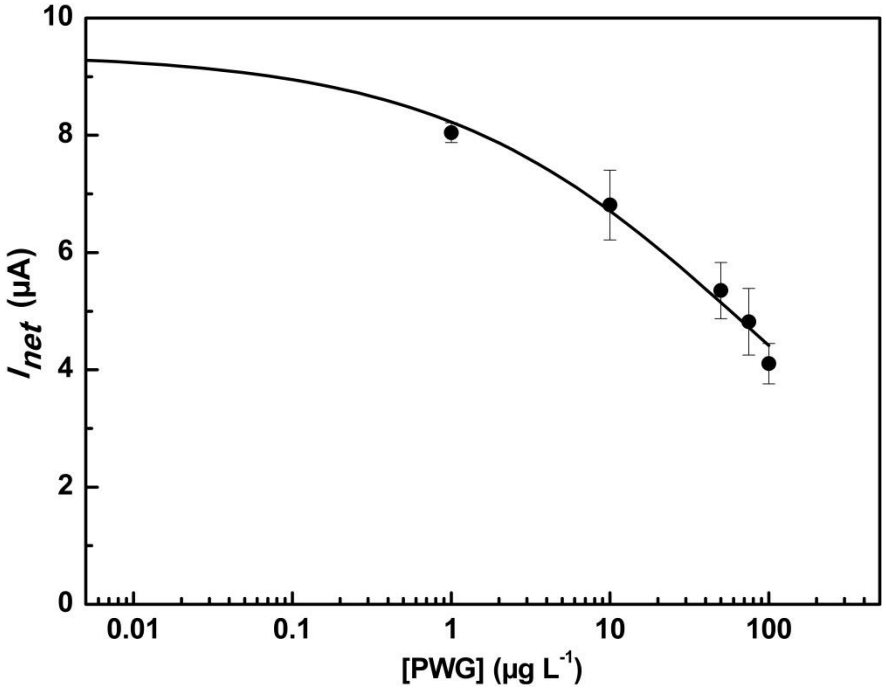


Figure 4

FIGURE 4



Author Biographies

Laura López López was a Master student at University of Oviedo during the academic course 2013-2014. During her master´s degree studies she has worked with electrochemical biosensors applied to allergens determination. Currently, she is working in an agro-food industry lab in UK.

Dr. Rebeca Miranda Castro received her PhD in Chemistry in 2009 from the University of Oviedo (Spain). She moved to the *Laboratoire d'Electrochimie Moléculaire* at the University of Paris Diderot (France) for three years, working under Dr. Limoges' supervision on the electrochemical study of enzymatic mechanisms for homogeneous and immobilized redox enzymes. She returned to Spain where she is currently a Postdoctoral Researcher in the Electroanalysis Research Group headed by Dr M.J. Lobo-Castañón. Her research interests focus on the development of electrochemical sensors for clinical diagnosis, food analysis and environmental monitoring using molecular recognition elements based on nucleic acids (genosensors and aptasensors).

Dr. Noemí de-los-Santos-Álvarez received her Ph.D. degree in Chemistry in 2002 from Universidad de Oviedo (Spain). She moved to Cornell University for two years under Prof. Abruña supervision working on characterization of solid and nanostructured intermetallic materials for their use as anodes in fuel cell. She moved back to Spain, where she is currently a Lecturer. Her research interest includes the development of electrochemical (bio)sensors for the detection of allergens and pathogens in food, clinical and environmental samples. She is focused on aptasensors and genosensors as well as SPR characterization of biorecognition events.

Dr. Arturo José Miranda-Ordieres received his Ph.D. in Chemistry from Universidad de Oviedo in 1985. Since 1988 he is a permanent Associate Professor in Analytical Chemistry at Universidad de Oviedo. His research interests have always been linked to electrochemistry, from fundamental studies to biosensing: enzymatic sensors, genosensors, aptasensors and biomimetic sensors. He is author of over 100 publications on peer reviewed journals.

Dr. María Jesús Lobo-Castañón holds a PhD in Chemistry and leads the Electroanalysis research group at the Department of Physical and Analytical Chemistry, University of Oviedo in Spain. Since 2001 she is Associate Professor in Analytical Chemistry at said university. Her research interests focus on the development of electrochemical sensors for clinical diagnosis and food analysis, using different molecular recognition elements such as enzymes, DNA, aptamers and molecularly imprinted polymers. She is the author of over 90 articles and book chapters in the field. All her written work is available at the group website http://www.unioviedo.es/electroanalisis/