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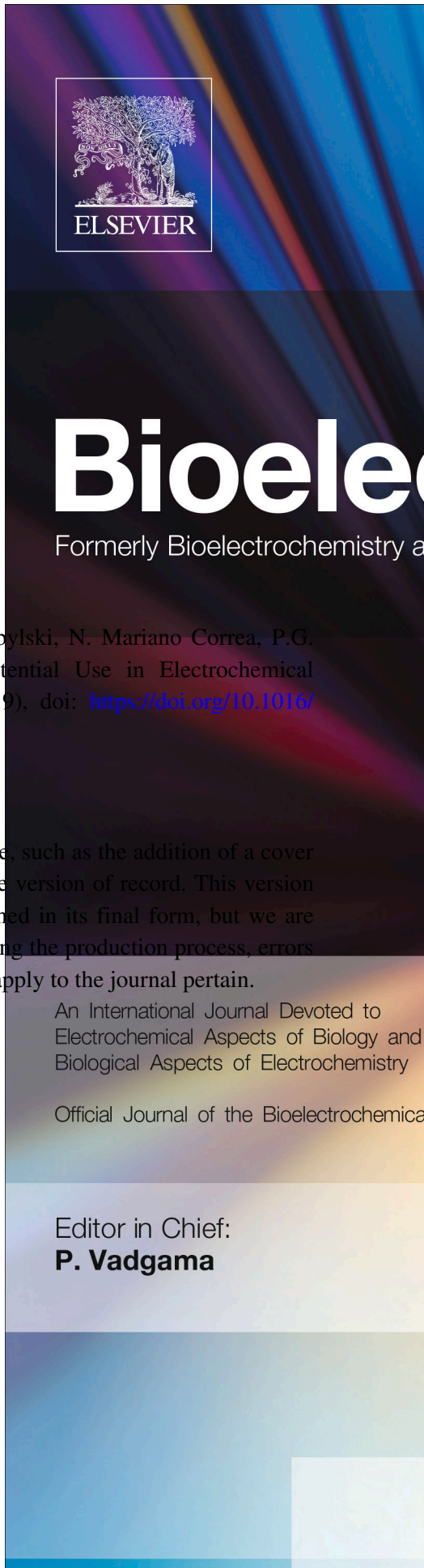

Polyclonal Antibody Production Anti Pc_312-324 peptide. Its Potential Use in Electrochemical Immunosensors for Transgenic Soybean Detection.

Marcos E. Farías[1,2], Mariela M. Marani[4], Darío Ramírez[5], Ana M. Niebylski[1,6], N. Mariano Correa[2,3], Patricia G. Molina[2,3]*.

[1] Departamento de Biología Molecular, Universidad Nacional de Río Cuarto, Fac. de Cs. Exactas, Fco-Qcas. y Naturales. Agencia Postal # 3. C.P. X5804BYA Río Cuarto. Argentina.

[2] Instituto para el Desarrollo Agroindustrial y de la Salud (IDAS). UNRC-CONICET. Facultad de Ciencias Exactas, Físico-Químicas y Naturales. Agencia Postal # 3. C.P. X5804BYA Río Cuarto. Argentina.

[.3] Departamento de Química. Universidad Nacional de Río Cuarto. Facultad de Ciencias Exactas, Físico-Químicas y Naturales. Agencia Postal # 3. C.P. X5804BYA Río Cuarto. Argentina.

[4] Instituto Patagónico para el Estudio de Ecosistemas Continentales (IPEEC), CONICET, CP U9120ACD, Puerto Madryn, Argentina.

[5] Instituto Multidisciplinario de Investigaciones Biológicas de San Luis (IMIBIO-SL), CONICET-Universidad Nacional de San Luis, Fac. de Quím., Bioquím. y Farmacia. C.P. 5700. San Luis. Argentina.

[6] Instituto de Biotecnología Ambiental y Salud (INBIAS) UNRC-CONICET. Facultad de Ciencias Exactas, Físico-Químicas y Naturales. Agencia Postal # 3. C.P. X5804BYA Río Cuarto. Argentina.

pmolina@exa.unrc.edu.ar

TEL 54 358 467 6111

*** Corresponding Author**

Abstract

A new polyclonal antibody that recognizes the CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4-EPSPS), which provides resistance to glyphosate in soybean (Roundup Ready®, RR soybean), was produced. New Zealand rabbits were injected with a synthetic peptide (Pc_312-324, (PEP)) present in the soybean CP4-EPSPS protein. The anti-PEP antibodies production was evaluated by electrophoresis (SDS-PAGE) and an enzyme-linked immunosorbent assay (ELISA) was developed in order to study their specificity. The ELISA showed that the polyclonal antibody was specific to PEP. In addition, the anti-–PEP was immobilized onto a gold disk electrode and the antigen-antibody interaction was evaluated using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Moreover, the EIS showed that the electron transfer resistance of the modified electrode increased after incubation with solutions containing CP4-EPSPS protein from RR transgenic soybean, while no changes were detected after incubation with no-RR soybean proteins. These results suggest that the CP4-EPSPS was immobilized onto the electrode, due to the specific interaction with the anti-PEP. These results show that this antigen-antibody interaction can be detected by electrochemical techniques, suggesting that the anti-PEP produced can be used in electrochemical immunosensors development to quantify transgenic soybean.

Keywords: polyclonal antibody; transgenic soybean; CP4-EPSPS protein; electrochemical immunosensor.

1. Introduction

During the last two decades, there has been an increasing trend for the commercial use and the worldwide proliferation of genetically modified (GM) plants [1]. World Health Organization (WHO) defines GM organisms (GMO) as those in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and / or natural recombination [2]. These organisms are generated to express a particular characteristic by introducing foreign genes that express the wanted protein [3]. In 2016, as much as 78% of soybean acres were planted with genetically engineered (GE) varieties resulting in the highest adoption rate of GE crops globally [4].

One of the most representative GM crops is the soybean GTS 40-3-2 event, which is commercially known as Roundup Ready® (RR) soybean since it was specially developed to be tolerant to the herbicide with the same name. This event contains an introduced construction with a gene of cp4 epsps (aroA:CP4) from the *Agrobacterium tumefaciens* strain CP4 expressing the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) which has a major role in some essential aromatic aminoacids synthesis [5,6]. CP4-EPSPS decreases the binding affinity to glyphosate, conferring increased tolerance to the glyphosate herbicide [5,7,8]. In plants with no resistance, glyphosate binds to EPSPS blocking the 5-enolpyruvylshikimate-3-phosphate synthesis depriving plants of essential aminoacids and secondary metabolites [9].

There is no scientific consensus when it comes to GMO safety or potential adverse effects on human health and environmental safety. However, the possible risk of transgenic components of RR soybean in related food products has become an important issue. To ensure food safety control, all the food stuffs derived from genetically modified technology must undergo a comprehensive evaluation before entering the market. Such assessment is of legal importance as part of the regulatory approval [10,11].

Since there are no concerns about the possible impact of GM foods on public health and on the environment, some governments have introduced regulations on food labeling [12,13]. This has generated interest in the development of analytical methods for the accurate, rapid, sensitive and low-cost detection of GM organisms in agricultural crops and food. Currently, different methods of detecting GM organisms have been developed in food samples, based mainly on the detection of proteins and DNA [14], using conventional methodologies (i.e., ELISAs and PCR), that usually result laborious, expensive, and time-consuming [15].

Currently, biosensors have become the most emerging and attractive alternative platforms for the detection of GMO in food and has been highlighted as one of the most promising ways to solve pertinent issues in relation to simple, fast, reproducible, and low cost multitarget detection, with special focus for potential automation, leading to a high demand for biosensing technologies in food sustainability [16,17].

In this sense, immunosensors, based on antigen-antibody reactions, are very sensitive, specific and, in addition, they have a primary role in the measurement of specific compounds in biological matrices, such as blood and plasma. Compared to traditional immunoassay methods, electrochemical immunosensors can offer analysis with multiple targets, with the advantage that they can be miniaturized, resulting in simple, fast, reliable and inexpensive devices being able to perform real time and *in situ* detections [18,19,20]. Moreover, considering that the working electrode is one of the main components of electrochemical immunosensors, the fabrication and modification

processes can be improved to amplify the response signal, thus increasing the sensitivity of the sensor [21]. One approach is to immobilize the antibodies or the antigens onto the electrode by using highly biocompatible materials, such as Au nanoparticles [22].

For the construction of an immunosensor, it is required an antibody (Ab) to recognizes the antigen of interest. Polyclonal antibodies (antibodies with varying affinities and specificities) can be produced by injecting the antigen into an animal (mouse, rabbit or goat). Rabbits are widely used for the development of a large variety of monoclonal and polyclonal antibodies due to the ability to respond to epitopes of human antigens that are not immunogenic in rodents and the ability to generate immune response to small molecules, among others [23,24].

In this sense, rabbit polyclonal antibodies have been used as analytical tools for immunological techniques development and as an important tool for food safety assessments [25]. Polyclonal antibodies are considered as more efficacious than monoclonal antibodies due to their ability to bind to multiple epitopes on an antigen [26,27].

For the specific detection of CP4-EPSPS, some monoclonal antibodies can be commercially acquired, but the CP4-EPSPS protein is currently unavailable, hindering the development of immunodiagnostic-based methods. Because of this, Marani et. al. proposed to synthesize a set of peptides that could be potential antigens for the production of new antibodies against CP4-EPSPS protein present in transgenic soybean by using bioinformatics [28].

In this work, we produced a new polyclonal antibody by using one of these candidates peptides proposed by Marani et al. [28] synthesized in our laboratory, to immunize the animals. This antibody was then probed to recognize the CP4-EPSPS protein, while linked into an electrode surface to evaluate the potential use in an electrochemical immunosensor for CP4-EPSPS sensitive, rapid and specific detection.

2. Experimental

2.1 Pc_312-324 peptide synthesis

Pc_312-324 Peptide (PEP), whose amino acid sequence is shown in Schematic 1, was selected by *in silico* evaluation based on their sequence and immunogenicity [28] Peptide was manually synthesized using a solid phase approach with Fmoc/t-butyl chemistry [29]. Peptide elongation was carried out in polypropylene syringes fitted with a polyethylene porous disk. Solvents and soluble reagents were removed by suction. A Rink amide MBHA resin (Peptides International) was used for the synthesis of the peptides. A cysteine residue was incorporated at the C-terminus to allow subsequent coupling. Sample was treated with trifluoroacetic acid/triisopropylsilane/water (TFA/TIS/H2O) (95:2.5:2.5) to remove the protecting groups. The formula ($A_{215}-A_{225}$) × 144 (μg/mL) was applied for peptide quantification [30]. Peptide synthesis was evaluated by high performance liquid chromatography-mass spectrometry (HPLC-MS).

2.2 Animals

New Zealand rabbits were used for immunization and antibodies production. During the immunization period the animals were housed in standard bioterium conditions with bedding and provided with water and feed *ad libitum*. Animal rooms were maintained

with a constant temperature (20 ± 2 °C) and relative humidity (40%–60%), in a 12:12 h light:dark photoperiod using artificial illumination. Procedures concerning animal treatments and experiments in this study were reviewed and approved by the Animal Use Ethics Committee of the UNRC (CoEdi) (Exp. 116/2015) being in accordance with the bioethics guidelines of the National Research Council, regarding care and use of Laboratory Animals, minimization of suffering, and number of animal used [31].

2.3 Antibody production and evaluation

For immunization the Pc_312-324 synthetic peptide was used. Keyhole Limpet Hemocyanin (KLH, ThermoFisher Scientific) was used as a carrier protein to guarantee the immunogenicity of the peptide, which was linked to it using m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS, Sigma-Aldrich). Freund's Complete Adjuvant (FCA, Sigma-Aldrich) was used to increase the immune response in the animals. KLH-PEP complex was formed following the protocol for coupling with heterobifunctional reagent according to Coligan [32].

The used immunization schedule is represented in Schematic 2. Before immunization, 2 mg/ml KLH-PEP solution was mixed with an equal volumen of FCA to make an emulsion. 1 ml of the emulsion was injected subcutaneosly in the back of each rabbit, in 4 different sites, on days 0 and 60. Animals were also boosted with KLH-PEP solution on days 14, 28, 72 and 156. Serum was obtained on days 0 (pre-immune serum, PIS), 28, 72 and 160 (immune serum, IS). The final doses used in each immunization was 1 mg KLH-PEP/ml/animal.

In all cases, blood samples were taken from marginal ear vein [33] after animals were anesthetized with ketamin/xylacin 50 mg/Kg and 5 mg/kg respectively [34]. Immune serum was obtained and immunoglobulin G (IgG) fraction was purified by precipitation using saturated ammonium sulfate solution (SAS). The serum was filtrated (nylon filter 0.45 μm) and SAS was added gradually up to a 40% concentration, precipitation was overnight at 4°C with continuous stirring. The suspension was centrifuged at 10000g for 30 min and the pellet was resuspended in phosphate buffer saline (PBS) and dialysed in PBS for 4 hours at 4°C in order to remove the $(NH_4)_2SO_4$. IgG concentration was determined using a commercial kit.

The production and purity of IgG was evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). We used acrylamide 12% resolving gel and 4% stacking gel. After 1-hour electrophoresis (MiniProtean, BioRad) the gel was stained with Coomassie Brilliant Blue.

2.4 Antibody specificity evaluation

ELISA was used in order to determine the specificity of the produced antibody against the antigen. The microtiter plate (Greiner Bio-oneTM MicrolonTM) was coated with antigen (PEP or solutions from RR and no-RR soybean seeds) by incubating the wells with 100 μl of solution containing 50 μg of antigen for 40 min at 37°C with gently stirring. After washing with PBST, the wells were blocked with 1% w/v skim milk in PBS for 40 min at 37°C. The wells were washed thrice and then were incubated with 50 μl of different dilutions of the obtained IgG for 40 min at 37°C. After washing 4 times, 50 μl of anti-rabbit antibody conjugated with horse radish peroxidase (Sigma-Aldrich) diluted 1:5000 v/v in washing buffer was added. The microtiter plate was incubated for another 40 min and the wells were washed 4 times. The assay was developed by adding 50 μl of 3,3',5,5'-tetramethylbenzidine (TMB) (1-step ultra TMB-ELISA substrate

solution, ThermoFisher Scientific), after 15 min incubation and addition of 50 µl of sulfuric acid (2 M) the absorbance was measured at 450 nm using a microplate reader.

2.5 Electrochemical measurements

2.5.1 AuNp synthesis

In order to immobilize the anti-PEP onto the working electrode, gold nanoparticles were synthesized according to Bethel et al. (1996) [35], with minor modifications by Monerris et al. (2012) [36]. $HAuCl_4$ solution in toluene (1.15×10^{-3} M) was added into a $NaBH_4$ aqueous solution (5.3×10^{-2} M) under constant strirring. Then the aqueous phase was recovered and the AuNp size was determined by UV-Vis spectroscopy [37] and atomic force microscopy (AFM).

2.5.2 Reagents, techniques and equipment

Electrochemical measurements were performed to evaluate the produced antibodies ability to detect the PEP used as the antigen, and the possibility to detect the recognition event between the anti-PEP and CP4-EPSPS. The working electrode used was a gold disk (area=0.126 cm^2). It was polished, sonicated and copiously rinsed with distilled water. With the purpose of obtaining good response the electrode was cycled several times in the H_2SO_4 (95-98% purity, Cicarelli) 0.5M solution prior to use. The counter electrode was a platinum foil of large area (2 cm^2). A freshly prepared Ag/AgCl KCl (3 M) reference electrode was used. In all experiments, lithium chloride (LiCl, Frederick Smith Chemical Company) solution (0.1 M) was used as supporting electrolyte. The pH values were measured with pH-meter Orion 720A calibrated with different buffer solutions.

For the modification of the electrode, cysteamine (Cys) (Sigma-Aldrich), AuNp nanoparticles synthetized in our laboratory and, anti-PEP purified from the IS by precipitation with SAS were used. For the synthesis of the AuNp, tetrachloroauric acid (HAuCl4) (Sigma-Aldrich), toluene and sodium borohydride ($NaBH_4$) (Sigma-Aldrich) were used. 3% skim milk solution was used as the blocking solution.

For electrochemical measurements potassium ferrocyanide ($K_4[Fe(CN)_6]$, Mallinckrodt) and hydroquinone (HQ, Sigma Aldrich) were used as the electroactive molecules.

The voltammograms were taken with AutoLab PGSTAT 204 potentiostat controlled by Nova 2.1. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used. The CV were performed at 100 mV / s. For the EIS, we worked in a frequency range from 0.1 Hz to 100 KHz, with an amplitude of 10 mV. The working potentials were 0.16 V for $K_4[Fe(CN)_6]$ and 0.3 V for HQ.

2.5.3 Electrode modification

The electrode was cleaned as indicated above. The preparation of the modified electrode is shown in Schematic 3 A. Firstly, the pre-cleaned Au electrode was incubated in 0.1 M cysteamine in ethanol for 2 hours at room temperature and rinsed with ultrapure water. Then it was immersed in AuNp solution for 30 min and rinsed again. Next, 10 µl of a polyclonal anti-PEP dilution (1:10) was dropped onto the electrode surface and left overnight in a moist chamber at 4°C. After modification, in order to exclude nonspecific adsorption, the electrode was incubated in a skim milk solution (3%) in PBS for 10 min at 37°C and rinsed extensively with ultrapure water. In order to verify the modification,

after every step of the modification procedure, CV and EIS were performed to a 1 x 10^{-3} M $K_4[Fe(CN)_6]$ with 0.1 M LiCl solution.

2.5.4 Peptide recognition test

Once modified, the electrode ability to detect PEP and lead to a measurable signal was probed. The modified electrode with anti-PEP was incubated in a PEP solution in PBS (1 mg/ml) for 30 min at 37°C and rinsed with ultrapure water. Then IES was performed to 1 x 10^{-3} M $K_4[Fe(CN)_6]$ with 0.1 M LiCl solution (Schematic 3 B).

2.5.5 CP4-EPSPS protein detection from RR and no-RR soybean samples

In order to evaluate the modified electrode with anti-PEP ability to recognize not only the pure PC_312-324 peptide but also to detect the CP4-EPSPS protein from a real sample, total proteins from RR and no-RR soybean seeds were extracted. The Instituto Nacional de Tecnología Agropecuaria (INTA) gently supplied both RR and no-RR soybean seeds. The proteins were extracted using the method described by Wu et al. [38] and Ocaña et al. [399].

Seeds were frozen in liquid nitrogen and ground to a fine powder using a mechanical grinder. The samples were mixed with cold acetone for 45 min and then centrifuged at 4000 *g* for 10 min at 4°C. This procedure was repeated twice to remove lipids, and left to dry overnight at 4°C. Extraction buffer TPBS (0.05% Tween-20, 2% SDS, PBS) was added to the dried defatted soybean powder and incubated for 30 min with continuous mixing, and then centrifuged at 20000 *g* for 15 min. The supernatant was recovered and dialyzed against PBS [38,39].

2.5.6 Electrochemical CP4-EPSPS protein detection

The modified electrode with anti-PEP was incubated with diluted no-RR (1:10) or RR soybean proteins (1:10 and 1:50) in PBS for 30 min at 37°C and rinsed with ultrapure water. After each incubation, IES was performed to a 1 x 10^{-3} M HQ with 0.1 M LiCl solution.

3. Results and discussion

3.1 Antibody production

The anti-PEP production and purification by precipitation were evaluated by SDS-PAGE. Figure 1 shows the SDS-PAGE of the serum proteins and the IgG fraction precipitated with ammonium sulphate of immunized rabbits. The IgG obtained from the gel image had a molecular mass of about 150–200 kDa, which is similar to the previously reported molecular mass [40,41]. A low concentration of IgG was observed in the pre-immune serum, when compared with the immune serum. Moreover, the crude serum contained an abundance of albumin, which could be seen as a thick band located at 57 kDa in the gel image, while this band intensity is lower when precipitated with ammonium sulphate. In addition, the intensity of IgG band in the samples obtained from precipitation was higher, indicating that SAS was enough for precipitating most of the IgGs from the immune serum. These results indicate that the immunization schedule generated an immune response in the animals, allowing to IgG production. On the other hand, a very good recovery and concentration of IgGs were achieved. Mariam et al. [40] evaluated different SAS concentrations for IgGs precipitation of rabbits immunized with hepatitis B antigen, obtaining a high IgGs purification factor with an optimal yield

(99%) when using 40% SAS, as used in this work. According to Mariam et al. [40] SAS precipitation is a simple, relatively low cost method that can be used in antibodies purification, allowing to a great recovery of these antibodies for posterior treatments in purification procedure.

3.2 Specificity evaluation: ELISA

The specificity of the produced anti-PEP was evaluated by ELISA. Figure 2 shows the absorbances obtained from different dilutions of immune serum samples taken at days 28, 72 and 160 of the immunization schedule. As can be observed, on day 28 an absorbance peak appears only in the 1:10 dilution. This indicate the presence of specific anti-PEP antibodies against the peptide in the sample, being 1:10 dilution the optimal concentration that increased the ELISA sensibility allowing to detect the anti-PEP presence. On the other hand, the absorbance at 450 nm (A_{450nm}) increased on serum samples corresponding to days 72 and 160. These results not only indicate that the obtained anti-PEP is specific against the PEP but also that the anti-PEP concentration in sera increased after every boost during the immunization schedule. As can be seen, there is no difference between the crude serum and the SAS precipitated IgGs, indicating that precipitation allowed to an excellent recovery of IgGs. Since 1:10 dilution showed the major A_{450nm} for all samples, it was taken as the working dilution in the following studies.

3.3 Electrochemical measurements

3.3.1 Electrode modification

In order to evaluate the antibodies ability to recognize and detect the PEP using electrochemical procedures, an Au disk electrode was modified with anti-PEP. The stepwise modification process of the Au disk electrode was characterized by CV. and EIS. The electrode response changes after each step were studied.

Figure 3 A shows the cyclic voltammograms of 0.01 M $K_4[Fe(CN)_6]$ with 0.1M LiCl solution recorded for each step of modifying the electrode. As can be seen, each step of the modification procedure exhibits changes in the cyclic voltammograms. For the bare electrode (Fig. 3A i) the highest current was observed, with well-defined anodic and cathodic peaks, characteristic of a one-electron reversible redox couple [42]. After modification with Cys and AuNp, both peak current and peak potential decreased (Fig. 3A ii). Moreover, after anti-PEP antibody assembling and skim milk blocking (Fig. 3A iii and iv, respectively) the peak currents of the redox couple further decreased, which would indicate that proteins layers onto the electrode surface retarded the electron transfer.

In EIS the impedance changes of the electrode surface can be analyzed through of a Nyquist plot. The semicircle of the plot defines the charge transfer resistance of the redox reaction while the straight-line represents the charge diffusion at the electrode surface. Interestingly, the charge transfer resistance value increased greatly after the accumulation of Ab or antigen on the surface, indicating that the step of modifying the electrode inhibits the access of the electron flow by acting as a biological barrier. Figure 3B shows the impedance changes of the different steps of the electrode surface modification. As can be seen in Figure 3B i, the bare Au electrode shows a linear plot, indicating a good conductive behavior. After Cys-AuNp self-assembling, the response exhibits no considerable changes in the impedance (Figure 3B ii) indicating that the electron transfer slightly decreased, compared to response of the bare electrode. This

could be due to the tunneling effect of the sandwich structure of metal-insulator-metal nanoparticles which achieves the electron transfer when compared with tunneling the electron between metal and redox species in solution [43,44]. It is also known that AuNP allow a better immobilization and orientation of proteins making the direct electron transfer more favorable [45]. The electrochemical impedance increased after immobilization of the anti-PEP antibody (Fig. 3B iii) showing an increased semicircle diameter, indicating an electron transfer resistance between the solution and the electrode surface, resulting from the immobilized anti-PEP. After blocking the electrode with skim milk, the resistance increased further (Fig. 3B iv) due to non-specific adsorption on the surface of the modified electrode. These results confirmed what was observed through CV, making it possible to ensure that the electrode was modified successively until the immobilization of the anti-PEP antibody.

In addition, the Au disk electrode morphology was studied during the different steps in the modification process by in situ high-resolution atomic force microscopy (AFM). Figures 4 A and B show the bare Au surface and the Cys-Au modified surface after AuNp assembly, respectively. It can be seen that the surface is covered with AuNP, which showed an average diameter of 40 nm, being this result in agreement with that obtained by UV-Vis spectroscopy where an absorbance peak was observed at approximately 526.8 nm, which would correspond to a diameter of 38 nm [37]. Figure 4 C shows the surface changes after the anti-PEP antibody was immobilized onto the AuNP-cys-Au surface. It can be observed, that the surface is covered with anti-PEP.

The EIS of the modified Anti-PEP-AuNp-Cys-Au electrode, after being immersed in a 1 mg/ml PEP solution for 30 min, is shown in Figure 5. As can be seen, an important increasing of the semicircle diameter is observed compared to the modified electrode response before peptide solution incubation. This would indicate that the anti-PEP antibody recognized the PEP in solution, leading to the immunocomplex PEP-anti-PEP formation, which difficult the electron transfer between the redox couple and the electrode interface.

3.3.2 CP4-EPSPS protein detection

EIS measurements were made after incubation of the Anti-PEP-AuNp-Cys-Au modified electrode with no-RR and RR proteins solutions at different concentrations. No difference in the EIS was detected before and after incubation of the modified electrode with no-RR soybean proteins solution (Figure 6 a and b, respectively). On the other hand, the electrochemical impedance increased after incubation of the modified electrode with RR soybean protein solution diluted 1:50. Moreover, after incubation with the solution diluted 1:10, the impedance increased further (Figure 6 c and d, respectively). These results not only indicate that the produced antibodies are able to recognize the CP4-EPSPS protein in a real soybean sample, but also suggest that these polyclonal antibodies are specific against the transgenic protein, without showing a cross reaction with the native no transgenic EPSPS protein. On the other hand, the changes observed in the electrode response indicates that the immunocomplex formation could be detected quantitatively by electrochemical methods, so the produced polyclonal antibody could be used for the construction of an electrochemical immunosensor for transgenic soybean detection or immunoaffinity columns development for the CP4-EPSPS protein extraction, which is not commonly available. Similar results were obtained by ELISA, showing no changes in the A_{450nm} when coating the plate with non-transgenic soybean, while an increment in the A_{450nm} was observed when using RR transgenic soybean. On this basis, future studies will be

carried out to find the optimal conditions to develop an immunosensor that allows the quantitative detection of transgenic soybean.

4. Conclusion

A new polyclonal antibody that recognizes the CP4-EPSPS protein from RR soybean was produced in rabbits. In addition, the presence and specificity of the antibody against the transgenic soy protein peptide was verified through an ELISA assay.

In addition, a gold electrode was successfully modified by immobilizing the anti-PEP antibody onto its surface. The electrochemical response of the modified electrode, confirm that the antibody is specific against the CP4-EPSPS protein, without cross reaction with the native no transgenic EPSPS protein, and, in addition, that the immunocomplex formation was able to be detected by electrochemical measurements, with different responses when different RR soybean proteins concentrations were used. This would allow to use the produced polyclonal antibody in the construction of a quantitative electrochemical immunosensor for transgenic RR soybean detection.

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Figure captions

Schematic 1: Amino acid sequence of the peptide Pc_312-324.

Schematic 2: Schematic illustration of the immunization schedule.

Schematic 3: Schematic illustration of (A) the fabrication process of the modified Au electrode, and (B) the PEP detection process.

Figure 1. Non-reducing SDS–polyacrylamide gel (12%) electrophoresis of: lane (M), protein molecular weight markers in kDa; lane (1) crude immune serum of rabbit 1 corresponding to day 160; lane (2), crude immune serum of rabbit 2 corresponding to day 160; lane (3), crude pre-immune serum of rabbit 1; lane (4), IgG precipitated with SAS 40%, from immune serum.

Figure 2. ELISA results of: immune serum obtained on day (a), 28; (b), 72; (c), 160; and of (d) SAS precipitated IgGs from immune serum obtained on day 160. HRP diluted 1:5000 was used as the secondary antibody.

Figure 3. (A) CV and (B) EIS of the gold electrode modification process in 1 x 10^{-3} M $K_4[Fe(CN)_6]$ with 0.1 M LiCl. (i) bare Au electrode, (ii) AuNp-Au, (iii) Anti-PEP-AuNp-Au, (iv) blocked Anti-PEP-AuNp-Au. Reference electrode: Ag/AgCl. CV: scan rate= 100 mV/s. EIS: working potential= 0.16 V, frequency range= 0.1–10 kHz.

Figure 4. AFM images of (a) bare Au surface (b) AuNP-cys-Au surface, and (c) Anti-PEP-AuNP-cys-Au surface.

Figure 5. EIS and of the CP4-EPSPS peptide detection by the modified electrode in 1 x 10^{-3} M $K_4[Fe(CN)_6]$ with 0.1 M LiCl. (a) modified electrode, (b) modified electrode incubated in PEP solution. Reference electrode: Ag/AgCl. Working potential= 0.16 V, frequency range= 0.1–10 kHz.

Figure 6. EIS and of the CP4-EPSPS soybean protein detection by the modified electrode in 1 x 10^{-3} M HQ with 0.1 M LiCl. (a) modified electrode, (b) modified electrode incubated in no-RR soybean proteins dilution (1:10), (c) modified electrode incubated in RR soybean proteins dilution (1:50), (d) modified electrode incubated in RR soybean proteins dilution (1:10). Reference electrode: Ag/AgCl. Working potential= 0.3 V, frequency range= 0.1–10 kHz.

Figure 1

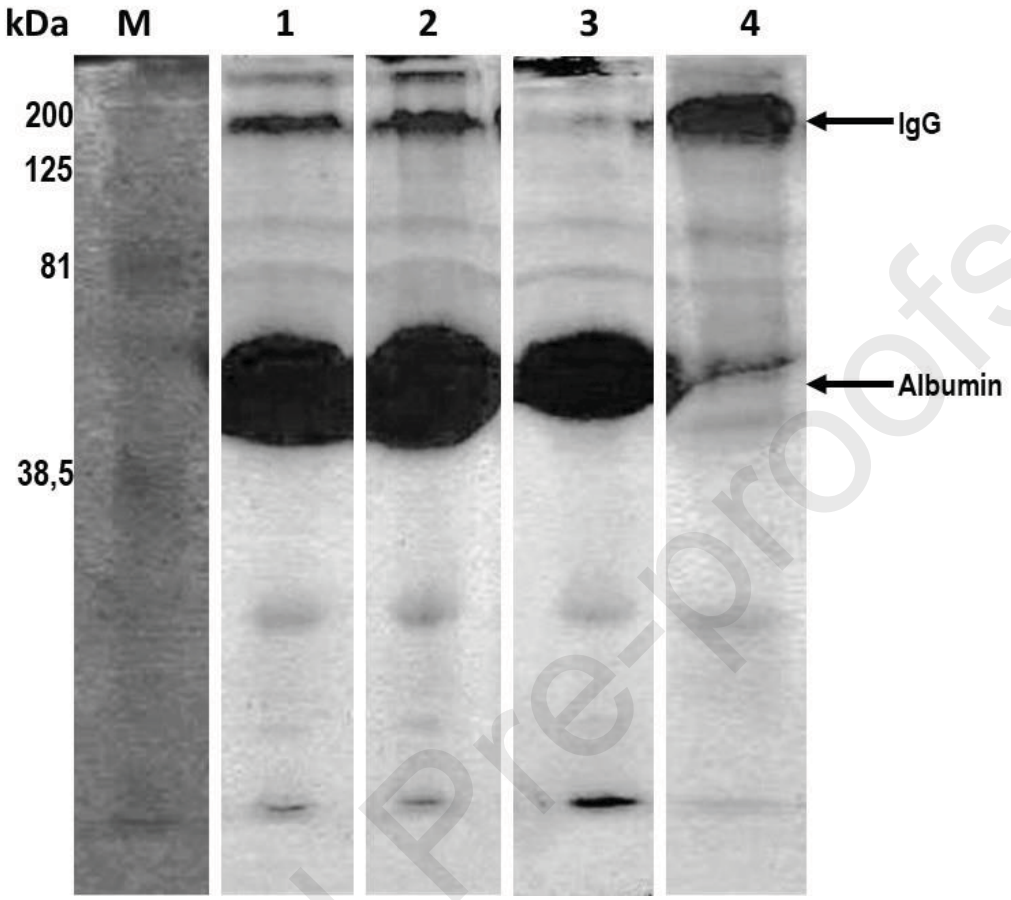


Figure 2

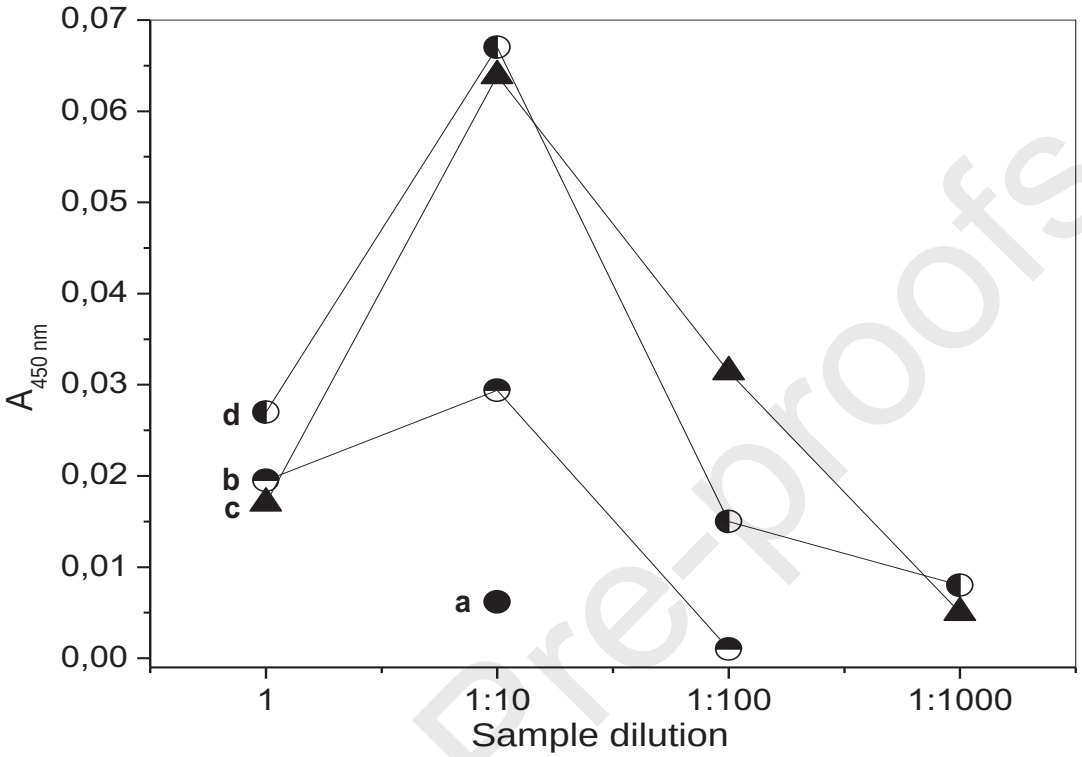


Figure 3

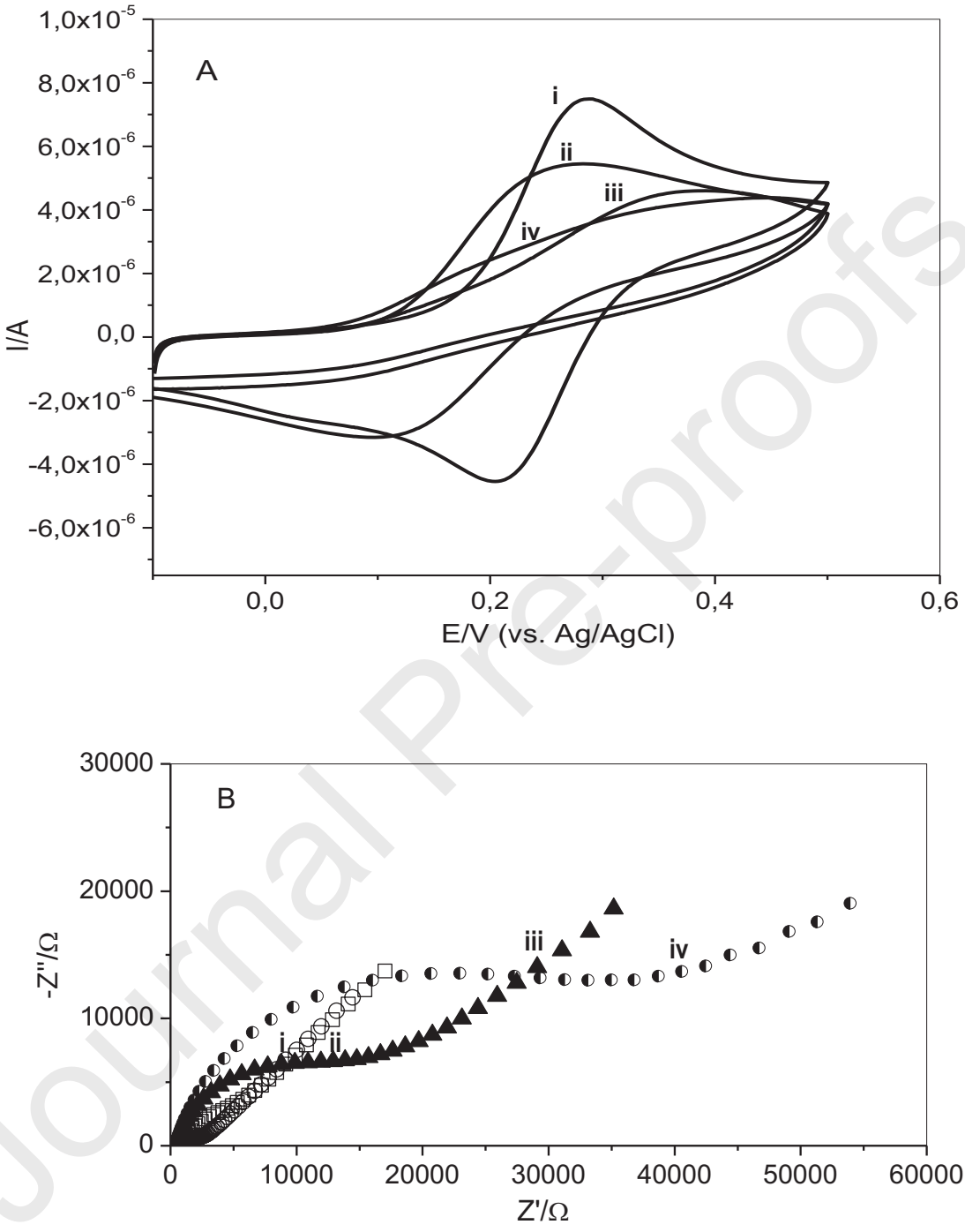


Figure 4

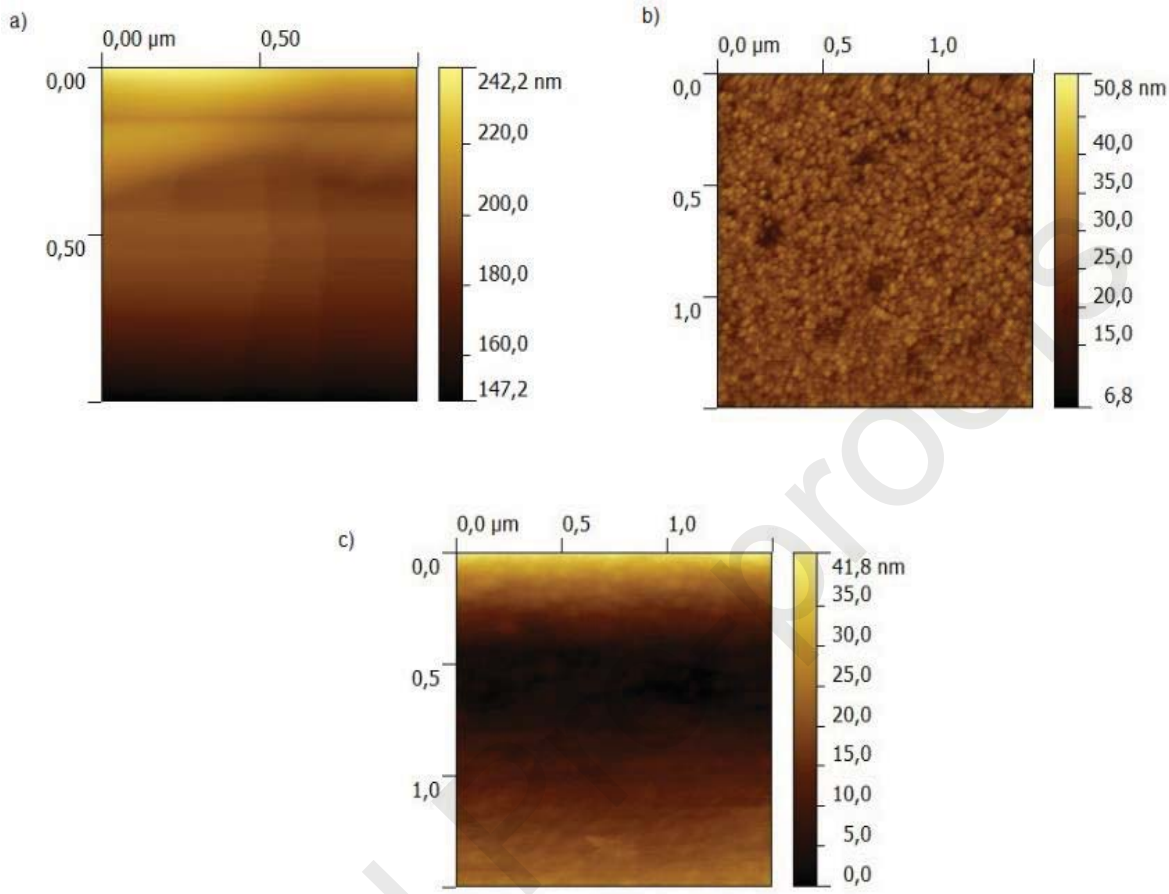


Figure 5

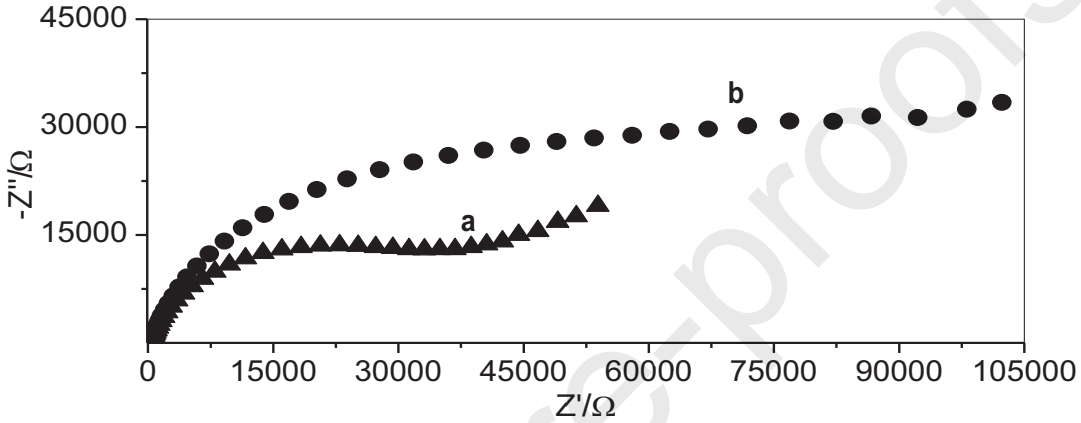
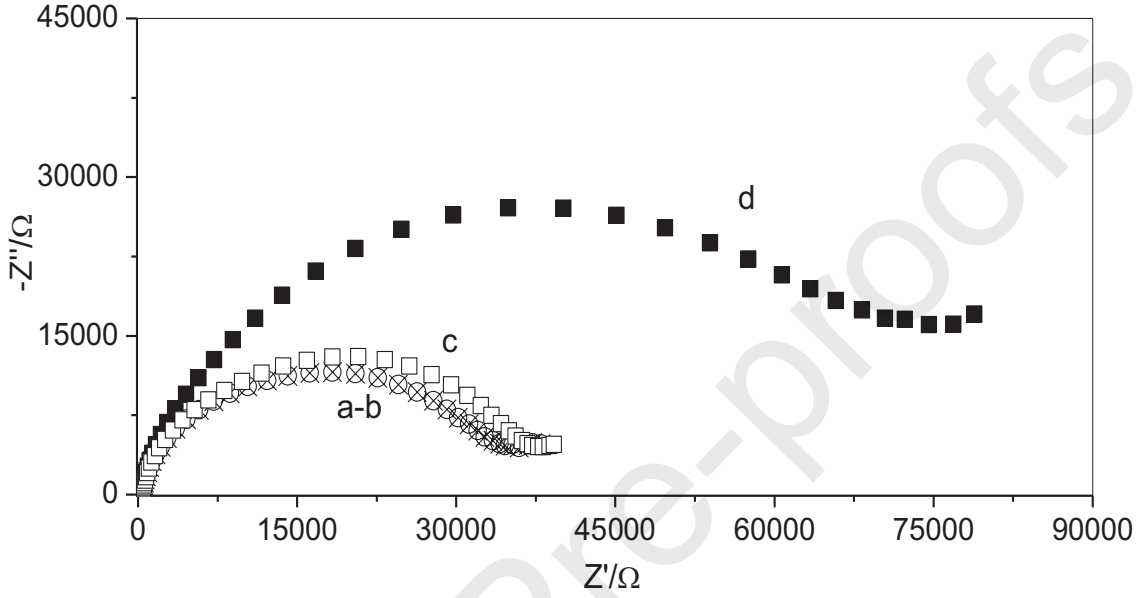


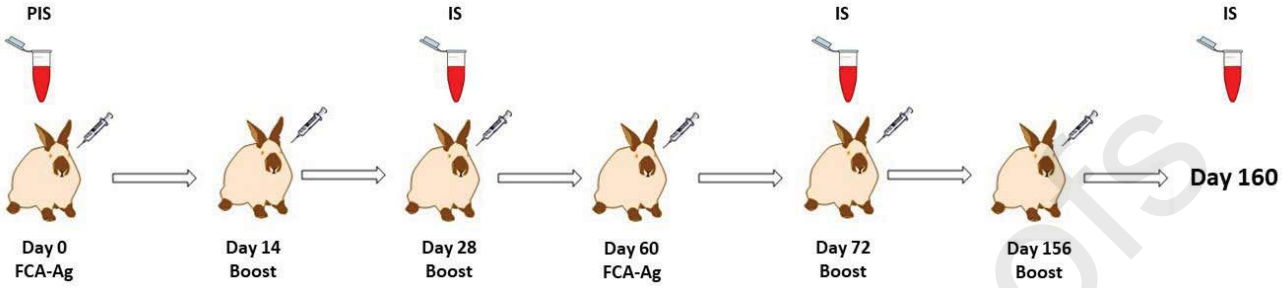
Figure 6



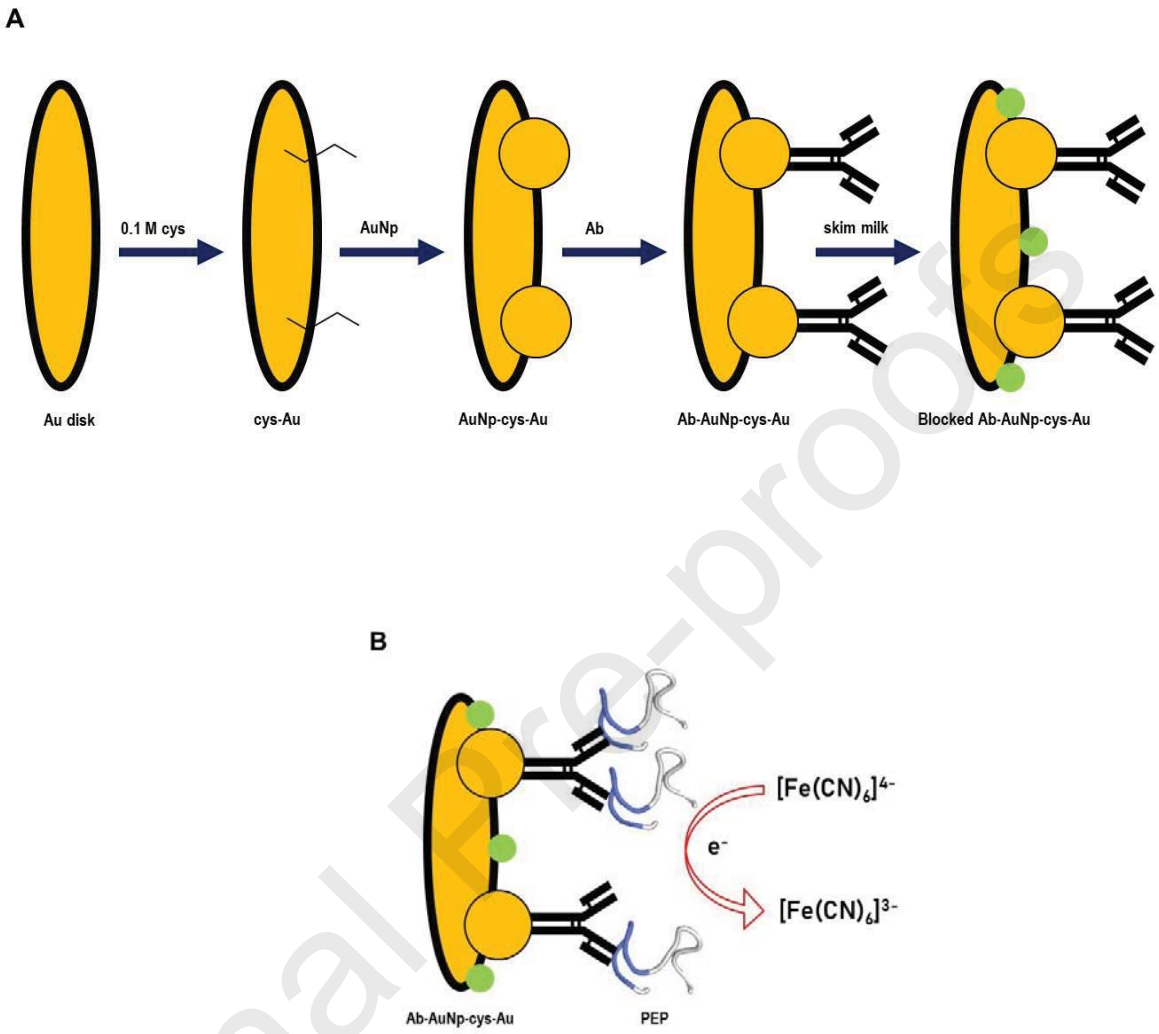
Schematic 1.

Lys-Gly-Val-Thr-Val-Pro-Glu-Asp-Arg-Ala-Pro-Ser-Met-Cys

Schematic 2.



Schematic 3.



Conflict of interest: none

Highlights

A Peptide from transgenic soybean CP4-EPSPS protein was synthetized.

The Peptide was used as antigen for a new Polyclonal Antibody production.

New Antibody specifically recognizes to synthetized Peptide and CP4-EPSPS protein.

The Antibody can be used in sensor development for transgenic soybean detection.