Carbohydrate Polymers 89 (2012) 586–591

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

A novel xyloglucan film-based biosensor for toxicity assessment of ricin in castor seed meal

R.F. Furtado[a,b], C.R. Alves[c], A.C.O. Moreira[d], R.M. Azevedo[d], R.F. Dutra[a,*]

[a] Biomedical Engineering Laboratory, Federal University of Pernambuco, Av. Prof. Moraes Rego 1235, 50670-901 Recife-PE, Brazil
[b] Embrapa Tropical Agroindustry, Fortaleza-CE, Brazil
[c] Department of Chemistry, State University of Ceara, Fortaleza-CE, Brazil
[d] Department of Pharmacy, Fortaleza University, Fortaleza-CE, Brazil

ARTICLE INFO

Article history:
Received 21 September 2011
Received in revised form 5 February 2012
Accepted 17 March 2012
Available online 26 March 2012

Keywords:
Castor seed meal
Ricin
RCA_{60}
Biosensor
Xyloglucan

ABSTRACT

Oil from the seed of the castor plant (*Ricinus communis* L.) is an important commodity for a number of industries, ranging from pharmaceuticals to renewable energy resources. However, the seed and subsequent seed meal contain ricin (RCA_{60}), a potent cytotoxin, making it an unusable product for animal feed. In order to investigate the efficiency of reducing the toxicity of the seed meal, a biosensor is proposed by exploring the lectin–carbohydrate binding. A gold electrode was assembled with a film of Xyloglucan (XG) extracted from *Hymenaea courbaril* L. The analytical response to RCA_{60} was obtained using a polyclonal antibody against RCA_{60} conjugated to peroxidase. The current responses were generated by reaction with H_2O_2 and amplified with hydroquinone as chemical mediator. Voltammetric studies showed that the XG film was tightly bound to the gold electrode. This biosensor allows discriminate lectins in native and denatured forms. The limit of detection of native RCA_{60} was 2.1 $\mu g\,mL^{-1}$. This proposed biosensor showed to be a potential and accurate method for toxicity assessment of the ricin in castor seed meal by simple polysaccharide film-electrode strategy.

1. Introduction

Ricin (RCA_{60}) is a harmful lectin found in castor seeds. This protein has about 66 kDa that makes up 1–5% by weight of the bean of the castor plant, *Ricinis communis*. Ricin is a type II-ribosome-inactivating protein (RIP) composed of an A-chain (32 kDa) that is disulfide-bonded to a B-chain (32 kDa). The B-chain has lectin-binding properties that allow it to bind to complex galactosides of cell-surface carbohydrates, while the A-chain has enzymatic activity (Olsnes & Koslov, 2001). The B-chain has about three carbohydrate-binding sites, each of which binds to the β-D-galactopyranoside (β-Gal) or β-D-N-acetylgalactosamine (β-GalNAc) residue in host glycoconjugated. The B-chain is considered to play an essential role in the internalization of the toxic A-chain (Olsnes, 2004; Venkatesh & Lambert, 1997). Because ricin acts rapidly and irreversibly (Audi, Belson, Patel, Schier, & Osterloh, 2004) post exposure therapy is more difficult than with other biologic agents (such as the botulinum toxins or bacterial agents) that can be treated with antibiotics.

The castor seed meal contains large amount of minerals and proteins. However, the presence of RCA_{60} makes it difficult for the castor seed meal to be used as feed ingredient for farm animals. Conventionally, prior to use, the castor meal goes through a detoxification process in order to neutralize the RCA_{60} (Barnes, Baldwin, & Braasch, 2009). After this procedure, it is required that the castor seed meal is subjected to a quality assessment of detoxification process. It is possible to prevent the death of animals and allow for safer use down the line. In this sense, the development of practical methods presenting lower cost for analysis that permit atoxicity evaluation of ricin in castor seed meal are desirable.

Several methods have been successfully developed for quantifying ricins and their isophorms in water or other mediums. These methods are based on detection by antibodies against ricin such as enzyme-linked immunoassay (Griffith, Newman, & Gee, 1986), chromatographic (Shyu, Shyu, Liu, & Tang, 2002) or based on recognizing by molecular weight as mass spectrometry (Lubelli et al., 2006). In order to minimize some difficulties such as cost of analysis and time consuming, biosensors have also been developed. Commonly, they are based on the specificity of antibodies (Narang, Anderson, Ligler, & Burans, 1997; Yin, Jia, Yang, Wang, & Zhang, 2012). However, the detection by exploring the interaction between carbohydrate–lectins has been performed. Uzawa et al. (2008) used synthetic carbohydrate chips for ricin detection and RCA120 differentiation based on

* Corresponding author. Tel.: +55 81 21268200; fax: +55 81 21268200.
E-mail addresses: rosa.dutra@ufpe.br, rosa.dutra@pq.cnpq.br, rfiremandutra@yahoo.com.br (R.F. Dutra).

0144-8617/$ – see front matter © 2012 Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2012.03.053

affinity for β-galactoside carbohydrate by SPR analysis. Gustafson (2003) evaluated the interaction between ricin and artificial cell membranes containing different glycolipids. Herein, since that the main objective is simply evaluating the toxicity of the ricins for a practical approach in animal feed, the carbohydrate–lectin interaction can be strategically explored using a film of natural polysaccharide. It well-knowledge that if ricins are denatured or detoxified they lose their carbohydrate ability to bind to β-Gal and β-GalNAc (Sharon & Lis, 2003). Xyloglucan (XG) is a natural polysaccharide characterized by a main chain of (1→4) linked β-D-glucan and side chains containing units of α-D-xylopiranosil and β-D-galactopiranosil (1→2)-α-D-xylopiranosil, each (1→6) linked to the main chain (Busato, Vargas-Rechia, & Reicher, 2001).

It has been widely used in affinity chromatography due to low cost, high extraction yield and ability to form gels at low concentrations (Amin, Rajabnezhad, & Kohli, 2009). XG is a biopolymer soluble in water as well as other natural gums. When this kind of polymer is used as biofilms, they commonly require crosslinking agents such as epichlohydrin to decrease the solubility and change other properties (Rioux et al., 2002). Thus, the use of natural polysaccharides with galactose units offers great potential for detection of ricin assessment. In this paper, a XG film on a gold electrode is proposed as sensor surface to assessment ricin toxicity. This anomeric characteristic results in high binding efficiency to the affinity matrices and biofilms containing these residues.

2. Materials and methods

2.1. Reagents and materials

Seeds of the *Hymenaea courbaril* L. (Jatobá or Brazilian cherry) were collected in northeastern Brazil. They contain about 40–45% of xyloglucan. Epichlorohydrin, horseradish peroxidase (HRP), hydroquinone and bovine albumin (BSA) were obtained from Sigma Chemical Co., St. Louis, MO, USA. All other reactants were of analytical grade and all solutions were prepared with deionized water.

2.2. Extraction and purification of XG from Hymenaea courbaril seeds

The quiescent seeds boiled for 1 h. Thereafter, the cotyledons were powdered and dissolved in distilled water (1:50, w/v). The material was centrifuged at 10,000 × *g* for 20 min at 4 °C. After centrifugation, the supernatant was precipitated with ethanol 96° GL (1:3, v/v) and the residue was added to distilled water for a new extraction. The pellet was dehydrated with acetone PA overnight, dried under hot air flow, reduced to fine powder (XG) and stored at 8 °C.

2.3. Purification of RCA_{60}

Endosperm of castor bean seeds, Nordestina cultivar from Embrapa-Brazil, was macerated and diluted in 0.15 M NaCl 1:10 (w/v). The samples of seeds were centrifuged at 32,981 × *g* for 20 min at 4 °C. The supernatant was collected and submitted to affinity chromatography according to Appukuttan, Surolia, and Bachhawat (1977). The peak fractions were collected and dialyzed against water, lyophilized, and kept at −20 °C until analysis. Then, the lyophilized material was dissolved in phosphate buffer (PB) pH 6.5 and protein quantification step was performed using the Bradford method (Bradford, 1976).

2.4. Production of the antibodies against RCA_{60}

Antibodies against RCA_{60} were produced in New Zealand rabbits according to Furtado et al. (2011). An amount of 100 μg RCA_{60} was analyzed by sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12% acrylamide). The bands corresponding to lectin were cut, macerated and diluted in 0.15 M NaCl. The antibodies were purified by Sepharose CL-4B activated with CNBr matrice immobilized with the lectin. The antibodies were eluted from matrices with 0.1 M glycine and 0.15 M NaCl, and they were dialyzed against distilled water and then lyophilized. Then the antibodies were diluted in 1% glutaraldehyde solution with four times the concentration of peroxidase for 1 h and they were dialyzed overnight against PB pH 7.0 (Avrameas, 1969). The antibodies against RCA_{60} after being conjugated to peroxidase were used in all remaining studies at 5 μg mL^{-1}.

2.5. Detoxification of castor seed meal

The castor seed meal was purchased from BOM Brasil Óleo de Mamona Ltda., Salvador, Bahia, Brazil. The detoxification treatment of castor seed meal was performed after adding into castor seed meal, 60 g of CaO diluted in water (1:10, w/v) according to Oliveira et al. (2010). The mixture remained for 8 h and after this period was dried for 48 h. The effectiveness of the detoxification chemical treatment was characterized using gel electrophoresis mini system Biorad, SDS-PAGE with 12% polyacrylamide gel (Laemmli, 1970) and haemagglutination test. The dry castor seed meal was diluted (1:8 g mL^{-1}) in NaCl 0.15 M for the evaluation of the biosensor performance.

2.6. Preparation of the XG gold electrode

First of all, the gold electrode (0.025 cm^2 area) acquired from Microquimica (Brazil) was cleaned in four steps: (a) a mechanical polishment of the electrode surface with alumina (0.3 μm); (b) a cleaning in deionized water in an ultrasonic bath for 5 min; (c) by immersing in piranha solution (1:3 – H_2O_2:H_2SO_4) for 3 min, and (d) finally, electrochemical cleaning by 20 successive scans in 0.5 M H_2SO_4 aqueous solution with potential sweep from 0 to 1.5 V.

The gold electrode surface was coated with a XG film containing galactose units. To prepare the film, the electrode surface was immersed (1 h) in 3 g L^{-1} XG solution prepared in 0.2 M epichlorohydrin and 0.014 M NaOH [1:1] previously centrifuged at 2500 × *g*. After this procedure, the coated electrode was rinsed with deionized water and incubated at 3% aqueous BSA solution for 1 h to block non-specific binding.

2.7. Analytical response

The modified electrode with XG film was incubated in samples containing RCA_{60} for 30 min at room temperature. Afterwards, the electrode was incubated for 30 min with antibodies against RCA_{60} conjugated to peroxidase (5 μg mL^{-1}). After rinsing with PB, the electrode was ready for the analytical measurements.

The assays were performed in a three electrochemical system using Ag/AgCl as reference and helical platinum wire as counter electrode immersed in an electrochemical cell (10 mL). The amperometric response was generated by HRP reaction with 60 μmol L^{-1} hydrogen peroxide in the presence of 35 μmol L^{-1} hydroquinone containing 0.1 mol L^{-1} NaH_2PO_4/Na_2HPO_4 (pH 6.5).

Cyclic voltammetry studies were performed at a scan rate of 100 mV s^{-1} with a potential range between −0.4 and 0.6 V in 4 mM $K_3Fe(CN)_6$ and 1 M KCl solution. All measurements were conducted at room temperature (25 °C) in solution purged with N_2 for 15 min. All electrochemical studies were performed using the Potentiostat/galvanostat Autolab® PGSTAT-12 (Eco Chemie B.V., The Netherlands) controlled by GPES software.

Amperometrical measurement was determined by cathodic peak at −100 mV. In order to correct baseline fluctuation or

differences among electrodes, the analytical responses (Δi) were obtained subtracted of the blank, i.e. electrode before ricin incubation step.

2.8. Scanning electron microscopy images

Scanning electron microscope (SEM) images to observe the surface structure of the modified gold electrode with xyloglucan film were obtained on a JEOL JSM 5900. The images were registered at an accelerating voltage of 5 kV.

3. Results and discussion

3.1. Preparation of the XG film

The mechanical strength of the XG matrices cross linked with epichlorohydrin (C_3H_5ClO) is well known and has shown excellent results in the commercial chromatographic methods (Appukuttan et al., 1977). The XG film on gold surface was successfully formed by epichlorohydrin in the presence of sodium hydroxide. When NaOH (7 mM) was added into the solution, the opened epoxy ring of C_3H_5ClO formed a bridge between polysaccharide chains (Silva, Feitosa, Maciel, Paula, & Paula, 2006). The interaction of the polysaccharide film with the gold surface is very complex (Crini, 2005). The process of polysaccharides cross linking with epichlorohydrin, besides forming an insoluble polysaccharides network, may also form other products such as glycerol and polymerized epichlorohydrin.

Chloride from the cross linking reaction of epichlorohydrin and hydroxide ion may influence the hydrophobic nature of the gold surface (Arrascue, Garcia, Horna, & Guibal, 2003; Baker, Friend, & Efthimios, 2009). Thus, the complexity that hydroxide and chloride ions give the gold surface lead to suppose that many types of interactions are involved in the adsorption of polysaccharide to the surface including covalent bonding ($Au(OH)_4^-$, $Au(OH)_3$ and/or $Au(OH)_3Cl^-$), hydrogen bonding and hydrophobic interactions (Amirkhani, Volden, Kaizhen, Glomm, & Nystrom, 2008). Cyclic voltammogram technique by use of a redox probe clearly shows that the dynamics of charge transfer at the electrochemical interface is strongly influenced by the nature of the electrode surface and also by the structure of the electric double layer. The modification of the surface can be demonstrated by comparing the electric charge per unit area of the modified and clean electrode. The coverage percentage of the surface (θ) was calculated from $[Fe(CN_6)]^{3-}/[Fe(CN_6)]^{4-}$ probe considering Eq. (1):

$$\theta(\%) = 1 - \frac{Q_m}{Q_c} \quad (1)$$

where Q_m is the electric charge per unit area of the modified electrode and Q_c is the electric charge per unit area of the clean electrode. The electric charge (Q) was obtained from the integrating redox peaks area of the cyclic voltammograms. According to the cyclic voltammograms performed by the redox probe solution in the clean and modified gold surface, the gold surface coverage was 13.1% ± 0.02 (n = 3) (Fig. 1a). The value found for the coverage percentage of the surface was quite satisfactory considering that the optimal solubility of the XG was achieved by hydroxide and chloride ions balance leading to a higher concentrations of XG film decrease the biosensor response in function of the increasing of its resistance electric.

The SEM technique was employed to characterize the XG film on the sensor surface. Fig. 1b exhibits micrograph of a film with heterogeneous and granular features. This granular formation is due to the random aggregation that can be attributed to the inherent tendency of the polysaccharides to form intermolecular hydrogen bonds between aggregate molecules (Jó, Petri, Beltramini, Lucyszyn, & Sierakowski, 2010, Sierakowski, Castro, Lucyzyn, & Petri, 2007).

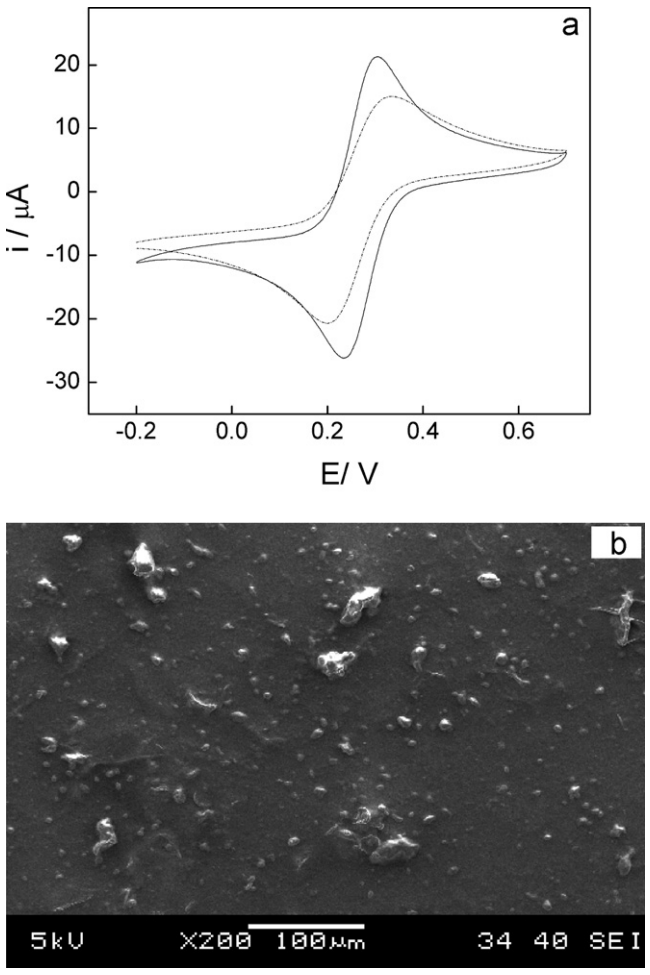

Fig. 1. Cyclic voltammograms of the (—) clean electrode and (– – –) modified electrode with XG film in solution of 4 mM $K_3Fe(CN)_6$ in 1 M KCl (a); scanning electron micrograph of the gold electrode with XG film (b).

Studies on XG film stability were done by scanning the electrode 25 consecutive voltammetric cycles in PB buffer. The voltammograms remained practically constant with a variation coefficient of 1.4%, determined from the difference among the redox peak amplitudes of each cycle.

3.2. Cyclic voltammetric studies

The stepwise of film obtaining and response to RCA_{60} were accomplished by cyclic voltammetries carried out in PB with 4 mM L^{-1} hydroquinone, $C_6H_4(OH)_2$, and 600 μmol L^{-1} H_2O_2 (pH 6.5, 0.1 M) (Fig. 2).

The response of the biosensor to RCA_{60} was measured after the electrode surface had been incubated with antibodies against RCA_{60} conjugated to peroxidase. There was a significant increase in the current cathodic peak (i = −6.25 μA) as shown in curve (c). This current peak around −0.1 V is associated with the peroxidase activity that catalyzes the oxidation of hydroquinone to p-quinone in the presence of hydrogen peroxide and, subsequently, the p-quinone formed is reduced electrochemically on the surface of the biosensor (Lei et al., 2004). A control experiment was carried out with antibodies against RCA_{60} conjugated to peroxidase incubation but without lectin, incubating the electrode surface with PB (curve b). This control was compared to electrode ready

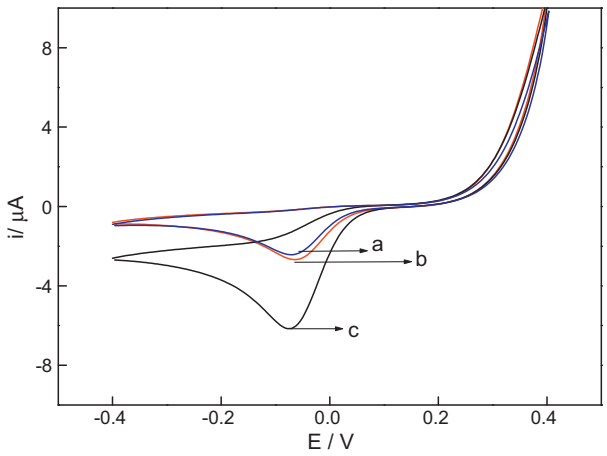

Fig. 2. Illustrative cyclic voltammograms of the biosensor response performed in 0.1 M phosphate buffer pH 6.5 with 4 mmol L^{-1} hydroquinone and 600 mmol L^{-1} H_2O_2. Curves represent the electrochemical signal of modified gold electrode: after formation of XG film and 3% BSA blocking (a), absence of RCA_{60} and incubation with antibodies anti-RCA_{60} labeled to peroxidase (b), and incubation with RCA_{60} and antibodies anti-RCA_{60} labeled to peroxidase (c).

with XG film without any incubation step (curve a). The curve (a) and (b) exhibited similar current peaks showing that the biosensor was responsive only when RCA_{60} was bound to XG film.

3.3. Optimization of the experimental conditions

Previous experiments were carried out to in order to establish the optimal pH, H_2O_2 and $C_6H_4(OH)_2$ concentrations, and the blocking agent (see Supplementary Material). The optimal concentrations of $C_6H_4(OH)_2$ and H_2O_2 were 35 μmol L^{-1} and 60 μmol L^{-1}, respectively, determined by a plateau reached on the biosensor response.

Polysaccharide film can bind to any protein depending upon the charge polymer chain in function of pH. According to Fujimoto, Reis and Petri (2002) protein was adsorbed in multilayers of chitosan and carboxymethylcellulose in a pH range from 3 to 6 based on the ellipsometry study. In this study, regarding to the pH, the optimum range was registered between 6.0 and 7.0 and reaches the maximum sensitivity at pH 6.5, which is close to optimum pH observed for peroxidase in solution (Gebelein & Dunn, 1990). The pH 6.5 was chosen for subsequent experiments.

Studies of blocking agent were conducted in order to minimize nonspecific adsorptions on the electrode surface. Here, different blocking agents were tested: 500 mM glycine, 3% BSA, 200 mM glycine and 1% BSA. The choice was evaluated after the electrode incubation with polyclonal antibodies against RCA_{60} conjugated to peroxidase without lectin step incubation. Then, the highest difference between reduction currents obtained in the absence and presence of RCA_{60} revealed that 3% BSA was the best blocking agent, subsequently 1% BSA, 500 mM and 200 mM glycine. Then, 3% BSA was adopted to subsequent studies.

3.4. Analytical response

The biosensor response in the presence of harmful lectin in PB pH 6.5 with 35 μmol L^{-1} hydroquinone and 60 μmol L^{-1} H_2O_2, is shown in Fig. 3(a). The calibration curve presented a good linearity ($r = 0.996$, $n = 7$) taking in consideration that many steps prior to the analytical readings have been established. A linear range was found between 7.0 and 500 μg mL^{-1} RCA_{60} (Fig. 3b) and a limit of detection of 2.1 μg mL^{-1} RCA_{60}. According to these results, the biosensor presented a satisfactory linear range considering that the median

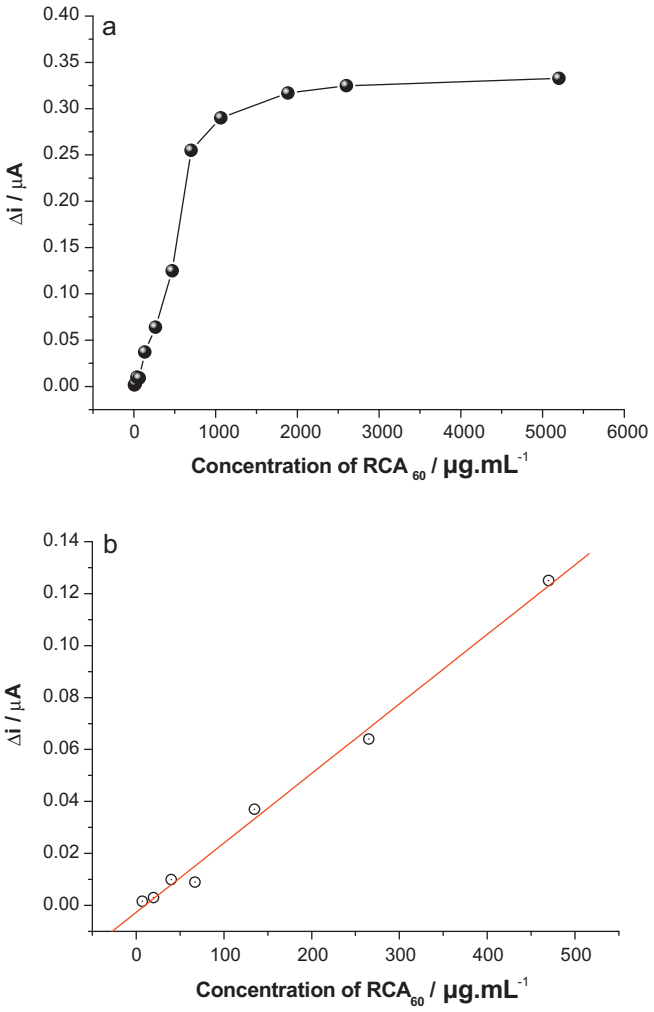

Fig. 3. (a) Typical analytical curve of the biosensor obtained for different concentrations of RCA_{60} in phosphate buffer pH 6.5 in 35 μmol L^{-1} hydroquinone and 60 μmol L^{-1} H_2O_2. The measurements were performed at −100 mV (vs. SCE). (b) Linear fit of calibration curve.

lethal dose (LD50) of ricin is around 22 μg kg^{-1} animal (Musshoff & Madea, 2009).

The reproducibility of the biosensor was evaluated from the amperometric response using three different modified electrodes with fresh XG solution cross linked with epichlorohydrin in the presence of 0.5 mg mL^{-1} RCA_{60}. The relative standard deviation of these electrodes was 6.5%. Thus the biosensor showed a good, reproducible behavior.

RCA_{60} has three recognized binding sites (Frankel et al., 1996). For this type of biosensor, it is interesting that the relative orientation of the C2 and C4 hydroxyls groups of galactose is kept considering that these lectins have quite specific structural requirements of the sugar (Venkatesh & Lambert, 1997). In this way, the crosslinking polyssacharide-epichlorohydrin film should not harm the recognition of the lectin nor its interaction with gold surface.

3.5. Reuse of the biosensor

Lectins bind to carbohydrates through weak hydrogen bonding, Van der Waals forces and hydrofobic interactions (Sharma, Srinivas, Adhikari, Vijayan, & Surolia, 1998). These bindings may be broken up when exposed to very strong acidic or alkaline solutions. Then, the reuse of the biosensor was performed through the exposure of the electrode to a solution of 0.1 M glycine–HCl (pH 2.6). The

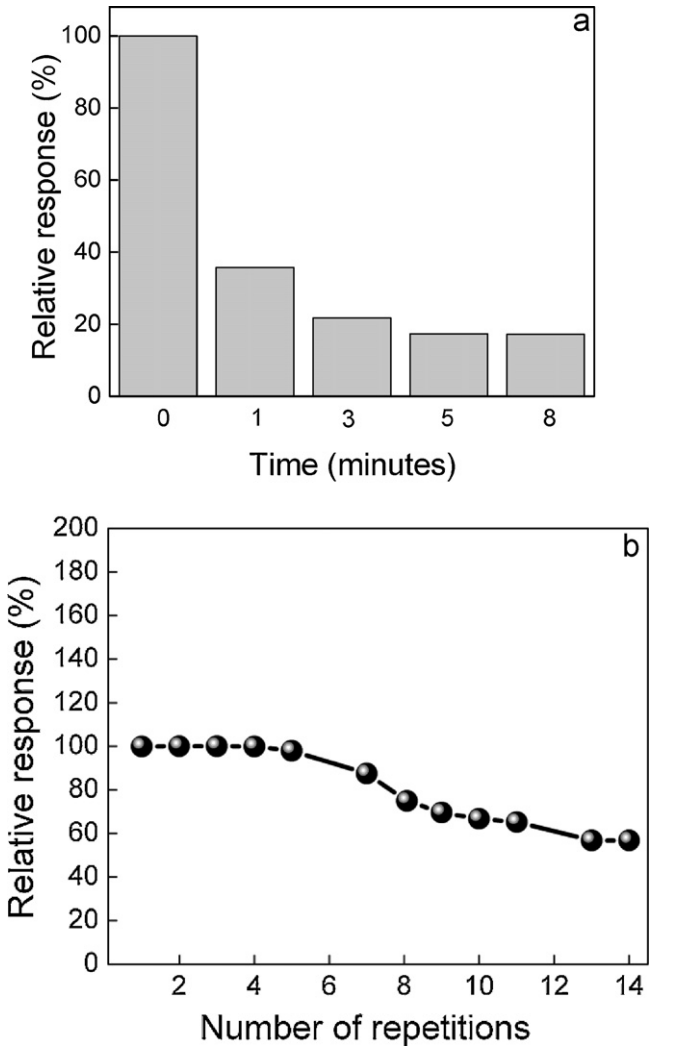

Fig. 4. Dissociation of RCA_{60} from XG film through time after immersion in 0.1 M glycine–HCl (pH 2.6) (a). Reuse of the biosensor after immersion in solution of glycine–HCl pH 2.6, for 5 min (b). Measurements performed in phosphate buffer pH 6.5 with 35 μmol L^{-1} hydroquinone and 60 μmol L^{-1} H_2O_2. Concentration of RCA_{60} 1 mg mL^{-1}. Applied potential of −100 mV (vs. SCE).

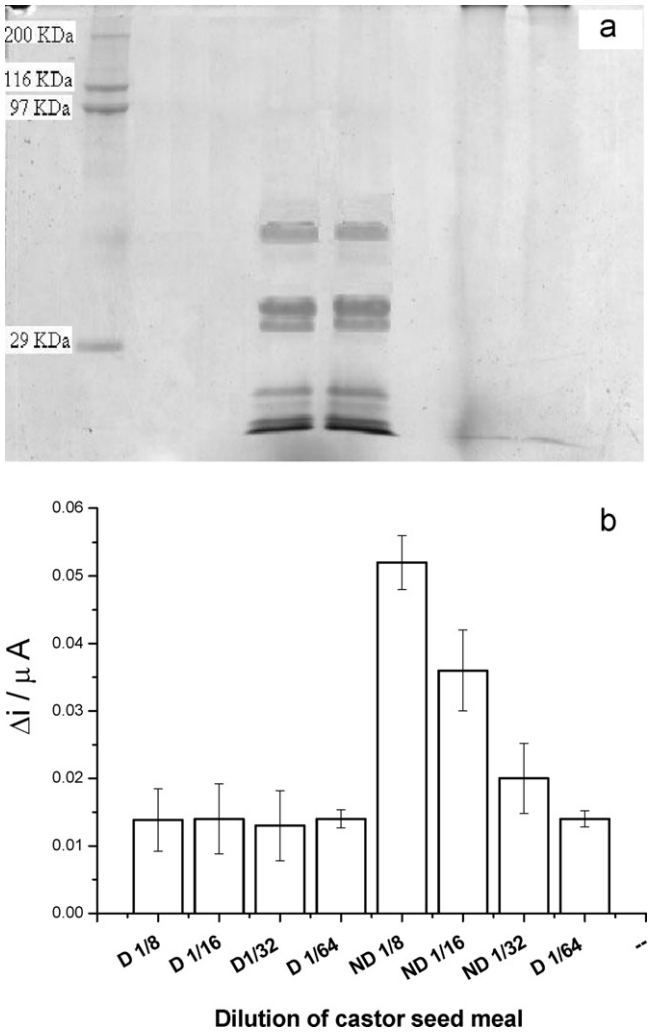

Fig. 5. (a) 12% Polyacrylamide gel SDS-PAGE of detoxified castor seed meal (right) and not detoxified castor seed meal (center) molecular weight marker (left). Each sample of castor seed meal was run in duplicate. (b) Determination of biosensor response in detoxified (D) and non detoxified (ND) samples of castor seed meal in different dilutions. The measurements were performed in phosphate buffer pH 6.5, 35 μmol L^{-1} hydroquinone and 60 μmol L^{-1} H_2O_2 at −100 mV (vs. SCE).

optimal exposure time in the 0.1 M glycine–HCl (pH 2.6) solution for releasing of the lectins bound to XG film was studied to assure the maximal reuse of the biosensor. When the time of electrode exposure was 1 min there was a release of almost 60% of the lectin bound. At 3 min, a loss of about 80% was achieved (Fig. 4a). Finally, at 5 min the maximal amount of lectin was unbound. Then this exposure time was chosen to remain experiments.

The biosensor stability was evaluated over seven days. All measurements were performed twice a day after reuse. When not in use, the electrode was kept in a refrigerator (2–8 °C) immersed in the PB solution pH 6.5. Under these conditions, the biosensor showed a good stability showed a good stability for five consecutive analyses with 5% of lost of the initial amperometric response when 0.5 mg mL^{-1} RCA_{60} was used (Fig. 4b). The lifetime of the electrode would be higher if lower concentrations of ricin were used. Thus, the biosensor presented a good stability and could be reused at least up to five times without calibration requirements.

3.6. Biosensor performance in castor seed meal

In order to evaluate the biosensor performance of discriminating non-detoxified (ND) and detoxified (D) castor seed meal firstly was used the gold standard polyacrylamide gel SDS-PAGE method. The lectin in the castor seed meal was denatured with CaO according to Oliveira (2010). As shown in Fig. 5a, the lectin detoxification process was efficient due to not observe the protein bands in the polyacrylamide gel in the second columns. The calcium oxide causes disruptions of non-covalent linkages that maintain the secondary and tertiary structures of proteins. Consequently, the hydrophobic groups are exposed and protein solubility decreases in aqueous solutions (Oliveira et al., 2010). However, complementary studies should be realized with the other isolated proteins from castor seed meal in order to determinate the effect of each one in the biosensor response.

Afterwards, the analytical performance of biosensor was tested in castor meal at different samples diluted in 0.15 M NaCl. It was used a stock solution of castor meal containing 35 μg mL^{-1} of RCA_{60}. According to Fig. 5b, the biosensor was capable to distinguish non-detoxified (ND) and detoxified (D) samples of the castor seed meal at 1:16 diluted that corresponds to concentration approximately 2.2 μg mL^{-1} RCA_{60}, similar to limit of detection previously mentioned. Taking account that the median lethal dose (LD50) of RCA_{60} is around 22 μg kg^{-1} animal (Musshoff & Madea, 2009), this method is interesting and reliable if the castor seed meal

is evaluated as feed for herd animals such as cattle, pigs and goats due to have high weight. However, more studies need to be made when lower detection limit of RCA_{60} is required, in animals of low weight or in others toxical via as inhalation. Finally, the proposed method is easy to use and can be portable which facilitates its use to assess the toxicity of castor seed meal.

4. Conclusions

The developed biosensor was able to quantify the RCA_{60} lectin and also assess its toxicity by simple exploring of the ricin specificity for galactose residues. Here, the XG film was for first time used to biosensor assembling. The results confirm a method that can be safely used to assess the toxicity of the castor seed meal for animal feed in order to avoid undesirable death of animals by toxic effects.

Acknowledgments

The authors acknowledge the financial support of the FINEP and CNPq Brazilian agencies.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2012.03.053.

References

Amin, S., Rajabnezhad, S., & Kohli, K. (2009). Hydrogels as potential drug delivery systems. *Scientific Research and Essay*, *3*, 1175–1183.

Amirkhani, M., Volden, S., Kaizhen, Z., Glomm, W. R., & Nystrom, B. (2008). Adsorption of cellulose derivatives on flat gold surfaces and on spherical gold particles. *Journal of Colloid and Interface Science*, *328*, 20–28.

Appukuttan, P. S., Surolia, A. E., & Bachhawat, B. K. (1977). Isolation of 2 galactose-binding proteins from *Ricinus communis* by affinity chromatography. *Indian Journal of Biochemistry & Biophysics*, *14*, 382–384.

Arrascue, M. L., Garcia, H. M., Horna, O., & Guibal, E. (2003). Gold sorption on chitosan derivatives. *Hydrometallurgy*, *71*, 191–200.

Audi, J., Belson, M., Patel, M., Schier, J., & Osterloh, J. (2004). Ricin poisoning: A comprehensive review. *American Medical Association*, *294*, 2342–2351.

Avrameas, S. (1969). Coupling of enzymes to proteins with glutaraldehyde: Use of the conjugates for the detection of antigens and antibodies. *Immunochemistry*, *6*, 43–48.

Baker, T. A., Friend, C. M., & Efthimios, K. (2009). Effects of chlorine and oxygen coverage on the structure of the Au (1 1 1) surface. *Journal of Chemistry Physics*, *130*, 084701–084702.

Barnes, D. J., Baldwin, B. S., & Braasch, D. A. (2009). Degradation of ricin in castor seed meal by temperature and chemical treatment. *Industrial Crops and Products*, *29*, 509–515.

Bradford, M. M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry*, *72*, 248–254.

Busato, A. P., Vargas-Rechia, C. G., & Reicher, F. (2001). Xyloglucan from the leaves of *Hymenaea courbaril*. *Phytochemistry*, *58*, 525–531.

Crini, G. (2005). Recent developments in polysaccharide-based materials used as adsorbents in wastewater treatment. *Progress in Polymer Science*, *30*, 39–70.

Frankel, A. E., Burbage, C., Fu, T., Tagge, E., Chandler, J., & Willingham, M. C. (1996). Ricin toxin contains at least three galactose-binding sites located in B chain subdomains 1α, 1β, and 2γ. *Biochemistry*, *35*, 14749–14756.

Fujimoto, J., Reis, E. A. O., & Petri, D. F. S. (2002). Formação de multicamadas de polissacarídeos e proteína. *Quimica Nova*, *25*, 757–761.

Furtado, R. F., Guedes, M. I. F., Alves, C. R., Moreira, A. C. O. M., Felix, W. P., & Dutra, R. F. (2011). Production of polyclonal anti-ricin antibodies. *Ciência e Agrotecnologia*, *35*, 124–130.

Gebelein, G. G., & Dunn, R. L. (1990). *Progress in biomedical polymers*. New York: Plenium Publishing Corporation.

Griffith, G. D., Newman, H., & Gee, D. J. (1986). Identification and quantification of ricin toxin in animal tissues using ELISA. *Journal of the Forensic Science Society*, *26*, 349–358.

Gustafson, I. (2003). Investigating the interaction of the toxin ricin and its B-chain with immobilized glycolipids in supported phospholipid membranes by surface plasmon resonance. *Colloids and Surfaces B: Biointerfaces*, *30*, 13–24.

Jó, T. A., Petri, D. F. S., Beltramini, L. M., Lucyszyn, N., & Sierakowski, M. R. (2010). Xyloglucan nano-aggregates: Physico-chemical characterization in buffer solution and potential application as a carrier for camptothecin, and anti-cancer drug. *Carbohydrate Polymers*, *82*, 355–362.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680–685.

Lei, C. X., Hu, S. O., Cun, X., Gao, N., Shen, G. J., & Yu, R. Q. (2004). An amperometric hydrogen peroxide biosensor based on immobilizing horseradish peroxidase to a nano-Au monolayer supported by sol–gel derived carbon ceramic electrode. *Bioelectrochemistry*, *65*, 33–39.

Lubelli, C., Chatgillaloglu, A., Bolognesi, A., Strochhi, P., Colombatti, M., & Stripe, F. (2006). Detection of ricin and other ribosome-inactivating proteins by an immunopolymerase chain reaction assay. *Analytical Biochemistry*, *355*, 102–109.

Musshoff, F., & Madea, B. (2009). Ricin poisoning and forensic toxicology. *Drug Testing and Analysis*, *1*, 184–191.

Narang, U., Anderson, G. P., Ligler, F. S., & Burans, J. (1997). Fiber optic-based biosensor for ricin. *Biosensors and Bioelectronics*, *9–10*, 937–945.

Oliveira, A. S., Oliveira, M. R. C., Campos, J. M. S., Lana, R. P., Machado, O. L. T., Retamal, C. A., et al. (2010). In vitro ruminal degradation of ricin and its effect on microbial growth. *Animal Feed Science and Technology*, *157*, 41–54.

Olsnes, S. (2004). The history of ricin, abrin and related toxins. *Toxicon*, *44*, 361–370.

Olsnes, S., & Koslov, J. V. (2001). Ricin. *Toxicon*, *39*, 1723–1728.

Rioux, B., Ispas-Szabo, P., Aït-Kadi, A., Mateescu, M., & Juhász, J. (2002). Structure properties relationship in cross-linked high amylose starch cast film. *Carbohydrate Polymers*, *50*, 371–378.

Sharma, V., Srinivas, V. R., Adhikari, P. A., Vijayan, M., & Surolia, A. (1998). Molecular basis of recognition by Gal/GalNAc specific legume lectins: Influence of Glu 129 on the specificity of peanut agglutinin (PNA) towards C2-substituents of galactose. *Glycobiology*, *8*, 1007–1012.

Sharon, N., & Lis, H. (2003). *Lectins* (2nd ed.). Amsterdam: Kluwer Scientific Publishers.

Shyu, R. H., Shyu, H. F., Liu, H. W., & Tang, S. S. (2002). Colloidal gold based immunochromatographic assay for detection of ricin. *Toxicon*, *40*, 255–258.

Sierakowski, M. R., Castro, L. B., Lucyzyn, N., & Petri, D. F. S. (2007). Assembly of xyloglucans and lectin onto Si wafers and onto amino-terminated surfaces. *Journal Brazilian Chemistry Society*, *18*, 1017–1023.

Silva, D. A., Feitosa, J. P. A., Maciel, J. S., Paula, H. C. B., & Paula, R. C. M. (2006). Characterization of crosslinked cashew gum derivates. *Carbohydrate Polymers*, *66*, 16–26.

Uzawa, H., Ohga, K., Shinozaki, Y., Ohsawa, I., Nagatsuka, T., Seto, Y., et al. (2008). A novel sugar-probe biosensor for the deadly plant proteinous toxin, ricin. *Biosensors and Bioelectronics*, *24*, 923–927.

Venkatesh, Y. P., & Lambert, J. M. (1997). Galactose-induced dimerization of blocked ricin at acidic pH: Evidence for a third galactose-binding site in ricin B-chain. *Glycobiology*, *7*, 329–335.

Yin, H.-Q., Jia, M.-J., Yang, S., Wang, S.-Q., & Zhang, J.-G. (2012). A nanoparticle-based bio-barcode assay for ultrasensitive detection of ricin toxin. *Toxicon*, *59*, 12–16.