Colloids and Surfaces B: Biointerfaces 66 (2008) 13–19

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Electrochemical evaluation of lectin–sugar interaction on gold electrode modified with colloidal gold and polyvinyl butyral

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ARTICLE INFO

Article history:
Received 29 February 2008
Received in revised form 14 April 2008
Accepted 8 May 2008
Available online 15 May 2008

Keywords:
Concanavalin A
Cratylia mollis
Gold nanoparticles
Impedance spectroscopy
Voltammetry

ABSTRACT

In this work, ConA and CramoLL lectins were immobilized on gold nanoparticles (AuNp) with polyvinyl butyral (PVB), and adsorbed on the surface of gold (Au) electrodes. Electrochemical impedance spectroscopy (EIS), in the frequency range from 100 mHz to 100 KHz, and cyclic voltammetry (CV), from −0.2 to 0.7 V, were performed on these electrodes, in phosphate buffer (PBS) solution containing 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture as a redox probe. EIS and CV measurements showed that redox probe reactions on the modified Au electrodes were partially blocked due to the adsorption of AuNp-ConA-PVB and AuNp-CramoLL-PVB. SEM images showed the presence of aggregates of AuNp-ConA on PVB spherules in a tridimensional structure on the surface of the Au electrode. Bovine serum albumin (BSA) was adsorbed on the AuNp-Lectin-PVB modified electrode in order to block the remaining free gold sites. Both EIS and CV techniques yielded results that confirm positive responses of the lectins to ovalbumin agglutination. These results indicate an improvement of the sensitivity for detection of sugars that can be applicable to construction of a biosensor sensitive to glycoproteins in solution.

1. Introduction

Lectins are proteins of non-immune origin that agglutinate cells and precipitate complex carbohydrates or polysaccharides. Their interaction with polysaccharides resembles the antibody–antigen and enzyme–substrate reactions. Lectins, which have a high degree of carbohydrate specificity, make a useful model for protein carbohydrate interactions [1,2]. They have been implicated in cell-to-cell interaction, ligand–receptor recognition, blood group typing, and transport of biological macromolecules, and to some extent in the immune recognition process [3–5]. The interaction of carbohydrates with proteins is a crucial step in many biological processes such as cell–cell recognition, adhesion, metastasis, bacterial and viral infection, and inflammation [6–8]. Lectins belong to a group of homologous proteins that bind carbohydrates but are not enzymes or antibodies. They have been used as models for the study of the molecular basis for protein–carbohydrate interaction and specificity [2]. Leguminous lectins, have a high level of primary structure identity and also present remarkable variations in carbohydrate binding [9,10]. This specificity is demonstrable not only in terms of the recognition of monosaccharide but also of different oligosaccharides by lectins with the same nominal monosaccharide specificity [11].

ConA is a lectin from jack bean and is a well-studied protein because of its molecular structure and many medical applications [12,13]. ConA is Gly/Man specific lectin, and is a tetramer at physiological pH, with each monomer possessing one saccharide site as well as a metal ion site [14]. CramoLL is a lectin isolated from *Cratylia mollis* seeds [15] from the same *Leguminosae* family and the same *Diocleinae* subtribe as ConA. Like ConA, CramoLL is specific for Gly/Man.

Gold nanoparticles are important nanomaterials that have been widely studied owing to their unique physical and chemical characteristics. They are excellent candidates for bioconjugation with proteins because amine groups and cysteine residues in the proteins are known to bind strongly with gold colloids [16,17]. Biomolecule–nanoparticle hybrid systems have, thus, been used in biosensors for monitoring the biomolecular interactions of nucleic acids [18,19] and proteins [20] as well as sugar–lectin interactions [21]. Electrochemical systems offer attractive advantages of miniaturization and low-cost (for meeting the demands of point-of-care diagnostics) and elegant ways for interfacing biorecognition events and signal transduction [22]. Electrochemical impedance spectroscopy has been used to be a sensitive and effective method to probe the interfacial properties of modified electrode [23,24]. In a previous work, we studied the adsorption of lectins directly

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doi:10.1016/j.colsurfb.2008.05.002

on gold electrodes [25]. It was shown that the proteins retained their activities towards small carbohydrates, although with pour sensitivity. In the present paper we describe a lectin (ConA and CramoLL) immobilization on colloidal gold and polyvinyl butyral using gold electrode for sensing the interaction between ConA/CramoLL and carbohydrates (ovalbumin and glucose). The results indicated selectivity for sugars tested and possible biosensor applications.

2. Experimental

2.1. Materials

Concanavalin A (ConA), bovine serum albumin (BSA, 99%), glucose, ovalbumin, polyvinyl butyral (PVB), and $HAuCl_4{\cdot}3H_2O$ were purchased from Sigma chemical (St. Louis, MO, USA), and *Cratylia mollis* lectin (CramoLL) was obtained from the Laboratory of Glycoproteins (UFPE), according to Ref. [15]. All chemicals and solvents used were of analytical-grade, and were used as received without further purification. Water used was obtained from Milli-Q plus (Billerica, USA).

2.2. Apparatus

Electrochemical measurements were carried out on an EG&G PAR 263A potentiostat interfaced with a 5210 EG&G lock in analyzer controlled by a computer. A three-electrode setup was employed throughout with an Ag/AgCl (saturated KCl) reference electrode. All potentials are referred to this electrode. A gold wire and modified gold disc ($d = 2$ mm) were used as auxiliary and working electrodes, respectively. The scanning electron microscopy images were obtained from a JSM 5900 (JEOL instruments, Japan) at an acceleration voltage of 10 kV and a working distance of 5 μm. Particle size distribution was obtained with a Zetasizer Nano ZS (Malvern Instruments, UK).

2.3. Preparation of colloidal gold

Colloidal gold was prepared according to the literature [26], by adding 2 mL of 1% (w/w) sodium citrate solution into 50 mL of 1.0% (w/w) $HAuCl_4$ boiling solution. The absorption maximum of the synthesized colloidal gold in the UV–vis spectrum was at 527 nm, and the solution was stored in a refrigerator in a dark-colored glass bottle before use.

2.4. Lectin immobilization and lectin–sugar interactions

The gold disc electrode was first mechanically polished with 0.05 μm α-Al_2O_3 powder, and washed ultrasonically in distilled water. The bare electrode was charged positively by holding it at a potential of + 1.5 V in a 0.1 M NaOH stirred solution for 2 min followed by +0.5 V for 45 s in the same medium [27]. A sol–gel method [28] was employed to modify the electrode. Either ConA or CramoLL solution (200 μg/mL) was mixed with 300 μL of colloidal gold into a beaker (4 °C) and kept under stirring for 10 min. Thereafter, 3 mL of polyvinyl butyral (PVB)-ethanol solution (2% v/v) was added to the beaker quickly. The electrode was dipped into the above solution for 30 min and then removed (see Scheme 1). Then, the modified electrode was incubated in a phosphate saline buffer (PBS) with 0.2% BSA for 20 min at 37 °C in order to block the remaining active groups. Finally, the lectin-modified electrode were rinsed with water to remove unbound lectin and incubated in 10 mM PBS (pH 7.4) containing carbohydrate (ovalbumin or glucose) for 15 min at room temperature (ca. 26 °C). In the case of ovalbumin as the carbohydrate, a study at varying concentration (25–200 μg/mL) was carried out. Glucose was investigated only at 200 μg/mL.

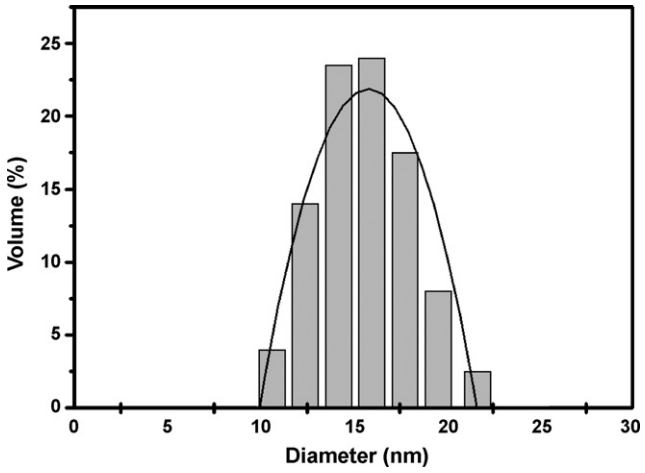


Fig. 1. Dynamic light scattering results showing narrow polydispersity and particle size distribution of AuNp prepared in sodium citrate 1%.

2.5. Electrochemical measurements

The impedance spectra were recorded in the frequency range of 100 mHz to 100 kHz. The amplitude of the applied sine wave potential was 10 mV, while the direct current (dc) potential was limited at the open circuit potential measured just before the application of the sine wave potential. Cyclic voltammetry (CV) experiments were performed with sweep potential between +0.7 and −0.2 V with scan rate of 50 mV/s. Impedance measurements and cyclic voltammetry were performed in the presence of a 10 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ (1:1) solution, used as a redox probe in PBS (pH 7.4). EIS and CV measurements were carried out at different stages for the preparation of the modified electrode described in Section 2.4.

3. Results and discussion

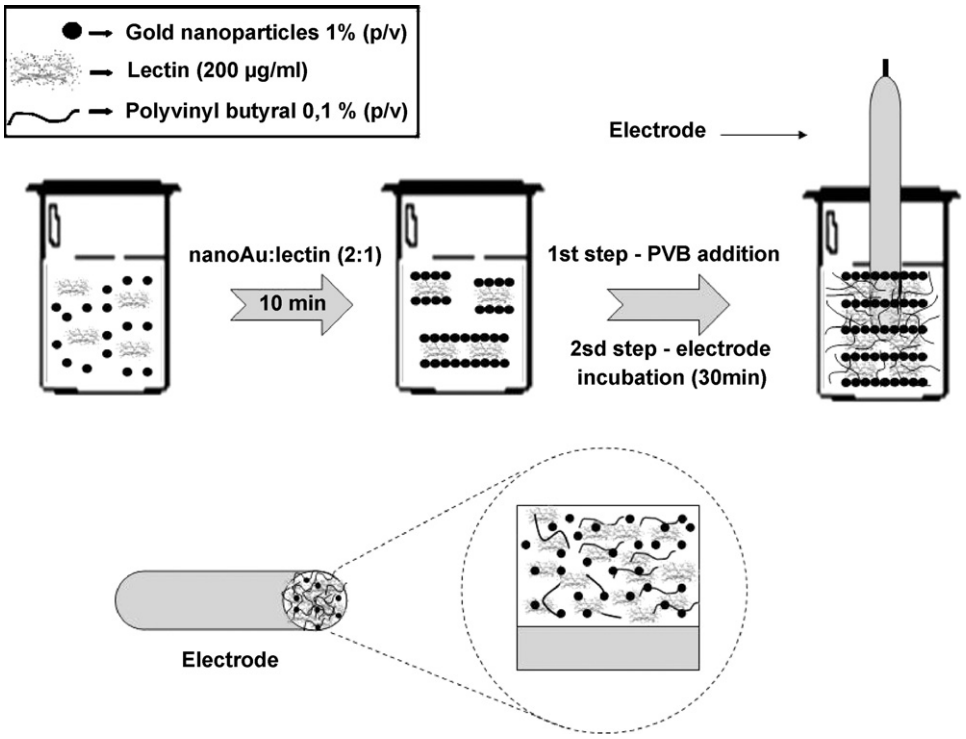
3.1. Characterization of AuNp and AuNp-ConA-PVB modified electrode

Fig. 1 shows the particle size distribution of AuNp. The distribution was greatly concentrated around 16 ± 1.3 nm. In addition, a negligible second population with an average value of 70 ± 4.7 nm can be observed. A relatively narrow polydispersity was obtained for the evaluated AuNp.

Fig. 2a shows the SEM images of gold electrode coated with AuNp and PVB. The nanoparticles are not seen in this magnification. The figure shows spherules of PVB on top of a PVB film with heterogeneous distribution. Fig. 2b was obtained for a gold electrode coated with AuNp, PVB and ConA. The presence of aggregates of AuNp-ConA on the PVB microparticles surface can be clearly seen in a tridimensional array. This film covered the entire surface and could be confirmed by EIS and CV, which indicated a resistive layer on the gold/solution interface.

3.2. Electrochemical impedance spectroscopy for the AuNP-Lectin-PVB modified electrode

Electrochemical impedance spectroscopy (EIS) is an effective tool for studying the interfacial properties of surface modified electrodes. The ferro-ferricyanide redox system is a valuable



Scheme 1.

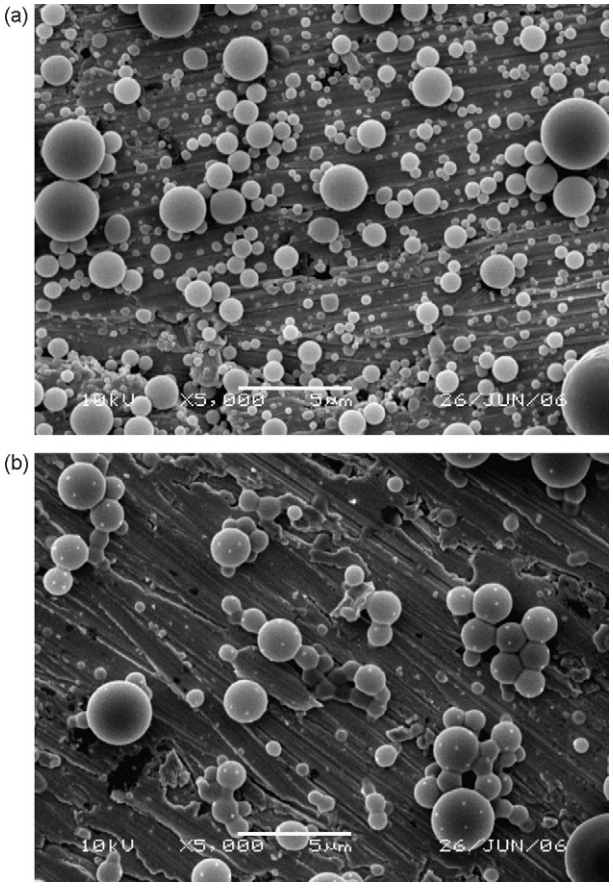


Fig. 2. SEM images of gold electrode coated with nanoAu-PVB (a) and nanoAu-ConA-PVB (b).

and convenient probe to monitor the barrier of the modified electrode, because the electron transfer between the solution species and the electrode must occur by tunneling either through the barrier or through the defects in the barrier. Therefore, ($K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$) was chosen as marker to investigate the changes of electrode behavior after each assembly step. Fig. 3a and b shows the impedance responses of the bare and modified gold electrode in PBS with $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$, at different stages of electrode modification.

All curves were characterized by a semicircular region (in the high frequency end of the spectrum) and a linear region (in the low frequency end of the spectrum). This behavior is well known [23,29] and can be easily modeled by a modified Randles equivalent circuit (Fig. 4). The circuit includes the following four elements: The ohmic resistance of the electrolyte solution, R_Ω, the Warburg impedance, Z_W, a constant phase element, Q, and the electron-transfer resistance, R_{CT}. The components, R_Ω and Z_W, represent bulk properties of the electrolyte solution and diffusion of the applied redox probe, respectively. Thus, they are not affected by chemical transformations occurring at the electrode interface. The other two components of the circuit, Q and R_{CT} depend on the dielectric and insulating features at the electrode/electrolyte interface. Whereas the charge transfer resistance is directly related to the kinetics of the redox reaction, the constant phase element has been related to the heterogeneous nature of the electrode/solution interface (its dispersion, caused, for instance, by the surface roughness) [30].

The insert of Fig. 3a, details the spectroscopic impedance behavior of the bare gold electrode. It exhibits an almost straight line that is characteristic of a diffusional limiting step of the electrochemical process. The EIS of AuNp modified gold electrode was similar to the bare gold electrode, indicating that the AuNp did not modified the interfacial electrochemical properties of the gold electrode. After being modified with AuNp and ConA lectin, the EIS shows a larger semicircle. The diameter of the semicircle is related to the interfacial electron-transfer resistance (R_{CT}). The large R_{CT}

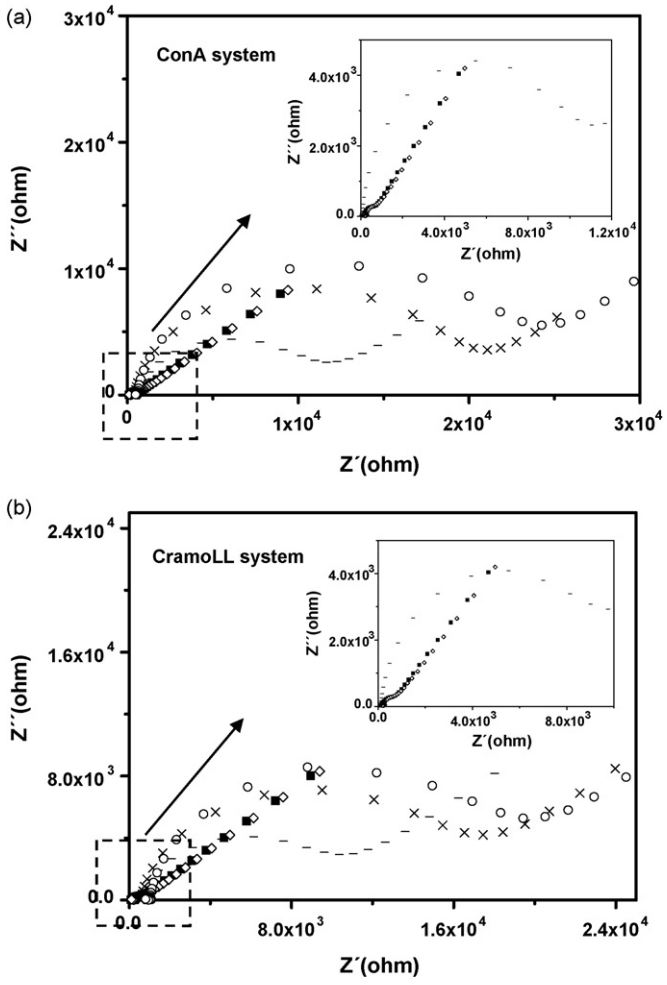


Fig. 3. Nyquist plots of the immobilization steps of lectins: (a) bare gold electrode (■), AuNp-gold electrode (◊), the AuNp-ConA (–), the AuNp-ConA-PVB (×), AuNp-ConA-PVB-BSA (○) modified electrode; (b) bare gold electrode (■), AuNp-gold electrode (◊), AuNp-CramoLL (–), AuNp-CramoLL-PVB (×), AuNp-CramoLL-PVB-BSA (○) modified electrode. The impedance spectra were taken in 10 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ 1:1 + 0.15 M NaCl in 10 mM pH 7.4 in the frequency range from 100 mHz to 100 kHz.

indicates that lectin molecules were blocking the electron transfer at the electrode/electrolyte interface. When the AuNp-ConA-PVB-gold electrode was obtained, the interfacial resistance increased even further. In this case, the impedance change of the process was ascribed to the PVB attached to the gold electrode surface. Finally,

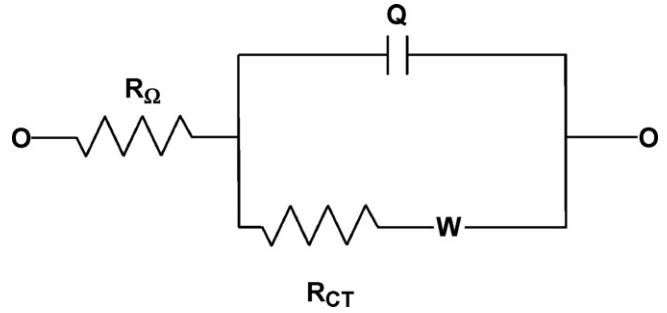


Fig. 4. Equivalent circuit applied to fit impedance measurements in the presence of redox pair of $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$. R_Ω, the ohmic resistance of the electrolyte solution; Q associated with the double layer capacitance; Z_W, the Warburg impedance; R_{CT}, the electron resistance.

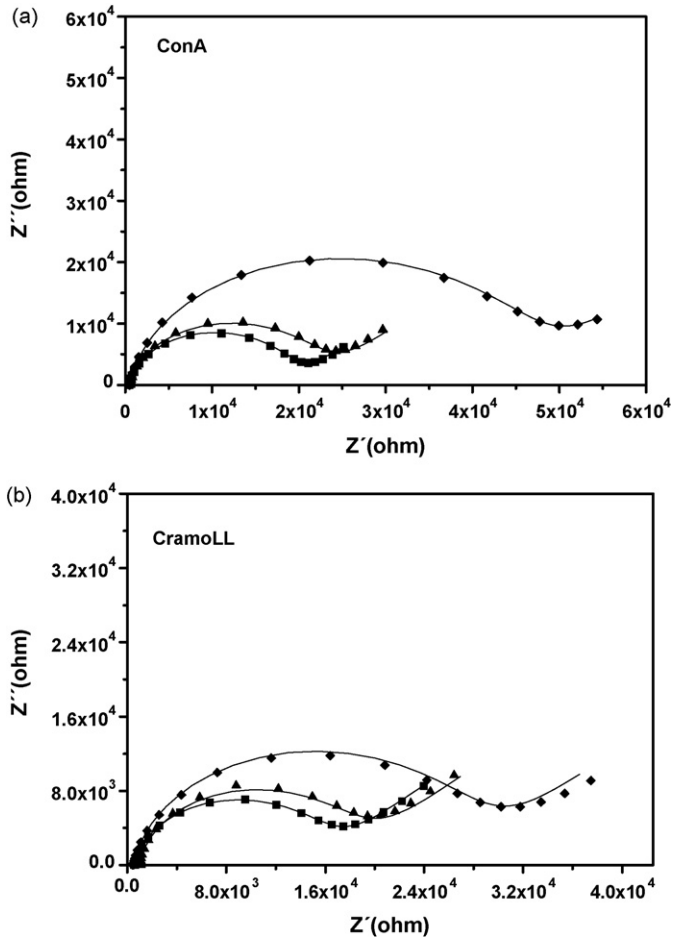


Fig. 5. Nyquist plots of the immobilization steps of lectins: (a) (■) AuNp-ConA-PVB; (▲) AuNp-ConA-PVB-BSA; (♦) AuNp-ConA-PVB-BSA-ovalbumin; (b) (■) AuNp-CramoLL; (+) AuNp-CramoLL-PVB-BSA; (♦) AuNp-CramoLL-PVB-BSA-ovalbumin. Solid lines in the figure represent the fitted lines.

in presence of BSA additional increase of resistance was observed, confirming the expected blockage of the remaining free sites on the gold surface.

The assembly of the AuNp-CramoLL-PVB-BSA system was evaluated by EIS in the same way as for ConA lectin, and the results are shown in Fig. 3b. The impedance response of this system showed similar behavior when compared to the AuNp-ConA-PVB-BSA system. In the sequence, starting with gold electrode and ending with the CramoLL systems (AuNp-CramoLL, AuNp-CramoLL-PVB, and AuNp-CramoLL-PVB-BSA electrode) a successive increase in the charge transfer resistance of the interface was obtained. As mentioned earlier, this response is a result of the successive blockage for electron transfer at the electrode/electrolyte interface, by the presence of elements on the electrode surface.

The impedance data were fitted with a Boukamp non-linear least square fitting program [31] using the modified Randles equivalent circuit described above. It was found to fit adequately the data, yielding more detailed information about the impedance behavior of lectin systems. The fitted curves shown in Fig. 5 indicate the excellent agreement between the circuit model and the experimental results, especially in the higher frequency range.

Table 1 lists the parameters of fitted impedance results. The changes in R_{CT} were the most significant among other impedance components. It can be observed that, in general, the charge transfer resistances for systems with ConA, are larger than those with CramoLL. This may be ascribed to the larger molecular weight of

Table 1
Fitted impedance results to modified electrodes from Fig. 2

Modified electrode	$R_{CT} \times 10^3$ (Ω)	Q (μF)	n
AuNp-ConA	10.26 ± 0.10	1.25 ± 0.05	0.89 ± 0.01
AuNp-ConA-PVB	18.80 ± 4.55	0.87 ± 0.18	0.92 ± 0.07
AuNp-ConA-PVB-BSA	21.70 ± 2.35	1.04 ± 0.22	0.92 ± 0.01
AuNp-CramoLL	9.10 ± 0.29	1.18 ± 0.37	0.89 ± 0.02
AuNp-CramoLL-PVB	15.30 ± 2.20	1.04 ± 0.15	0.91 ± 0.05
AuNp-CramoLL-PVB-BSA	17.20 ± 1.05	1.13 ± 0.01	0.92 ± 0.01
AuNp-PVB	6.77 ± 0.15	0.99 ± 0.18	1.00 ± 0.03
AuNp-PVB-BSA-ovalbumin	6.83 ± 0.08	0.91 ± 0.45	0.65 ± 0.01
AuNp-PVB-BSA-glucose	5.43 ± 0.49	0.73 ± 0.49	0.70 ± 0.06

ConA (55 kDa) as compared to CramoLL (31 kDa). The sequence of measurements AuNp-PVB, Au-Lectin, and AuNp-Lectin-PVB, roughly shows the additive blockage of the interface, confirming that the amount of material immobilized and/or adsorbed on the electrode surface directly correlates with the impedance. Additionally, attention is called for the small increase in R_{CT} associated with glucose (5.4 kΩ) and ovalbumin (6.8 kΩ) in the absence of lectins, as compared to the lectins themselves (10.9 and 9.1 kΩ). The system with glucose shows the lowest resistance value. It seems to indicate that glucose is causing an increase in the permeability of the redox probe system at interface. BSA does not seem to adsorb strongly on the electrode, and its role as blocking agent for non specific sites on the surface, for the system being studied needs further investigation.

From these results we observed that ConA interaction with ovalbumin was stronger than CramoLL. They demonstrate that these lectins remain with their biological recognize after exposition onto electrode surface. These differences can be explained by the studies that were performed to determine the relevance of the neighborhoods of the monosaccharide binding site and this way explain the reported binding differences between CramoLL and ConA, their experiments were carried out by superimposing CramoLL structure on the crystallographic structures of ConA complexed with several oligosaccharides [32]. There were identified two regions containing differences that could account for the differences in oligosaccharide and glycoprotein binding between proteins. Substitutions of Thr226 and Ser168 in ConA for Gly226 and Asn168 in CramoLL in the extended binding are reasonable to conclude that CramoLL should have a lower affinity for oligosaccharide when compared with ConA, due to a loss of contact (Gly226) and steric hindrance (Asn168) with saccharide close to the monosaccharide binding site [32].

With the present results, comments on the constant phase element parameters (Q and n) would be very speculative. However, some remarks should be made about the variation of n. This parameter is close to 1 in most experiments, and close to 0.5 only for the systems AuNp-PVB-BSA-ovalbumin and AuNp-PVB-BSA-glicogen. This is an indication of some diffusional aspect of the charge transfer process in the interface. In other words, besides bulk diffusional mass transport limitations, there is some diffusional limitations inside the material imobilized on the electrode surface. The bulk mass transport, is accounted for by the Warburg impedance in the equivalent circuit, and appears in the low frequency part of the EIS results. The diffusion limited charge transport in the interface is indicated by the n value close to 0.5 in these two systems, mentioned above.

3.3. AuNP-Lectin-PVB modified electrode as a biosensor

ConA and CramoLL bind to different glycoproteins present in human plasma [33], and they show differential binding to normal and transformed human mammary cells [34]. They exhibit high affinity for α-D-glucose, α-D-mannose, glycogen [15,35] and ovalbumin [15]. These lectins recognize monosaccharide and glycoprotein, which are the inhibitor of the agglutination of erythrocyte precipitation of carbohydrate polymers, by the lectin [15,36].

Because the protein–carbohydrate interaction is a common phenomenon in biological systems, the study of interaction between ConA/CramoLL with glucose and ovalbumin will provide a better understanding of this interaction [37]. Moreover, these lectins can bind to the carbohydrate moiety of glycoconjugates on the cellular membranes. The interaction between them, which resembles antibody–antigen reaction, can be used to probe the properties of the cellular surface, and their study has great significance in immunology.

These lectins recognize a fraction of ovalbumin containing Man7 and Man8 oligomannose chains, and it has been verified that its interactions with the ConA and CramoLL are relatively unaffected by the protein matrix of ovalbumin [38].

The results presented in Fig. 5a (for ConA) and Fig. 5b (for CramoLL), clearly show that both lectins were capable to recognize the ovalbumin glycoprotein as can be seen by the increase of charge transfer resistance, R_{CT}. Hence, the performance of the modified electrode for detection of sugars and glycoproteins was evaluated through the relative variation of this parameter (ΔR_{CT}). This variation can be calculated according to the following equation:

$$\Delta R_{CT}(\%) = \frac{R_{CT(\text{lectin-sugar})} - R_{CT(\text{lectin})}}{R_{CT}} \times 100\%$$

where, $R_{CT(\text{lectin})}$ is the value of the electron-transfer resistance of the AuNp-Lectin-PVB-BSA modified electrode. $R_{CT(\text{lectin-sugar})}$ is the value of the electron-transfer resistance of the AuNp-Lectin-PVB-BSA modified electrode after exposing it to solutions containing glucose or ovalbumin.

In the case of ovalbumin, a study was carried out for different concentrations. The results are shown in Fig. 6a and b. The ΔR_{CT} increases clearly with ovalbumin concentration, which indicated the interactions between lectin and sugar can be sensed by the modified electrode. This increase in ΔR_{CT} is similar for both lectins; however, for higher ovalbumin concentrations, ConA shows more scattering of ΔR_{CT} (Fig. 6a), whereas CramoLL seems to displays a plateau (Fig. 6b). We observed that no changes of the

Table 2
Fitted impedance results to lectin–sugar interaction from Fig. 2

Modified electrode	Lectin–sugar interaction			ΔR_{CT} (%) (Ovalbumin)	ΔR_{CT} (%) (Glucose)
	Before[a] (R_{CT}, Ω)	After[b] (R_{CT}, Ω)	After[c] (R_{CT}, Ω)		
AuNp-ConA-PVB-BSA	21,700	45,900	12,230	112	−43.6
AuNp-CramoLL-PVB-BSA	17,200	27,530	13,120	60.1	−23.7

[a] Before contact with sugars.
[b] After contact with ovalbumin.
[c] After contact with glucose.

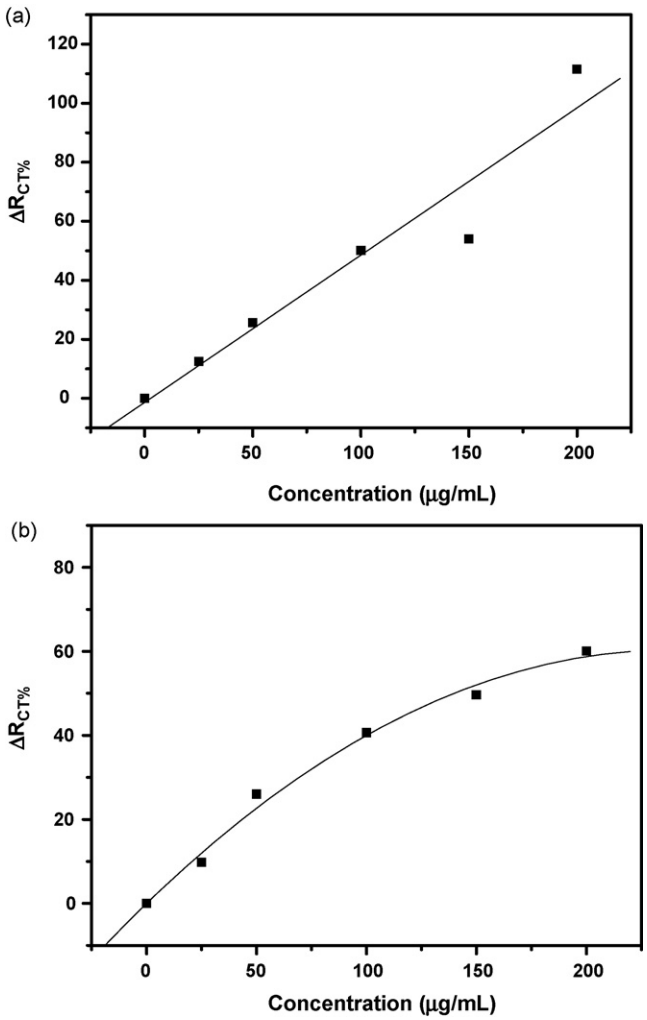


Fig. 6. Diagrams show $\Delta R_{CT\%}$ for the systems that corresponded the to the AuNp-ConA-PVB-BSA (a) and AuNp-CramoLL-PVB-BSA (b) after incubating with different concentrations of ovalbumin, in PBS (pH 7.4), represented 200, 150, 100, 50 and 25 μg/mL.

electron-transfer resistance were detected with experiments using lactose. Since both lectins are known to have no interaction with this carbohydrate, and its derivates, this indicates that the changes of the electron-transfer resistance observed with ovalbumin were due to specific lectin–sugar interaction. Moreover, the different behavior seen between the two lectins, shows that the variation in impedance is related to the ovalbumin–lectin interaction, rather than the ovalbumin adsorption alone.

A summary of these results is given in Table 2, for fixed concentrations of ovalbumin and glucose. These results demonstrate that the lectins are retaining their capabilities to recognize these complex carbohydrates after adsorption. However, there is a striking effect observed with glucose. The charge transfer resistance of the interface decreases upon addition of glucose (indicated by the negative value of ΔR_{CT}). As mentioned earlier, it seems that the interaction of glucose with the electrode increases the permeability of the interface for the redox probe system. Nonetheless, the results are a clear indication that this system could be applicable to the construction of a biosensor for glycoproteins present in human blood serum.

As can be seen, ΔR_{CT} increased with the presence of sugar, indicating lectin recognition. ConA and CramoLL lectins are specific for ovalbumin glycoprotein and glucose monosaccharide.

This way the modified electrode AuNp-ConA/CramoLL-PVB-BSA can be used as a device to disease diagnosis by immunoglobulin detection in serum blood human.

3.4. Voltammetry measurement of the biosensor

Cyclic voltammograms (CVs) of ferricyanide are valuable and convenient tools to monitor the barrier of the modified electrode, because the voltammogram characteristics are sensitive to the presence of adsorbed species on the electrode. Therefore, in analogous way as was done for the impedance measurements, it was chosen as marker ($K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$) to investigate the changes of electrode behavior after each assembly step.

Fig. 7a (ConA) and b (CramoLL) show the CVs for a bare electrode, AuNp-Lectin-modified electrode, and AuNp-Lectin-PVB-modified electrode. As shown in the figure, the stepwise assembly of different materials on the gold electrode is accompanied by a decrease in the amperometric response of the electrode and an increase in the peak-to-peak separation between cathodic and anodic waves of the

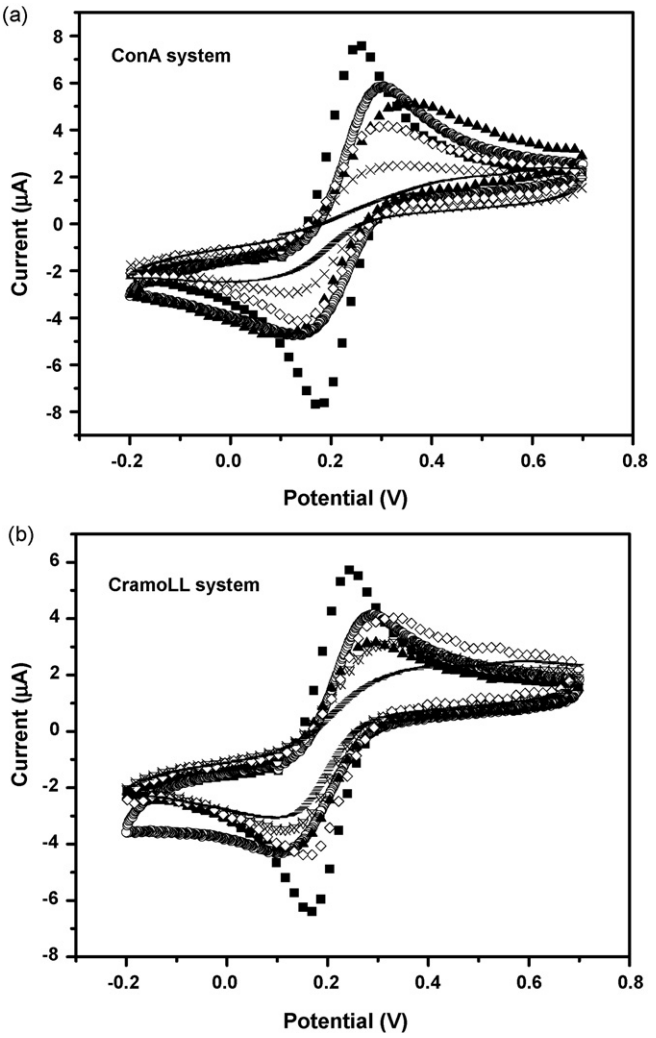


Fig. 7. Cyclic voltammograms (CVs) of the electrode at different stages: (a) (■) bare gold electrode; (○) AuNp-ConA; (▲) AuNp-ConA-PVB; (×) AuNp-ConA-PVB-BSA; (◊) AuNp-ConA-PVB-BSA-glucose and (▬) AuNp-ConA-PVB-BSA-ovalbumin; (b) (■) bare gold electrode; (○) AuNp-CramoLL; (▲) AuNp-CramoLL-PVB; (×) AuNp-CramoLL-PVB-BSA; (◊) AuNp-CramoLL-PVB-BSA-glucose and (▬) AuNp-CramoLL-PVB-BSA-ovalbumin. Supporting electrolyte 10 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ 1:1 + 0.15 M NaCl in 10 mM pH 7.4 solution; scan rate, 50 mV/s.

Table 3
Amperometric anodic shift for the nanoAu-ConA/CramoLL-PVB-modified electrode treated with 200 μg/mL sugars

Modified electrode	Lectin–sugar interaction			ΔI (%) (Ovalbumin)	ΔI (%) (Glucose)
	Before[a] ($1/I_b$, μA^{-1})	After[b] ($1/I_a$, μA^{-1})	After[c] ($1/I_a$, μA^{-1})		
AuNp-ConA-PVB-BSA	0.262	0.781	0.265	198	1.14
AuNp-CramoLL-PVB-BSA	0.315	0.474	0.272	50.5	−13.65

[a] Before contact with sugars.
[b] After contact with ovalbumin.
[c] After contact with glucose.

redox probe. Analogous to what was observed by impedance; in the presence of glucose the currents are higher than in the presence of ovalbumin. Mainly, after ovalbumin is coupled onto the lectin molecules, peak separation is considerably increased and an almost total disappearance of the peak shape was observed. The reason is that the lectin–sugar complex can act as an inert electron blocking layer, in such a way the voltammograms shift from a diffusion controlled process to an activation controlled one.

Since the adsorption of species decreases the current, an analysis analogous to the one carried out for the impedance results can be done with the reciprocal of the peak currents. The extent of adsorption can be expressed in a relative percent deviation as:

$$\Delta I(\%) = \frac{[(1/I_b) - (1/I_a)]}{(1/I_b)} \times 100\%$$

where, I_b is the anodic peak current before binding the lectin with ovalbumin and glucose and I_a is the anodic peak current after carbohydrate interaction.

Table 3 shows the result of this analysis for the AuNp-ConA/CramoLL-PVB-BSA modified electrode before and after the reaction toward 200 μg/mL of ovalbumin and glucose. All the measurements were repeated three times and the results were nearly the same. As seen from Table 3, the relative percent deviations of both lectins with ovalbumin were higher than with glucose. Indeed, glucose caused a decrease in percent deviations. Moreover, the ConA system displayed a larger deviation than the CramoLL system, for both sugars. Finally, it is clear that the sensitivity of ConA towards ovalbumin was the largest of all systems investigated. These results are in agreement with those presented above for the impedance data.

4. Conclusions

In the present work, ConA and CramoLL were immobilized on a gold electrode modified with AuNp-PVB. Cyclic voltammetry and electrochemical impedance techniques were used to investigate the immobilization of ConA and CramoLL on AuNp colloid. The electrochemical response of a redox probe system ($K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$) was effective as a signal for the presence of protein as well as agglutination reactions on the electrode. Through this we could observe that both lectins retained their biological activities after contact with electrode surface, since ConA and CramoLL interacted with ovalbumin, demonstrating, this way, that these systems can be used to detect the presence of complex glycoconjugates and glycoproteins in solution. Different structural arrangement of ConA and CramoLL in the electrode surface displayed a concentration dependence on ovalbumin, which was linear for ConA and with a saturation response for CramoLL.

Acknowledgements

The authors are grateful for the partial support from the Brazilian National Council for Scientific and Technological Development (CNPq). Oliveira MDL would like to thank CNPq, an agency of the Brazilian Ministry of Education, for a graduate scholarship.

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