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Effect of metal in Schiff bases of chitosan adsorbed on glassy carbon electrode in the inhibition of sphingomyelinase C toxin

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ABSTRACT

This study was conducted to assess the catalytic electrode surface adsorption and capture properties of different metal chitosan derivatives in aqueous phosphate buffer solution (pH = 7.3). Early, recent work showed that the response of Iron chitosan complex with R = -CH_3 on the periphery, over blood red cells in presence of sphingomyelinase C was protected. The effect of others substituent (R = -Br, -Cl, -F, NO_2, -OCH_3, -H) on the periphery of the Schiff base ligand did not show correlation with the oxidation of sphingomyelinase C and its biological response. For this reason, various adsorbed metal (M = Fe of recent work, Cu, Ni and Co) complexes of chitosan and Schiff bases on glassy carbon electrode for the oxidation of sphingomyelinase C were investigated and compared, each one with -CH_3 group on the periphery of the Schiff base. UV–Vis and IR-TF spectroscopies, electrochemistry and microscopy assay were performed; then, the metal effect underlying. For the Schiff base, cobalt and copper complexes did not proved to be a remarkable cellular protector in presence of the enzyme, but the nickel complex showed to be a cellular protector at short time, this conclusion help to proposal a reaction mechanism for the electrochemical and biological studies.

1. Introduction

Sphingolipids are critical constituents and modulators of cell membranes functions, given support to the cell form and the selective permeability (Kleinhans, 1998), but it is know that, toxins and poisons from *Loxosceles* spiders like sphingomyelinase C (SMASE) and D isomers hydrolyze these sphingolipids to ceramides (Rivera et al., 2015). The complex bi-lipydic and proteinic membrane of the erythrocyte is destroyed, inducing a severe inflammatory response and cell death or dermonecrosis (Zhou and Blom, 2015). About 46% of patients bitten by spiders in some Latin American country are associated to *Loxosceles*, developing clinical signs such as hemoglobinuria, headache, nausea, vomiting, tachycardia, hypotension, renal failure, and eventually causing death (Ríos et al., 2007).

Based on the above considerations, found new molecular materials of potential interest for the inhibition of SMASE is necessary. The early work explored a large variety of iron chitosan complexes (-CH_3, -OCH_3, -NO_2, -Cl, -Br, -F and -H) voted to the search some inhibition in the activity of SMASE. The results showed that the highest electrocatalytic activity was exhibited by the iron complex substituted with -CH_3 group on the periphery (Caro et al., 2017).

The chitosan then, a natural linear polysaccharide of 2-amino-2-deoxy-D-glucose repeating units obtained from chitin (Sánchez et al., 2007), can be a good material for inactivate the SMASE. In addition this polymer and its derivatives are interesting material in electrochemistry because these adsorbs strongly on graphite and other electrode materials like a gel made of polypropylene (Saxena et al., 2010) at monolayer levels. This kind of modified electrode has been used in the study of nitrite's oxidation (Jiang et al., 2005), in studies of molecules such as hydrogen peroxide (Xu et al., 2006), in the potentiometric determination of anionic species (Bakker et al., 1994], immobilization of glucose oxidase and as stabilizer on gold electrodes to immobilize some proteins (Feng et al., 2005), etc. This polymer also has been widely used in paintings, pesticides, vaccines, as a skin regenerator, as adsorption of metal ions like Co(II), Cu(II), Zn(II), Pb(II), and food additive (Shahidi et al., 1999; Baba et al., 1996; Chen et al., 2008; Chang and Chen, 2005a; 2005b; Chang et al., 2006; Gudjónsdóttir et al., 2015). But the main interest to use this polysaccharide is due to its bioavailability, which is presented as a biodegradable particle with remarkable affinity with acid dyes, proteins (Zeng and Ruckenstein, 1998; Chion and Li, 2002; Guibal et al., 1998; Evans et al., 2002; Cheng et al., 2003) and many biomacromolecules (Ying et al., 2011).

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The properties of the complexes are related to the structure of ligands, because it is known that its biological activity is due to the presence of amino groups that generate positives charge which interact with part of macromolecules that are found in the cell surface, inhibiting their growth (Kurita, 2001).

In recent work the iron chitosan complex with R = -CH_3 adsorbed in a surface of glassy carbon electrode was active in front of SMASE's oxidation, this was agree with the erythrocytes counting (SMASE plus complex in blood solution) because this complex was the only one inside the family of studied complexes that protected to the erythrocytes, although the UV–Vis spectroscopy indicated that there was not binding formation in solution between the complex and the SMASE (Caro et al., 2017). According to this, it is interesting change the metal in the complex amongst other things in this kind of chitosan complexes for obtain more information.

2. Materials and methods

2.1. Reagents

SMASE of *Bacillus cereus* (10 units), Chitosan with a degree of *N*-acetylation of 30% approximately (Lillo and Matsuhiro, 1997) of average MW: 1.11×10^6 Da (Chang et al., 2012) were obtained from Sigma Chemical Co. The Schiff base of 5-CH_3-chitosan-salicylaldehyde (SB- CH_3) was used for synthesize chitosan complexes of Fe(II), Ni(II), Cu(II) and Co(II), these species were characterized using, UV–visible and IR-TF spectroscopies and electrochemistry in previous work except nickel chitosan (Caro et al., 2013, 2014).

Other chemicals: KH_2PO_4-Na_2HPO_4, NaCl, sulfuric acid, isopropanol, dimethyl sulfoxide (DMSO), acetone, ethanol, trypan blue solution and nitrogen (N_2) were of analytical grade from Merck and used as received.

2.2. Nickel chitosan synthesis and characterization

A suspension of 0.5 g of chitosan with 30 mL of methanol and 0.38 g of 5-methyl salicylaldehyde was stirred for 10 h in reflux at 60 °C. The product was filtered in vacuum and washed with methanol. The product was dried and weighted and then, 0.096 g of this solid was dissolved in methanol (30 mL) at ambient temperature. Nickel acetate (0.018 g) was added with stirring for 12 h and then was concentrated in vacuum. The solid obtained was filtered and washed with methanol obtaining 0.10 g of green nickel complex. The Schiff base and nickel complex were analyzed by IR-TF, UV–Visible and electrochemistry. A FTIR-4100 Jasco spectrophotometer recorded spectra with an accumulation of 64 scans and resolution of 4 cm^{-1} from 4000 to 200 cm^{-1}. For IR spectra, the samples (100.0 mg) were dried for 24 h at 30 °C and were mechanically mixed with 20 mg of KBr. Derivation, including the Savitzky–Golay algorithm with 25 smoothing points, was performed using the OPUS/I.R. version 1.4 software incorporated into the hardware of the instrument (Costamagna et al., 2003; Da Silva et al., 2011).

2.3. Electroanalytical measurements and UV–visible spectroscopy

For electrochemical measurements, the treatment of working electrode (0.196 cm^2) to obtain a reproducible surface before its modification implies, the clean in sulfuric acid 0.5 M at negative potentials and the polished its surface with 1200 and 4000 grit emery paper, finally, the surface of the electrode was cleaned in water by ultrasonic equipment. Complexes were adsorbed on glassy carbon by placing a drop of complex in DMSO (1.5×10^{-5} M) solution on the electrode surface for 20 min, then the electrode surface was treated with the same solvent, after this, the surface was cleaned with ethanol (Caro et al., 2004). A spiral wire exposing an area of 14 cm^2 was used like auxiliary electrode. All solutions were prepared using ultra-pure Milli-Q water (Millipore© UP system). Oxygen was eliminated by purging solutions with nitrogen for 25 min. SMASE was introduced to cell with 25 mL of buffer solution by adding 0.01 units of SMASE (10 μL). The electrochemical cell contain an auxiliary and work electrode, where its potential value were quoted versus calomel electrode of over saturated solution of KCl (SCE), like reference electrode.

The electrochemical cell, electrodes and all the reported potential values were obtained from an Electrochemical Analyzer potentiostat/galvanostat Model 600 D Series of CH Instruments.

For the UV–Vis spectroscopy measurement, the complexes were dissolved in phosphate buffer solution at physiologic pH and the spectra were measured in the 200–1000 nm range, in a Perkin Elmer Model Lambda 25 UV–Vis spectrophotometer with 1 nm resolution. The UV–Vis spectra, give information of ligation between the SMASE and different complexes in solution, were done by the addition of aliquots of 10 μL of SMASE toxin.

2.4. Microscopy: determining the survival of erythrocytes

The erythrocyte's count was done with blood samples collected and following dilutions just as early work (Caro et al., 2017). After three dilutions a solution of 3.833×10^6 cell/mL was obtained like mother solution. The control 1 solution had 10 μL of mother solution of red cells, 90 μL of saline solution and 1 μL (0.4%) of trypan blue solution (3.795×10^5 cell/mL). The control 2 solution was treated with blood solution, trypan blue and chitosan complex solution of 1.76×10^{-8} M, the control 3 solution had blood solution, trypan blue solution and 10 μL of SMASE. Finally the solution of the reaction had the same red cells concentration, SMASE and complex concentration that control solutions (Caro et al., 2017). The membrane of dyed cells was permeable and destroyed, then, counting of live cells was done in four external quadrants of the camera with Neubauer rule in a Motic AE31 Inverted phase contrast Microscope with a digital microscope camera Moticam 5+.

3. Results and discussion

3.1. FT-IR spectroscopy

The most significant bands found in the FT-IR spectra of Schiff base and nickel-complex of chitosan are present (see Table 1). The FT-IR spectra of Schiff base showed high absorption level at 1637 cm^{-1} attributed to the C=N stretching characteristic of the imino group. The second derivative FT-IR spectra of nickel complex showed a tendency to lower wavenumbers in the C=N absorption relative to the free ligand, indicating the coordination of the nitrogen atom of the Schiff base to the metal ion (Costamagna et al., 2003). Its appearance indicates that the alcoholic groups do not participate in the coordination to the metal.

3.2. Chitosan metal-complexes adsorbed on glassy carbon electrode

The Co, Fe and Cu (II) chitosan derivatives were characterized by elemental analysis (C, H, N), IR spectroscopy, UV–Vis spectroscopy and

Table 1
IR bands of imino group of the Ni-SB, Schiff bases (Caro et al., 2013), Co and Cu complexes (Caro et al., 2014).

Sample	$\nu_{C=O}$ (amide)	δ_{N-H} (amine)	$\nu_{C=N}$ (imine)	$\nu_{antisC-O-C}$ (bridge)	ν_{O-H} (phenol)	$\nu_{C=C}$ (aromatic ring)
Chitosan	1658.8	1594.9	Nd	1152	3370	Nd
Schiff Base	Nd	Nd	1637	1160	3424	1594
Ni-SB	nd	Nd	1635	1148	3290	1580
Co-SB	nd nd	Nd	1630	1150	3300	1573
Cu-SB		Nd	1629.7	1145	3270	1582

ν; streching vibrations. δ; bending vibrations. nd: not detected.