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Short Communication

A novel model to characterize the electric double layer of lectins from *Cratylia mollis* (Camaratu bean) and *Canavalia ensiformis* adsorbed on metallic surface

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Abstract

The seed lectin from *Cratylia mollis* (Cra) is glucose/mannose specific, similarly to Concanavalin A, Con A, a well characterized lectin from *Canavalia ensiformis*. The equilibrium properties of an adsorbed layer may be examined based on the redox potentials of the constituents of this layer. The potential for Con A and Cra was obtained by potentiostatic techniques, using a saline solution as support to control the distribution of charges between saturated calomel electrode and platinum electrode as the working electrode, in aerated environment. These potentials are modelled using a Chi-square statistical distribution ($p < 0.05$); greater stability for Con A was obtained with 1.0 mg/ml, at 10°C. The positive redox potential determined for Cra (+94 mV) and Con A (+88 mV) indicated a high sensitivity of our electrodes which will give information on the kinetic behaviour of the biological interface.

Keywords: *Cratylia mollis*; Redox potential; Potentiostatic technique

1. Introduction

Lectins are proteins that bind carbohydrates with high degree of specificity and have similar or distinct structures (Correia & Coelho, 1995; Sharon & Lis, 1990; Tavares et al., 1996; Seetharaman & White, 1996). The physicochemical characterization of lectins is important to explain their behaviour in different biological properties. The Concanavalin A, Con A, obtained from *Canavalia ensiformis* seeds is the most studied glucose/mannose lectin. Molecular forms, with glucose/mannose recognition have also been purified from *Cratylia mollis* lectin, Cra (Paiva & Coelho, 1992). Structural differences and distinct biological activities have been identified between Cra and Con A (Tavares et al., 1996). Electrochemical techniques are able to give a direct insight into the interface containing electrically charged groups adsorbed to electrode surface. This adsorption determines subsequent behaviour of the interface, and hence the biocompatibility of the material. The kinetics behaviour and redox potential of adsorbed molecules depend on a variety of factors: the pH near the electrode, the temperature in the system and the properties of a double layer at the interface. This potential is important for the application of cyclic voltammetry techniques in the kinetics investigation of a biological interface. In this work, the change capacity in the interface electrode/solution for Con A and Cra was monitored by potentiostatic techniques, with the purpose of determining the electrochemical redox potential of the lectins.

2. Materials and methods

All lectin solutions were prepared in 0.15 M NaCl. The Con A was obtained from Sigma and Cra was purified on Sephadex G-75, according to Correia and Coelho (1995).

2.1. Apparatus and experimental

To carry out the experiments, an electrochemical system was used with a cell containing a working and a reference

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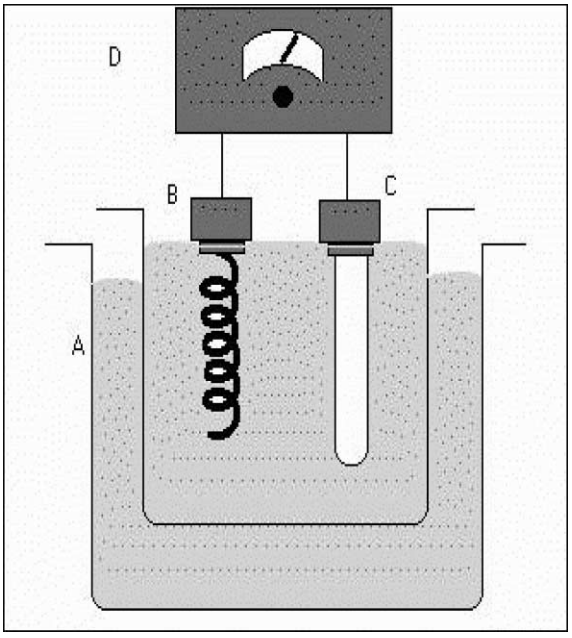

Fig. 1. Electrochemistry cell coupled to a multimeter of high impedance to measure the equilibrium potential. Electrochemistry cell (A), work electrode (B), Calomel electrode (C) and multimeter (D).

electrode, coupled to a high impedance multimeter (ICEL IK-1500), to register electrochemical potentials. The working electrode was a sheet of platinum in a spiral form, measuring 7 cm^2. The system was immersed in an ice bath (Fig. 1) and different temperatures (5, 10 or 20°C) were maintained. The Con A electrochemical potential was determined in the following concentrations: 0.6, 0.9 and 1.0 mg/ml, at the different temperatures; for Cra the electro-chemical potential was determined for a concentration of 1 mg/ml, at 10°C. The redox potential behaviour was modelled using a Chi-square statistical distribution ($p \ll 0.05$) obtained through separate exponential equations; all the treatments were derived from at least five repetitions. The following equation $[Y = Y_\theta + A\exp(-x/t)]$ described the redox potential; where Y is the redox potential at time t, after immersion of the electrode in the electrode in the lectin solution and Y_0 is the measured potential at time 0.

3. Results and discussion

The exponential equations of Con A and Cra, as well as the decay time for Con A are shown in Fig. 2. Temperature affects several variables that control the change capacity at the electrode/electrolyte interface (Fricquelmont-Loïzos, Takenouti, & Kanté, 1997), at 10°C a significant slower decay was observed for all treatments (Fig. 2). The best

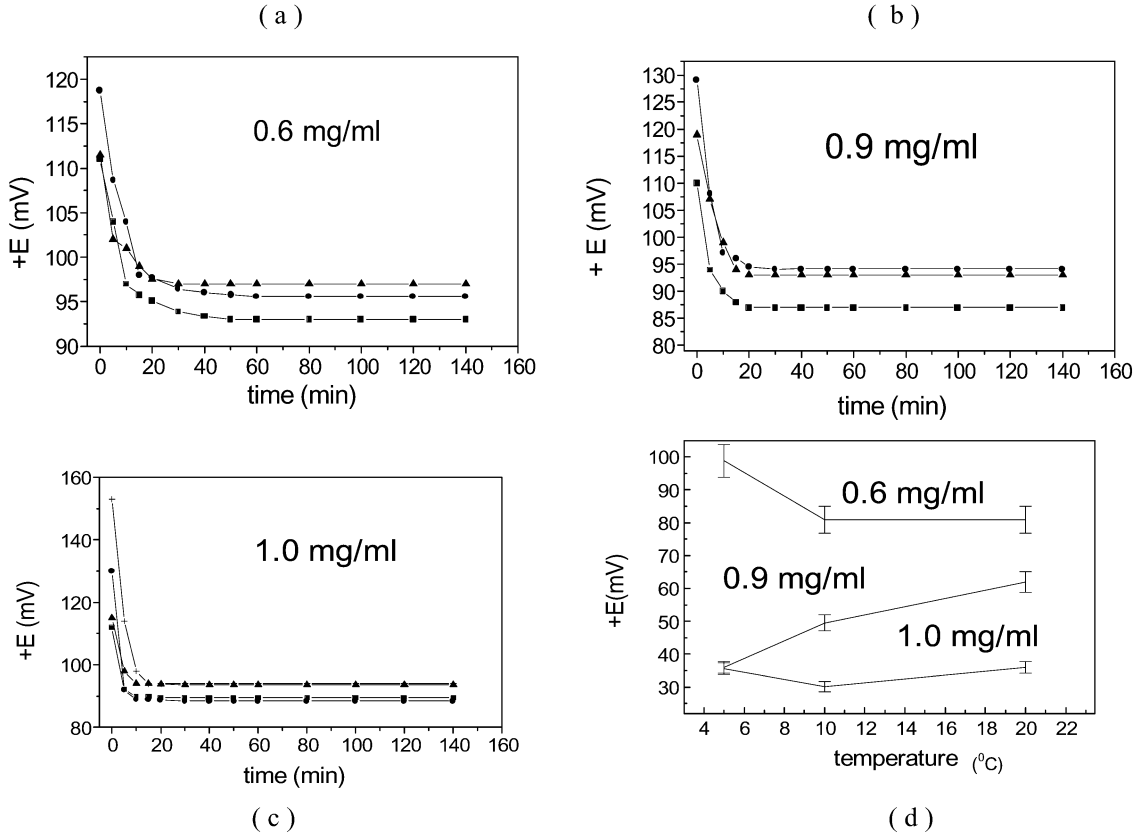

Fig. 2. The redox potential obtained experimentally was modelled by application of Chi-square ($p \ll 0.05$). The experiments were performed with Con A at: (a) 5°C (■); (b) 10°C (○); and (c) 20°C (▲) and Cra in the concentration of 1 mg/ml at 10°C (— + —) in (c).

result for Con A was obtained with 1.0 mg/ml, at 10°C, with greater stability of the redox potential (Fig. 2c), in a short-time (Fig. 2d). The decay time of the conformational changes on the metal surface provides information that are well suited to kinetic studies using electrochemical and spectroelectrochemical methods (Fricquelmont-Loïzos et al., 1997). These physicochemical approaches have been used to analyse protein structures in the electrode interface (David, King, & Hawkridge, 1998). The Cra-redox potential was at +94 mV, defined on the later condition (Fig. 2c), which was more positive than Con A (+88 mV); they were stabilized at 30 and 20 min, respectively. The electrical potential difference between the two phases, the electromotive force (EMF), was the resultant of transference of electron from a metal conductor to an electron acceptor in the second phase. On the basis of the EMF data and the known potential of the reference electrode, the overall redox potential was obtained (Nikitas, 1996). It depends on various factors, such as conformation of the adsorbed molecule, adsorption strength, and stabilization time of the double layer at the interface electrode/solution (David et al., 1998; Fricquelmont-Loïzos et al., 1997). All factors depend up on the material from which the electrode is made (Cohen, Leve, Rubin, & Willner, 1996; Kim & Seung, 1996; Nikitas, 1996). The redox potential for Con A in the concentration range of 2–20 μM on an Au-electrode, with a surface area of 0.03 cm^3, was −150 mV and had a small change capacity in the electrode/electrolyte interface (Cohen et al., 1996). In our work, the potential for Cra (Fig. 2c) and Con A (Fig. 2–c) was observed to be positive. The observed results suggested that a high surface of the electrode increases the stabilization of the double electric layer at the electrode/solution interface. The area of the electrode is important to obtain high and more stabilized potentials (Kim & Seung, 1996). The knowledge of the redox potential is needed to obtain cyclic voltammograms of the interaction between lectin and carbohydrate (Cohen et al., 1996), and for analysis of the redox-active biological complexes on metal electrodes (Kayesta & Hajela, 1996; Kim & Seung, 1996; McLeod, Freeman, Harvey, Lay, & Bond, 1996). The range of redox potential from −300 to +300 mV in cyclic voltammetric experiments to cytochrome *c* in an HS–$(CH_2)_9$-functionalized Au electrode vs Ag|AgCl|3 M KCl, was within the range reported for native cytochrome *c* in contact with a mediator layer (Niaura, Gaigalas, & Vilker, 1996). The investigation of redox potential for Cra and Con A will make the development of cyclic voltammograms with initial redox potential of +94 and +88 mV, respectively, possible. The knowledge of the electrical double layer in the electrode/solution interface for the studied lectins is of great importance for understanding the reaction mechanism of these proteins. A very small change, at the electrode/solution interface could result in high or low ability of the redox proteins to participate in the electrons transference reactions.

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