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Electrochemical Impedance Spectroscopy Study of Concanavalin A Adsorption on Glassy Carbon Electrode: An Analysis of Capacitance Dispersion

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ABSTRACT

Electrochemical impedance spectroscopy has been used to investigate the adsorption of concanavalin A lectin on glassy carbon electrode in 0.15 M NaCl solution as a function of electrode potential in the range of 0.2 to 0.8 V vs. Ag/AgCl. Under such conditions a maximum coverage of 0.23 is obtained. A graphical procedure is described to extract relevant information on the electrical behavior in terms of capacitance dispersion (Constant Phase Element, CPE). It is found that the protein does not desorb in the potential range investigated. Potential dependent capacitance dispersion is observed, being highest at more cathodic potentials. With additional information from electrostatic potential calculations the results indicate that the protein is adsorbed with a side-on orientation having its dipole moment oriented parallel to the surface.

1. Introduction

Proteins adsorption onto solid/liquid interfaces plays a key role in essential biological processes [1], and has several implications on medical (dentistry, implants) [2–5], materials [6–8] and industrial research (cosmetics, pharmaceutics, food) [9–11].

For analytical purposes, the development of biosensors is deeply related to studies to control protein adsorption, in order to avoid problems like difficult microfluid transport on Lab-on-chip devices [12,13], loss of sensibility of enzyme-linked immunoassays due to denaturation [14,15] or uncontrolled orientation [16], biofouling at *in vivo* biosensors [17], non-specific interactions in protein microarrays [18], and others. Electrochemical Impedance Spectroscopy (EIS) detection is often incorporated in those devices [19,20], but can also be used to study the adsorption process *per se*. The electrical response of the surface to protein adsorption is frequently related to the capacitive component [21], in general associated with changes in thickness or relative dielectric constant of the boundary phase [22].

Concanavalin A is a lectin with well characterized structure [23,24] and with a variety of proposed applications on biosensors, especially for glucose detection [25–27]. The protein can be found as dimer or tetramer depending on solution parameters, especially pH [28]. Although adsorption studies concerning this protein were already performed in surfaces such as mica [29], polystyrene [30], germanium [31] and other semiconductors [32], electrochemical investigations are poorly found on literature, except for platinum surfaces [33,34].

Capacitance is a relevant parameter in protein adsorption characterization. In some conditions, it can be used to estimate protein coverage θ according Eq. (1), based on Frumkin model of two parallel capacitors [35–37]:

$$\theta = \frac{C_{\theta=0} - C}{C_{\theta=0} - C_{\theta=1}} \quad (1)$$

where $C_{\theta=0}$ and $C_{\theta=1}$ are the capacitances when $\theta = 0$ and 1, respectively, and C is the capacitance for $0 \leq \theta \leq 1$. The relation can be used if $d\theta/dE \sim 0$ [38], that is, potential regions where there is no induced desorption.

By using Eq. (1) it is possible to monitor the amount of protein adsorbed with time and in equilibrium conditions as a function of several variables. For some proteins, electrostatic interaction has great influence in adsorption process, so a knowledge of capacitance vs. potential profile offers a way to control the amount adsorbed by changing the potential [16,39,40].

The capacitance is commonly obtained from EIS data by considering the equivalence between an electrical circuit and the electrochemical interface. The circuit chosen depends on the

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behavior of experimental results. For a *blocking electrode*, i.e. when electrons are blocked and do not cross the interface, the impedance may be expressed as:

$$Z(j\omega) = R + \frac{1}{j\omega C} = R - \frac{j}{\omega C} = Z' + jZ'' \quad (2)$$

where $j = (-1)^{1/2}$, ω the angular frequency, R the solution resistance, C the double layer capacitance, Z' and Z'' the real and imaginary part of impedance, respectively.

In capacitance studies from impedance data involving protein adsorption it is common to use the imaginary part of impedance as a way to obtain C for a given frequency ω by means of Eq. (3):

$$Z'' = -\frac{1}{\omega C} \quad (3)$$

In most situations, a frequency range where C is independent of ω may be chosen to represent the capacitance of the system, presumably the double-layer capacitance. This can be verified by a $\omega Z''$ vs. ω plot, which usually gives a decrease in capacitance until a pseudo-plateau is reached. Then one frequency in that plateau range is picked to obtain the capacitance from measurements of Z''. This approach has been largely used to characterize kinetics of protein adsorption [41–43]. However, for this purpose, many frequency values have been used, either based on capacitive plateau or without a clear justification [44–48].

For an ideal *blocking electrode* the Nyquist plot is a vertical straight line over the whole frequency range. This is due to the nature of the capacitor in the circuit which has a constant phase angle of 90°. In many cases this behavior is not observed, and the result is a tilted lined with a less than 90° slope. In these cases, the capacitor of the RC circuit is then substituted by a new circuit element, a constant phase element (CPE, or Q), which has a phase angle related to the parameter α. Therefore, the impedance of this RQ circuit is described by Eq. (4):

$$Z(j\omega) = R + \frac{1}{(j\omega)^{\alpha} Q} \quad (4)$$

and leads to a more general relation between the imaginary part of impedance and frequency [49]:

$$Z'' = -\sin\left(\frac{\alpha\pi}{2}\right)\frac{1}{\omega^{\alpha} Q} \quad (5)$$

This behavior has been interpreted in terms of a so called capacitance dispersion. According to Brug et al. [50] α is related to dispersion of relaxation time $\tau = RC$ through the surface due to capacitance inhomogeneity. They developed, based on dielectric distribution used by Cole-Cole [51], a well known relation for this capacitance as a function of Q, α and R for blocking electrodes:

$$C = \left(QR^{1-\alpha}\right)^{1/\alpha} \quad (6)$$

And, naturally, if $\alpha = 1$ the equation above implies $C = Q$. Some effects which can interfere on α values (in different conditions) are roughness [52], fractal geometry of surface [53], adsorption [54,55], 2D and 3D geometric distributions [56] and heterogeneities in atomic scale [57,58].

It is clear that when $\alpha = 1$ Eq. (5) yields Eq. (3), and $Q = C$. Orazem et al. [49] applied Eq. (5) in order to obtain α from the slope of a linear segment of a log $|Z''|$ vs. log ω plot. Hence, the Q values obtained are dependent on frequency, but are constant in the high frequency side. They applied this procedure to a reactive (non-blocking) interface. For a blocking electrode only a simulation of this procedure was presented, indicating that Q should be constant over the whole frequency range.

In this work we performed an evaluation/comparison of some methods to obtain Q, α and C, and also investigated the behavior of those parameters on adsorbed concanavalin A as a function of potential. Moreover, to increase our understanding of protein adsorption phenomena we performed some calculations using molecular mechanics approach. From the results of Bouckaert et al. [59] available in PDB server, we calculate protein surface electrostatic potential, which was useful in the discussion of the role of adsorbed protein orientation on C and α variation with potential.

2. Methods

2.1. Solutions

All the solutions were prepared using Milli-Q water (resistivity 18.2 MΩ cm), which was also used in the cleaning and rinsing procedures. NaCl (Vetec, reagent grade) was used to prepare 0.15 M electrolyte solution (pH 6). Concanavalin A (Sigma-Aldrich, Type IV) was used without further purification.

2.2. Cell

For impedance measurements a two-compartment electrochemical glass cell, with three electrodes, was employed. A glassy carbon disk electrode (BAS, 3 mm diameter) was used as working electrode, a gold wire was used as counter electrode and Ag/AgCl/saturated KCl as reference electrode, to which all the potentials E_{DC} are referred. All experiments were carried out at the temperature of 24 °C.

2.3. Adsorption procedure

The working electrode was polished for 3 minutes with alumina slurry (1 μm) using a polishing cloth, rinsed with water and placed in ultrasonic bath (Unique Ultrasonic Clean, model USC-800) for 7 minutes to remove excess alumina. Then it was placed in H_2SO_4 98% (F. Maia, reagent grade) for 5 minutes and it was electrochemically cleaned by potential cycling between 0.9 to -0.2 V (50 cycles at 200 mV s^{-1}) in 0.5 M H_2SO_4 solution in a single compartment cell. The cleanliness of the surface was evaluated by cyclic voltammetry in 4 mM $Fe(CN)_6^{3-}$ (Vetec, reagent grade)/0.5 M KNO_3 (Nuclear, reagent grade) solution, yielding a peak difference between anodic and cathodic potentials around 63 mV. All voltammetric measures were made with a Gamry potentiostat (potentiostat/galvanostat/ZRA reference 600™) with the cell inside a Faraday cage (Gamry Vista Shield). After cleaning the electrode was placed in protein solution (0.8 mg mL^{-1} in 0.15 M NaCl) for 40 min (established to maximize adsorption), followed by a rinse with water and then impedance measurements were performed.

2.4. Impedance measurements

The same apparatus employed on cyclic voltammetry experiments was used to obtain impedance data. The impedance spectra were acquired in the frequency range of 10^{-1} to 10^{4} Hz (10 points per decade). A sinusoidal potential wave with amplitude E_{AC} = 5 mV was superimposed on constant potentials E_{DC} between 0.2 and 0.8 V. The experiments for different E_{DC} were executed randomly. Impedance data was fitted using Z-*view* software (2.3d, Scribner Associates, Inc.). Impedance measurements were performed both on the bare electrode and on the electrode with adsorbed protein, in 0.15 M NaCl. All statistical analysis were carried out at the 95% confidence level.

2.5. Electrostatic potential calculations

The .pdb file (code 1DQ2) describing the apo-concanavalin A dimer atomic coordinates was converted in .pqr file at pH 6 using PDB2PQR [60] and then the electrostatic potential was calculated