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Short communication

Impedance-derived electrochemical capacitance spectroscopy for the evaluation of lectin–glycoprotein binding affinity

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ABSTRACT

Characterization of lectin–carbohydrate binding using label-free methods such as impedance-derived electrochemical capacitance spectroscopy (ECS) is desirable to evaluate specific interactions, for example, ArtinM lectin and horseradish peroxidase (HRP) glycoprotein, used here as a model for protein–carbohydrate binding affinity. An electroactive molecular film comprising alkyl ferrocene as a redox probe and ArtinM as a carbohydrate receptive center to target HRP was successfully used to determine the binding affinity between ArtinM and HRP. The redox capacitance, a transducer signal associated with the alkyl ferrocene centers, was obtained by ECS and used in the Langmuir adsorption model to obtain the affinity constant $(1.6 \pm 0.6) \times 10^8$ L mol^{-1}. The results shown herein suggest the feasibility of ECS application for lectin glycoarray characterization.

1. Introduction

Electrochemical approaches such as amperometry (Poorahong et al., 2011), voltammetry (Compton and Banks, 2007), and impedance spectroscopy (EIS) (Lisdat and Schäfer, 2008; Bueno and Gabrielli, 2009) are reproducible and suitably sensitive to transduce biological signals inherent in binding affinity events, e.g., antigen–antibody (Bryan et al., 2013) and enzyme–substrate (Gonçalves et al., 2014). Additionally, EIS has been used successfully to characterize lectin–carbohydrate interactions and for the design of glycoprotein biosensors (Bertok et al., 2013; Loaiza et al., 2011; Yang et al., 2013).

Faradaic capacitive approaches comprising impedance-derived electrochemical capacitance spectroscopy (ECS) have not yet been employed for glycoprotein biosensoring. ECS biosensoring has the advantage of eliminating the need for a redox probe in the biological matrix or electrolyte. The differences between EIS and ECS are associated with the electrochemistry communication between the redox probe and the metallic electrode surface (Fernandes et al., 2014). The transducer signal in EIS measures the change in the impedance caused by the target binding on the biosensor surface, specially associated with the charge transfer resistance (R_{ct}) element in a redox probe in the biological matrix or electrolytic solution (Fernandes et al., 2013, 2014;). However, the ECS transducing signal measures the change in the redox capacitance (C_r) of the redox tethered receptive interfaces (Fernandes et al., 2013, 2014; Lehr et al., 2014; Bueno and Davis, 2014a, 2014b) and does not require a redox probe in solution. This causes the ECS biosensor approach to be advantageous for multiplexing point of care applications.

Briefly, in ECS the transduction signal, C_r, is related to the redox density of states (DOS) whose occupancy, for one single state level, is given by Bueno et al. (2012, 2013), Bueno and Davis (2014a, 2014b):

$$C_r = \frac{e^2 \Gamma}{k_B T} f(1-f) \tag{1}$$

where $f = n/\Gamma = F(E_r, \mu_e) = \{1 + \exp[(E_r - \mu_e)/k_B T]\}^{-1}$ is the occupation function given by the Fermi Dirac function, n is the electron density occupancy, e is the elementary charge, k_B is the Boltzmann constant, T is the absolute temperature, Γ is the redox molecular surface coverage, E_r is the half-wave potential and μ_e electron chemical potential (Bueno et al., 2012, 2013).

Affinity interaction is transduced to the redox capacitance mechanistically as proposed by Bueno et al. (2012) and Bueno and Davis (2014a, 2014b). The hypothesis is that a redox probe tethered in a SAM has a charging fingerprint (energy storage from

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a combination of the electrostatic field associated with charge separation and a quantized term resulting from resonant access to specific confined molecular states – C_r or redox capacitance) sensitively dependent on the redox probe environment. A change in the environment, due a biorecognition event such as antibody–antigen and lectin–glycoprotein bindings, disturb C_r, thus allowing transduce of the signal. This idea has been used to detect successfully relevant biomarkers (Lehr et al., 2014; Fernandes et al., 2013, 2014).

We use C_r as the transducer signal to characterize the ArtinM lectin binding with HRP glycoprotein using the binding affinity constant calculated from the Langmuir isotherm model. ArtinM was selected since it is an important mannose binding lectin from *Artocarpus heterophyllus* seeds; ArtinM is a tetrameric non-glycosylated protein composed of identical 16 kDa protomers, each one with a carbohydrate recognition domain (CRD) (Rosa et al., 1999). Glycoarray analysis of ArtinM specificity revealed that subsets of complex-type bi-antennary N-glycans containing Manα1-3 (Manα1-6)Manβ1 are well recognized by the lectin (Nakamura-Tsuruta et al., 2008), which accounts for the selectivity of ArtinM binding to certain N-glycans such as those linked to some protein cell receptors (Carvalho et al., 2011; Pereira-da-Silva et al., 2012). Furthermore, the interaction of ArtinM–N-glycan can be explained using the ArtinM–HRP model, i.e. using crystallography analysis (Jeyaprakash et al., 2004) and a label-free sensor, such as Quartz Crystal Microbalance (QCM), with dissipation factor (Pesquero et al., 2010; Giménez-Romero et al., 2013).

2. Material and methods

2.1. Reagents

All reagents described in this section were purchased from Sigma-Aldrich.

Lectin ArtinM was extracted from *Artocarpus heterophyllus* seeds by affinity chromatography with immobilized D-mannose as previously described (Santos-de-Oliveira et al., 1994).

All solutions used in the analytical procedures were prepared with Milli-Q purified water (Millipore Corp., Simplicity System, Bedford, MA, US) with 18.2 MΩ cm at 25 °C. The protein solutions were prepared and used in a 10 mM PBS (phosphate buffered saline) solution at pH 7.4.

2.2. Biosensoring surface engineering

The self-assembled monolayer (SAM) approach was used to immobilize both the lectin (ArtinM) and alkyl ferrocene (redox probe) onto the gold surface and obtain the transducer capacitive signal proportional to the ArtinM–HRP binding. The gold electrode (2 mm, Metrohm) was cleaned and the surface area was determined as previously described (Fernandes et al., 2014; Lehr et al., 2014). These determinations (~0.036–0.038 cm^2) were used to normalize the absolute recorded capacitance.

The electroactive SAM was constructed by immersing the cleaned gold surface for 16 h at 25 °C in a mixed solution of 0.2 mM 16-mercaptohexadecanoic acid (for covalent lectin attachment) and 2.0 mM 11-(ferrocenyl)undecanethiol (redox probe) in ethanol. The carboxyl groups of the 16-mercaptohexadecanoic acid were activated with an aqueous solution containing 0.4 M N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) and 0.1 M N-hydroxysuccinimide (NHS) for 30 min. Subsequently, the electrode was washed using PBS, dried with nitrogen gas, and immersed in 0.15 mg mL^{-1} ArtinM solution in PBS for 1 h. Finally, the ArtinM electrodes were immersed in a 0.1% gelatin solution for 30 min to block unspecific sites (Fig. 1a).

2.3. Electrochemical capacitance spectroscopy measurements

An AUTOLAB potentiostat (PGSTAT30) with a FRA module controlled by NOVA software was used for all electrochemical measurements. A three-electrode setup was used for the measurements consisting of a 2.0 mm diameter gold working electrode from METROHM, a platinum mesh counter electrode, and an Ag|AgCl (saturated KCl) reference electrode.

Electrochemical characterization was conducted using the supporting electrolyte of 20 mM $TBAClO_4$ (tetrabutylammonium perchlorate) dissolved in acetonitrile and H_2O (20:80) without a redox probe. EIS measurements were conducted in the frequency range 10 mHz to 100 kHz with peak to peak amplitude of 10 mV and verified for compliance with the Kramers–Kroning linear systems theory using NOVA software. The ECS analysis was performed by determining the capacitance using the following relationship $C^*(\omega) = 1/i\omega Z^*(\omega)$, where ω is the angular frequency and i is the complex number, $i = \sqrt{-1}$ (Bueno et al., 2013). In processing $Z^*(\omega)$ it is possible to obtain the imaginary $C'' = \varphi Z'$ and real $C' = \varphi Z''$ part of capacitance noting that $\varphi = 1/(\omega|Z|^2)$.

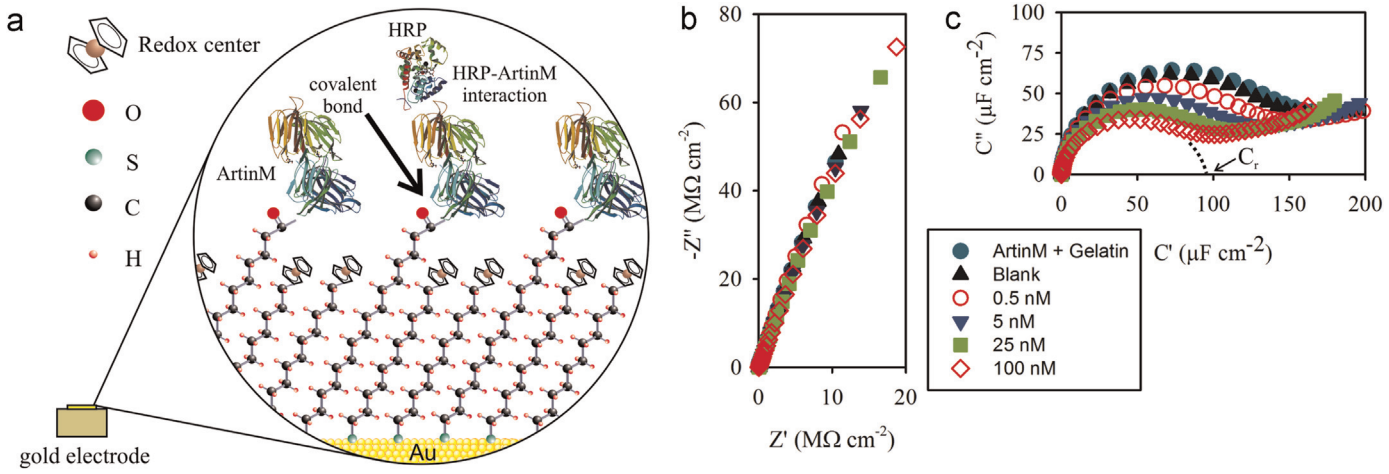

Fig. 1. (a) Schematic representation of functional SAM to characterize ArtinM–HRP binding by ECS. The C_r signal obtained from ECS is sensitive to the HRP target concentration and can be used as a transducer signal to design label-free glycoprotein sensing. Examples of (b) impedance and (c) capacitance Nyquist plots of the ArtinM-HRP interface at different concentrations of HRP. The higher responsiveness exhibited by the capacitance in comparison to the impedance plots underlines the utility of capacitive redox glycoprotein sensing. C_r is obtained from the diameter of the semi-circle in capacitive Nyquist diagrams (as exemplified in c). The data were obtained at the E_r, i.e. 0.48 V vs Ag|AgCl.