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Application of electrochemical impedance spectroscopy for studying of enzyme kinetics

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ABSTRACT

The application of electrochemical impedance spectroscopy (EIS) in theory and experiment for investigation of redox enzyme kinetics has been described. As a model system an enzyme horseradish peroxidase adsorbed on graphite electrode has been chosen. Three different mathematical models based on generalized mechanism of horseradish peroxidase catalyzed hydrogen peroxide reduction have been formulated and used for derivation of theoretical electrochemical impedances. In this way, mechanistic details related to bioelectrochemical reaction including all relevant kinetic parameters are obtained. The presented approach overcomes limitations of classical equivalent circuit approach, since it does not rely on phenomenological elements, but on particular reaction mechanism. We have shown that the EIS is more sensitive for parameter estimation and model discrimination than a steady state response.

1. Introduction

Redox enzymes can be efficiently coupled with an electrode surface giving prospect of highly efficient and selective bio(electrochemical) transformations for energy conversion and/or production of commodities or fine chemicals. One example is glucose oxidase which coupled with an electrode in presence of glucose and providing for oxygen reduction cathode generates electricity and D-glucono-1,5-lactone with applications in different industries [1]. Other examples might comprise whole enzymatic cascades performing complex sequences of biochemical reactions, turning for example such inert and environmentally polluting substances (like CO_2) into useful commodities (e.g. methanol) [2,3]. These processes have a significant potential for development of new enzyme based production systems, with electrochemistry playing an important role, especially regarding electrochemical regeneration of redox enzymes (redox co-factors). Although the electrochemical regeneration is feasible, its efficiency is still too low to be considered competitive for industrial applications [4]. Part of the reason is complexity of such electrochemical systems, commonly involving enzymes, mediators, and electron conductive materials such as carbon nanomaterials as well as some additives. All these components are interacting with an enzyme in a certain way, influencing its catalytic activity. To design, optimize and improve an efficient bio(electrochemical) system detailed knowledge and understanding of the involved steps (enzyme kinetics, electron transfer kinetics, mass transfer) and their interactions are required. In this respect, advanced experimental methods which can enable distinction of involved processes at the different time scales and which can provide quantitative system characterization can be helpful.

An example of such a method is electrochemical impedance spectroscopy (EIS), with broad range of applications in different fields of electrochemical science and engineering. EIS has also been used for studying of bioelectrochemical systems. Major application here was monitoring of the immobilization of biomaterials such as enzymes, or antigens/antibodies on electrodes by recording the changes in the Faradaic impedance of a redox probe (e.g. ferri/ferrocyanide) [5–9]. The data evaluation was mainly phenomenological; for example by using an equivalent circuit approach [5], where kinetic data related to the Faradaic impedance of a redox probe on protein modified surfaces were obtained.

Unlike these previous reports, the focus of the present work is the Faradaic impedance of bioelectrochemical event itself. The analysis in the present paper includes the derivation of theoretical impedance of enzyme/electrode system based on reaction mechanism of enzyme catalyzed electrochemical reaction and the experimental validation. In this way mechanistic details related to bioelectrochemical reaction including all relevant kinetic parameters can be obtained. The presented approach overcomes limitations of classical equivalent circuit approach, since it does not rely on phenomenological elements, but on particular reaction mechanism.

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In following, three mathematical model variants based on primary catalytic cycle of HRP catalyzed hydrogen peroxide reduction have been formulated. The models have been implemented in MATLAB software and used to simulate theoretical steady state and electrochemical impedance responses. The parameter values are obtained by fitting of the experimental data. While all three models were able to describe experimental steady state data, only the model taking into account enzyme kinetics in accordance to Michaelis–Menten treatment was able to describe impedance response under all investigated conditions. It was shown that EIS is more sensitive for parameter determination and model discrimination than a steady state analysis.

2. Derivation of theoretical electrochemical admittance

Analytical expressions of electrochemical admittance are developed, based on nonlinear frequency response (NLFR) approach [10,11], restricted in the present case to its linear part. This method was initially developed for analysis of nonlinear electrical circuits and in chemical engineering for investigation of adsorption equilibrium and kinetics [11,12]. Its application in the case of electrochemical systems has been demonstrated in our recent papers on an example of methanol and ferrocyanide oxidation kinetics [13–15]. In general, this method provides a set of frequency response functions (FRFs), where the first order FRF contains the linear part of the response, while the higher order FRFs contain nonlinear fingerprint of the system. We have shown that the first order FRF is identical to the electrochemical admittance (reciprocal of the electrochemical impedance). The procedure for derivation of the FRFs consists of the following steps (for further details please refer to [13–15]):

(a) Definition of nonlinear mathematical model including basic kinetic, mass transport equations as well as dynamic material and charge balances.
(b) Definition of input and output variables.
(c) Taylor approximation of the reaction rate expressions around the steady state.
(d) Substitution of the Taylor polynomials into the mass balance equations.
(e) Substitution of the input and outputs into the equations gained in step "d" and application of harmonic probing.
(f) Solution of the equations derived in step "e".

In following some details related to model definition as well as final expressions for steady state current and theoretical electrochemical admittance (reciprocal of impedance) are provided. For additional information please see Appendix B.

2.1. Model definition

As a model system horseradish peroxidase (HRP) adsorbed on graphite electrode has been chosen. First report of direct electron transfer (DET) of this enzyme on carbon surface in presence of hydrogen peroxide dates back to 1979 [16]. Since then extensive research on this enzyme, with evidences of DET on different materials has been conducted [17]. The mechanism of HRP catalyzed hydrogen peroxide reduction has been also studied extensively [18–20]. According to literature primary catalytic cycle, can be represented as follows [17,20]:

$$\underbrace{H_2O_{2bulk}}_{S_{bulk}} \xrightarrow{diff} \underbrace{H_2O_{2,0}}_{S(0,t)} \quad (1)$$

$$H^+_{bulk} \xrightarrow{diff} H^+_0 \quad (2)$$

$$\underbrace{H_2O_{2,0}}_{S(0,t)} + \underbrace{HRP\left[-Fe^{3+}\right]}_{\text{Native Ferric, } E_{red}} \overset{k_1/k_{-1}}{\rightleftarrows} \underbrace{HRP\left[-Fe^{3+} - H_2O_2\right]}_{ES} \quad (3)$$

$$\underbrace{HRP\left[-Fe^{3+} - H_2O_2\right]}_{ES} \xrightarrow{k_{cat}} \underbrace{HRP\left[-Fe^{4+} = O,\ \overset{\bullet}{P}^{+}\right]}_{CPI} + H_2O \quad (4)$$

or

$$\underbrace{H_2O_{2,0}}_{S(0,t)} + \underbrace{HRP\left[-Fe^{3+}\right]}_{\text{Native Ferric, } E_{red}} \xrightarrow{K_1} \underbrace{HRP\left[-Fe^{4+} = O,\ \overset{\bullet}{P}^{+}\right]}_{CPI} + H_2O \quad (5)$$

$$\underbrace{HPR\left[-Fe^{4+} = O,\ \overset{\bullet}{P}^{+}\right]}_{CPI} + H^+_s + e^- \xrightarrow{k_{e1}} \underbrace{HRP\left[-Fe^{4+} - OH\right]}_{CPII} \quad (6)$$

$$\underbrace{HRP\left[-Fe^{4+} - OH\right]}_{CPII} + e^- + H^+_s \xrightarrow{k_{e2}} \underbrace{HRP\left[-Fe^{3+}\right]}_{\text{Native Ferric, } E_{red}} + H_2O \quad (7)$$

where S refers to substrate (in this case hydrogen peroxide H_2O_2), E_{red} to the native form of enzyme, ES, to the enzyme substrate complex and CPI, and CPII, to the oxidized forms of the enzyme, the so-called compound I (CPI), and compound II (CPII). The subscript "bulk" refers to the bulk of the solution and the subscript "0" to the electrode surface.

According to this scheme, HRP reacts initially with the substrate forming an enzyme substrate complex (ES). The complex decomposes further to compound I (CPI) which is then electrochemically reduced in presence of proton H^+ giving compound II (CPII). The initial state of HRP is regenerated by a further electrochemical step followed by H^+ incorporation.

In addition to above presented steps, which correspond to primary catalytic cycle of HRP, more generalized catalytic cycles include also effects of HRP inhibition [17,21]. According to literature the effect of inhibition becomes significant at higher substrate concentrations and longer time of experiment (e.g. in [21] inhibition was reported for concentrations higher than 500 μM). To avoid this, analysis in the present paper is restricted to lower concentration range and shorter time of experiments where it can be assumed that the inhibition is significantly small and therefore can be neglected.

Based on reaction mechanism presented above and by introduction of different assumptions three model variants have been formulated in the present work.

In the model 1, steps 3 and 4 are lumped (step 5) and all mass transfer effects (substrate and proton diffusion, steps 1 and 2) are neglected. The model 2 is alike model 1, but it includes mass transfer effects. The model 3 is the most general one considering formation and disproportion of ES complex in accordance to steps 3 and 4 and all mass transfer effects. Similar assumptions have been also considered in literature, e.g. steps 3 and 4 were lumped in [18,19,22]. Besides, two electrochemical steps are often lumped in one step [18,22]. This assumption is perfectly justified in the case of steady state model formulations, but in the case of dynamic system characterization cannot be adopted.

The rate expressions for enzymatic steps Eqs. (3)–(5) are formulated as follows:

$$r_1(t) = k_1 \times \Gamma_{E_{red}}(t) \times c_S(0,t) - k_{-1} \times \Gamma_{ES}(t) \quad (8)$$

$$r_2(t) = k_{cat} \times \Gamma_{ES}(t) \quad (9)$$

in model 3 or

$$r_1(t) = K_1 \times \Gamma_{E_{red}}(t) \times c_S(0,t) \quad (10)$$

in models 1 and 2.