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Computational Modeling of the Amperometric Bioanalytical System for Lipase Activity Assay: a Time-Dependent Response

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Abstract. This paper presents computational modeling of response kinetics of bioelectroanalytical system based on the interfacial action of enzyme lipase. The model also assumes that the substrate of enzyme is located on the surface of micelles which are spread in the solution under study. Two distinct mathematical models have been developed and evaluated through computational simulation series. The results of simulation demonstrate that diffusion is important factor for the sensitivity of bioelectroanalytical system, and it is important to take this process of mass transfer into account in all system areas.

Keywords: computer simulation, biosensors, amperometric bioanalytical system.

1 Introduction

Recently, the amperometric detection method of *Thermomyces lanuginosus* lipase activity has been published [1]. Lipases, triacylglycerol hydrolases (EC 3.1.1.3) that cleave triacylglycerols at the oil/water interface, have extensive applications in the food, paper, pharmaceutical, cosmetic, detergent, leather, and textile industries [2, 3]. Widespread practical use of these enzymes requires fast and reliable analytical routines to assess their activity. The electrochemical technique, described in [1], presents the method of this kind.

In the work under discussion, a lipid-like synthetic compound O-palmitoyl-2,3-dicyanohydroquinone (PDCHQ), that contains both the ester and the electroactive hydroquinone-based groups, was used as a lipase substrate. The PDCHQ molecules were solubilized in the Triton X-100 micelles, while the product of enzymatic hydrolysis, 2,3-dicyanohydroquinone, was readily oxidized on the electrode in a diffusion-controlled

process. Under the diffusion control, the magnitude of the electrode current is determined solely by the concentration and diffusion coefficient of the electroactive species (in the case of work [1], 2,3-dicyanohydroquinone) and the effective thickness of the diffusion layer [4].

The authors of the paper [1] have performed experiments under the steady-state conditions. The aim of the present work is computational modeling of response kinetics of this bioelectroanalytical system.

2 Model

In a simplified one-dimension model (Fig. 1), the working space of bioelectroanalytical system described in [1] could be divided into two parts: the first one – wide area, where enzymatic reaction and molecular/particle (convective-)diffusion occur, the second one - narrow area of a diffusion layer, where the diffusion of hydrolysis product occurs. The latter model assumes that area 2 experimentally could be made inaccessible (e.g., by covering the electrode surface with dialysis membrane) for other components of the system.

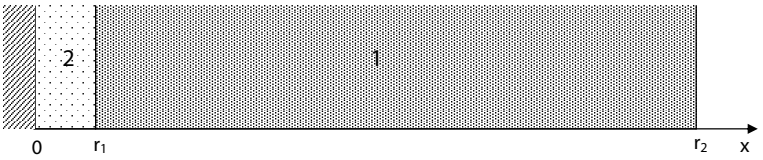

Fig. 1. Scheme of the model used in the present study: 1 – area, where reaction and diffusion and/or convection occur, 2 – area, where reaction product diffusion occurs, $x = 0$ – the electrode surface, $x = r_1$ – outer surface of area 2.

The processes in area 1 could be written in the following schematic form which is most commonly used for the description of lipase interfacial activation [5]:

$$E \underset{k_d}{\overset{k_p}{\longleftrightarrow}} E^*, \tag{1}$$

$$E^* + S \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} E^*S \xrightarrow{k_{cat}} E^* + P, \tag{2}$$

where E is the enzyme in solution, E^* is the enzyme penetrated in the surface of micelle, S is the substrate on the micelle surface, E^*S is the enzyme-substrate complex, and P represents the reaction product. According to the model, only P diffusion takes place in area 2, generating amperometric response of the system. The electrical signal is proportional to the derivative of reaction product concentration $\frac{\partial P}{\partial x}|_{x=0}$. The change of this parameter with time is the object of our computational simulations.

The system under discussion can be described by two different mathematical models:

1. Assuming that area 1 is large enough and substances are distributed evenly, e.g., by convection process. Thus, it may be inferred that the concentrations of all substances

are uniform across all area, and reaction equations can be solved in single space point without taking diffusion into account. Also it is assumed that there is no special separation between areas 1 and 2, therefore all substances (except the reaction product P) are uniformly distributed across area 2;

2. It is assumed that substances are distributed non-uniformly in area 1 and diffusion should be taken into account. It is also assumed that there is special separation between areas 1 and 2 (e.g., area 2 represents dialysis membrane of thickness r_1 on the electrode surface), so only reaction product diffusion occurs in area 2.

For both models it is true that beyond zone 1 ($x > r_2$), there is large volume uniformly filled with the same substances and where the same reactions occur. All these substances and reaction product flow to zone 1 through boundary $x = r_2$.

First model is described by the following system of non-linear differential equations for single area 1 space point [5]:

$$\frac{dE}{dt} = -k_p \frac{I}{V} E + k_d \frac{I}{V} E^*, \tag{3}$$

$$\frac{dE^*S}{dt} = k_1 E^* \times S - (k_{cat} + k_{-1}) E^* S, \tag{4}$$

$$\frac{dE^*}{dt} = k_p E + (k_{cat} + k_{-1}) E^* S - (k_d + k_1 S) E^*, \tag{5}$$

$$\frac{dS}{dt} = k_{-1} E^* S - k_1 E^* \times S, \tag{6}$$

where symbols E, E^*, E^*S and S represent concentrations; I is the total interfacial area of micelles; V is the total volume; k_p, k_d, k_1, k_{cat}, k_{-1} are the rate constants shown in equations (1) and (2); t – time. The following initial conditions ($t = 0$) were applied:

$$\begin{aligned} &E(0) = E_0, \\ &E^*(0) = 0, \quad E^*S(0) = 0, \\ &S(0) = S_0. \end{aligned} \tag{7}$$

Additional equation for area 2 (product diffusion plus gain from reactions in this area):

$$\frac{\partial P}{\partial t} = d_P \frac{\partial^2 P}{\partial x^2} + k_{cat} \frac{I}{V} E^* S, \quad x \in (0, r_1), \tag{8}$$

where symbol P represents reaction product concentration; d_p is the diffusion coefficient of P; x – distance, E^*S is calculated from solution of equation system (3)–(6). Initial condition ($t = 0$) for the second part of calculations:

$$P(0, x) = 0, \quad x \in [0, r_1], \tag{9}$$

whereas boundary conditions:

$$P(t, 0) = 0, \quad t > 0 \tag{10}$$

and

$$\frac{\partial P}{\partial t} = k_{cat}\frac{I}{V}E^*S, \quad x = r_1,\ t > 0, \tag{11}$$

which is calculated from solution of equation system (3)–(6).

The second model is described by the system of non-linear partial differential equations [5]:

$$\frac{\partial E}{\partial t} = -k_p\frac{I}{V}E + k_d\frac{I}{V}E^* + d_E\frac{\partial^2 E}{\partial x^2}, \tag{12}$$

$$\frac{\partial E^*S}{\partial t} = k_1E^* \times S - (k_{cat} + k_{-1})E^*S + d_{E^*S}\frac{\partial^2 E^*S}{\partial x^2}, \tag{13}$$

$$\frac{\partial E^*}{\partial t} = k_pE + (k_{cat} + k_{-1})E^*S - (k_d + k_1S)E^* + d_{E^*}\frac{\partial^2 E^*}{\partial x^2}, \tag{14}$$

$$\frac{\partial S}{\partial t} = k_{-1}E^*S - k_1E^* \times S + d_S\frac{\partial^2 S}{\partial x^2}, \tag{15}$$

for area $x \in (r_1, r_2)$. Definitions are the same as for the first model, and d_E, d_{E^*S}, d_{E^*}, d_S are the diffusion coefficients of free enzyme, micellar enzyme-substrate complex, micelle with penetrated enzyme (in fact, $d_{E^*S} = d_{E^*}$), and substrate, respectively. Reaction product generation and diffusion equation is as follows:

$$\begin{aligned} &\frac{\partial P}{\partial t} = qk_{cat}\frac{I}{V}E^*S + d_P\frac{\partial^2 P}{\partial x^2}, \quad x \in (0, r_2),\\ &q = \begin{cases} 0, & x \in (0, r_1];\\ 1, & x \in (r_1, r_2). \end{cases} \end{aligned} \tag{16}$$

Initial conditions ($t = 0$):

$$\begin{aligned} &E^*(0, x) = 0, \quad E^*S(0, x) = 0,\\ &E(0, x) = E_0, \quad S(0, x) = S_0, \quad x \in [r_1, r_2];\\ &P(0, x) = 0, \quad x \in [0, r_2]. \end{aligned} \tag{17}$$

Boundary conditions:

$$P(t, 0) = 0, \quad t > 0; \tag{18}$$

no flow condition for subregions boundary point $x = r_1,\ t > 0$:

$$\left.\frac{\partial E}{\partial x}\right|_{x=r_1}(t) = 0, \ \left.\frac{\partial S}{\partial x}\right|_{x=r_1}(t) = 0, \ \left.\frac{\partial E^*S}{\partial x}\right|_{x=r_1}(t) = 0, \ \left.\frac{\partial E^*}{\partial x}\right|_{x=r_1}(t) = 0 \tag{19}$$

and boundary condition for point $x = r_2, t > 0$:

$$\begin{aligned} &P|_{x=r_2}(t) = P_{r_2}(t), \quad E|_{x=r_2}(t) = E_{r_2}(t),\\ &S|_{x=r_2}(t) = S_{r_2}(t), \quad E^*S|_{x=r_2}(t) = E^*S_{r_2}(t),\\ &E^*|_{x=r_2}(t) = E^*_{r_2}(t). \end{aligned} \tag{20}$$

P_{r_2}, E_{r_2}, $E^*_{r_2}$ and $E^*S_{r_2}$ are calculated from solution of equation system (3)–(6).

3 Computer simulation setup and results

The series of computational simulations were performed to investigate how electrode readings would differ if bioelectroanalytical system worked under the first or second model.

The first simulation experiment was designed according to the first model of bioelectroanalytical system. Calculations were divided into two steps: in the first step, calculations were performed according to equations (3)–(6), and in second step, the diffusion of P was calculated for area 2 according to Eq. (8). The following values were used in calculations (all parameters, except kinetic constants, are from [1]): $d_P = 5.49 \cdot 10^{-5}$ cm^{2}s^{-1}, $r_1 = 4 \cdot 10^{-3}$ cm, $I = 7.5 \cdot 10^5$ cm^2, $V = 10$ cm^3, $E_0 = 2.35 \cdot 10^{-8}$ mol cm^{-3}, $k_{cat} = 75$ s^{-1}, $k_1 = 1.12 \cdot 10^9$ cm^{2}mol^{-1}s^{-1}, $k_{-1} = 10$ s^{-1}, $k_p = 100$ cm s^{-1}, $k_d = 0.025$ s^{-1}, $S_0 = 6.7 \cdot 10^{-12}$ mol cm^{-2}. The system of differential equations was discretized using the implicit finite difference scheme and non-linear equation system, corresponding to this scheme, was solved using simple iterations method [6]. Integration steps in space and time were as follows: $h_x = 5 \cdot 10^{-6}$ cm, $h_t = 1$ s; integration in time interval was $T = [0..3000]$. The results of computational experiment are presented in Fig. 2.

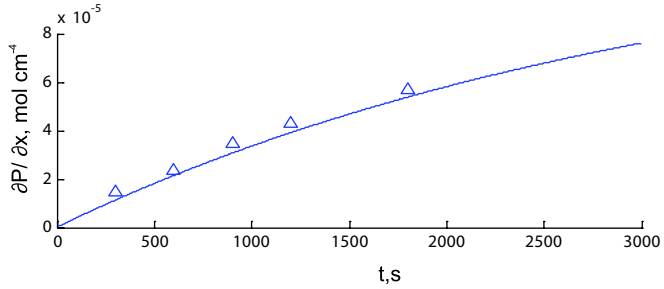

Fig. 2. △ – recalculated experimental data points from [1] ($\frac{\partial P}{\partial x}|_{x=0}$ (in mol cm^{-4}) dependency on time); solid line – computed $\frac{\partial P}{\partial x}|_{x=0}$ (in mol cm^{-4}) dependency on time (in seconds) according to the first model of bioelectroanalytical system.

Fig. 2 also contains the recalculated data points from ref. [1]. For the rotating disk electrode employed in [1], $\frac{\partial P}{\partial x}|_{x=0} = I/(nFAd_P)$ [4], where I is the experimental current values, $n = 2$ is the number of electrons transferred during the oxidation of P, F is the Faraday constant, and $A = 0.07$ cm^2 is the electrode surface area. As can be seen from Fig. 2, the mathematical model (model 1) and a set of kinetic constants used in the computational experiment enabled us to attain good agreement between the experimental and modeling results. It is believed that systematically slightly higher values of experimental data points in Fig. 2 result from imperfect subtraction of background current in work [1]. Unfortunately, because of complexity of interfacial lipase action (see Equations (1) and (2)), individual kinetic constants for this enzyme are not reported in the literature. This fact presents difficulties to check the validity of the kinetic constants used

in our calculations.

The second experiment was designed according to the second model of amperometric system. Constant values were the same as for the first experiment, additionally the second stagnant diffusion layer was defined: $r_2 = 8 \cdot 10^{-3}$ cm; $d_{E^*S} = 10^{-7}$ cm^{2}s^{-1}, $d_{E^*} = 10^{-7}$ cm^{2}s^{-1}, $d_E = 10^{-6}$ cm^{2}s^{-1}, $d_S = 10^{-6}$ cm^{2}s^{-1}, when $r_1 \leq x \leq r_2$ and, for the diffusion coefficients, zero in other cases. Differential equation system was transformed into implicit finite differences scheme and non-linear equation system corresponding to this scheme was solved using simple iterations method [6]. The results are presented in Fig. 3 (continuous line). The same experiment was repeated two more times with following boundary location pairs a) $r_1 = 8 \cdot 10^{-4}, r_2 = 4.8 \cdot 10^{-3}$; b) $r_1 = 4 \cdot 10^{-4}, r_2 = 4.4 \cdot 10^{-3}$ (all values in cm).

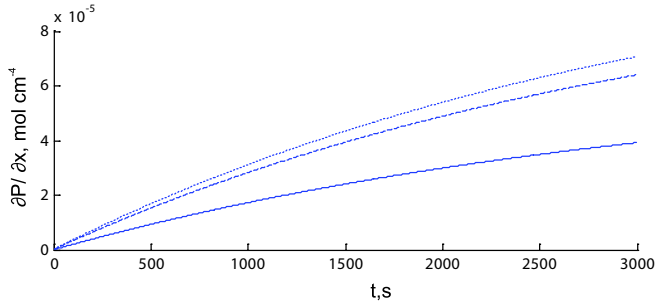

Fig. 3. Solid line – $\frac{\partial P}{\partial x}|_{x=0}$ (in mol cm^{-4}) dependency on time (in seconds) according to the second model of analytical system and assuming $r_1 = 4 \cdot 10^{-3}$ cm and $r_2 = 8 \cdot 10^{-3}$ cm; dashed line – $r_1 = 8 \cdot 10^{-4}$ cm and $r_2 = 4.8 \cdot 10^{-3}$ cm; and dotted line – $r_1 = 4 \cdot 10^{-4}$ cm and $r_2 = 4.4 \cdot 10^{-3}$ cm.

4 Conclusions

The results of foregoing computational experiments enable us to make the following conclusions:

1. Assuming a simple model of single stagnant diffusion layer at the electrode surface as well as three-step interfacial activation and action of enzyme, we were able to compute the performance of electroanalytical system for the determination of *Thermomyces lanuginosus* lipase activity described by Ignatjev et al. [1].

2. A set of individual kinetic constants for *Thermomyces lanuginosus* lipase with respect to the synthetic substrate, O-palmitoyl-2,3-dicyanohydroquinone, is suggested in the computational experiments.

3. As expected, modeling of the analytical system with additional stagnant diffusion layer (the diffusion layer in model 1 is replaced by the dialysis membrane of the

same thickness, and the second diffusion layer of constant thickness in model 2 is formed, for instance, by electrode rotation) demonstrates a decreased initial rate of system response (i.e., rate of current increase upon enzyme injection in the system). Computational experiments also show that significant decrease of dialysis membrane thickness and increase of electrode rotation rate (leads to the decreased thickness of the second diffusion layer) should improve the performance of the analytical system. Electrochemical experiments along these lines are in progress.

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