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Macrokinetic Model of Catalase Electrode with Biphasic Enzyme Inhibition*

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Abstract. Macrokinetics of catalase based enzyme electrode was investigated in presence of enzyme inhibitor – hydroxylamine. The modeling of the electrode was performed using biphasic scheme of enzyme inhibition and external diffusion limitation. The maximal enzyme electrode sensitivity was indicated at transition from diffusion to kinetically controlled mode. The fitting of experimental data demonstrated that the enzyme electrode had 70% of maximal sensitivity.

Keywords: enzyme electrode, catalase, Aspergillus niger, hydroxylamine, inhibition.

1 Introduction

Native enzymes are widely used for inhibitors determination [1]. The enzyme electrodes (biosensors) utilizing immobilized enzymes are also applied for the determination of irreversible enzyme inhibitors. Among such electrodes the most known might be cholinesterase-based biosensors for high toxic phosphorous organics determination [2].

The investigations of the action of reversible inhibitors in enzyme electrodes are rather seldom at least for two reasons. The first one is related to difficulties of

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enzyme electrodes modeling and the second one – to limiting amount of practically interesting reversible inhibitors. Even inhibition of the enzymes with heavy metals is often irreversible [3].

The aim of presented study was to build enzyme electrode based on *Aspergillus niger* catalase (ANC) and to investigate its action in presence of hydroxylamine (HA). It was shown that in solution the HAHA exhibits biphasic inhibition of ANC with intermediate, probably nitric oxide-ferrocatalase, formation [4].

2 Mathematical model

2.1 Outline of the model

The enzyme electrode to be considered below consists of thin layer of enzyme solution fixed with dialysis membrane on electrode surface (Fig. 1). The substrate and inhibitor diffuses through inert (catalytically non active) dialysis membrane. The concentration of substrate in bulk solution is S_b and on surface is S_s. The concentration of substrate on surface is always less in comparison to S_b due to enzyme reaction. The concentration of inhibitor in bulk solution is I_b and on surface of the electrode – I_s.

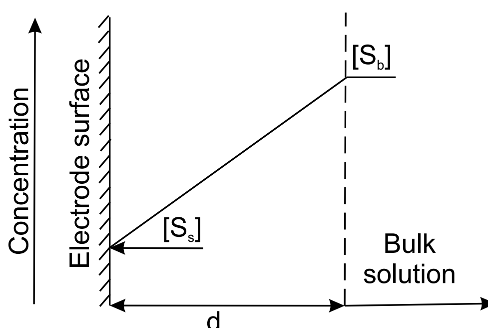

Fig. 1. The scheme of enzyme electrode at steady state conditions. S_b corresponds to bulk and S_s – to surface substrate concentration. The thickness of inert membrane is d.

2.2 Modeling of the enzyme electrode action

The scheme of catalase inhibition with hydroxylamine in solution was analyzed in [4], and can be described by (1)–(4).

$$\mathrm{Ferricatalase} + \mathrm{H_2O_2} \rightarrow \mathrm{Compound\,I} + \mathrm{H_2O} \qquad k_1 \qquad (1)$$

$$\mathrm{Compound\,I} + \mathrm{H_2O_2} \rightarrow \mathrm{Ferricatalase} + \mathrm{O_2} + \mathrm{H_2O} \qquad k_2 \qquad (2)$$

$$\mathrm{Compound\,I} + \mathrm{HA} \rightarrow \mathrm{Intermediate} \qquad k_3 \qquad (3)$$

$$\mathrm{Intermediate} \rightarrow \mathrm{Ferricatalase} \qquad k_4 \qquad (4)$$

where k_1, k_2, k_3 and k_4 corresponds to the kinetic constants of the reaction (1), (2), (3) and (4), respectively.

According to this scheme, the HA acts like as reversible inhibitor. At steady state the reaction rate of the second phase of the inhibition may be expressed by the following equation:

$$V_b = \frac{k_1 \cdot E_s \cdot k_4 \cdot S_b \cdot (2 \cdot k_2 \cdot S_b + k_3 \cdot I_b)}{k_2 \cdot S_b \cdot k_4 + k_4 \cdot k_3 \cdot I_b + k_1 \cdot S_b \cdot k_4 + k_3 \cdot k_1 \cdot S_b \cdot I_b}. \qquad (5)$$

During the action of the enzyme electrode the enzyme reaction proceeds together with mass transport of substrate and inhibitor. To describe the model the concentration of substrate on the surface of electrode should be determined. According to Fick's law the steady state substrate flux (J) through inert membrane may be expressed as:

$$J = D \cdot \frac{S_b - S_s}{d} \qquad (6)$$

where D – diffusion coefficient of substrate; d – thickness of membrane.

The equalization of enzyme rate on the surface (5) and substrate flux (6) at steady state conditions gives equation:

$$\frac{D \cdot (S_b - S_s)}{d} = \frac{k_1 \cdot E_s \cdot k_4 \cdot S_s \cdot (2 \cdot k_2 \cdot S_s + k_3 \cdot I_b)}{k_2 \cdot S_s \cdot k_4 + k_4 \cdot k_3 \cdot I_b + k_1 \cdot S_s \cdot k_4 + k_3 \cdot k_1 \cdot S_s \cdot I_b}. \qquad (7)$$

Solution of this equation for S_s, gives expression of surface substrate concentration as a function of S_b, I_b, E_s and mass transport parameters. There this bulky expression is not shown.

The surface concentration of inhibitor (I_s) at steady state conditions may be expressed in analogous to (7). However, our calculations show that difference between bulk and surface inhibitor concentrations is insignificant in comparison to analogous change of substrate concentration due to low k_4 rate. Thus in further modeling $I_s = I_b$ was assumed.

The diffusion module ρ was expressed as:

$$\rho = \frac{V_b}{S_b}\frac{d}{D}. \tag{8}$$

The value of this dimensionless parameter is always positive. Moreover, when value of ρ is less than 1, the total process rate is controlled by enzymatic reaction. At $\rho > 1$ the process is controlled by diffusion.

The modeling of th enzyme electrode action was performed calculating "sensitivity":

$$Sensitivity = \frac{S_{s(I=I_b)} - S_{s(I=0)}}{I_b} \tag{9}$$

where $S_{s(I=I_b)}$ and $S_{s(I=0)}$ is the surface substrate concentration at fixed S_b and it can be calculated from (8) at $I = I_b$ and $I = 0$, respectively. (It is worth to notice that normally the enzyme electrodes sensitivity is calculated differencing the electrode current against inhibitor concentration, i.e. $\partial i/\partial c$).

2.3 Representation of the model

Kinetic constants used for calculations were taken from [4]. Their values are: $k_1 = 1.4\,\mu\text{M}^{-1}\text{s}^{-1}$, $k_2 = 5.7\,\mu\text{M}^{-1}\text{s}^{-1}$, $k_3 = 0.86\,\mu\text{M}^{-1}\text{s}^{-1}$, $k_4 = 2.6 \cdot 10^{-2}\,\text{s}^{-1}$. Diffusion coefficient of H_2O_2 was assumed $2.7 \cdot 10^{-5}\,\text{cm}^2/\text{s}$ [5]. The thickness of membrane was accepted $d = 0.01$ cm. The concentration of substrate varied in the range from 0 to $1000\,\mu$M, inhibitor concentration was $10\,\mu$M. The dependence of electrode sensitivity on enzyme and substrate concentrations is depicted in Fig. 2.

The calculations show that the sensitivity of enzyme electrode increases linearly with increasing bulk substrate concentration (Fig. 2). However, the dependence of sensitivity on enzyme concentration was not linear in the range 0–$0.1\,\mu$mol/cm^2. As it is possible to notice from Fig. 2 there are two regions of

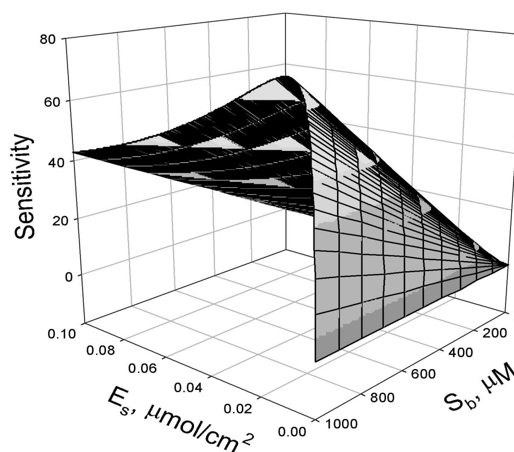

Fig. 2. The dependence of enzyme electrode sensitivity on bulk substrate (S_b) and enzyme (E_s) concentration. Inhibitor concentration $I_b = 10\,\mu$M.

enzyme concentrations: the sensitivity is increasing at E_s less than 9.8 nmol/cm^2 and decreasing at higher E_s concentrations. At $E_s = 9.8$ nmol/cm^2 the enzyme electrode shows maximal sensitivity. This enzyme concentration was named as $E_{s(max)}$. The $E_{s(max)}$ does not depend on hydrogen peroxide concentration. The values of diffusion module calculated at $E_{s(max)}$ and inhibitor concentration $0\,\mu$M and $10\,\mu$M were 8.1 and 0.13, respectively. This means that the maximal enzyme electrode sensitivity is achieved if the electrode acts at diffusion limiting conditions but the regime changes to kinetic limitations in presence of inhibitor.

3 Fitting experimental data by the model

The description of the enzyme electrode preparation and response measurements is described in [6]. The response was analyzed as a function of bulk substrate (H_2O_2) and inhibitor concentration. The signs in Fig. 3 show experimental data whereas curves represent calculated values at fixed bulk H_2O_2 concentration. It is possible to notice good agreement of experimental and calculated value if E_s is equal to 1.6 ± 0.4 nmol/cm^2. The comparison of calculated sensitivity at $E_{s(max)}$ and at fitted E_s values showed that the enzyme electrode response corresponded to 70% of maximal sensitivity.

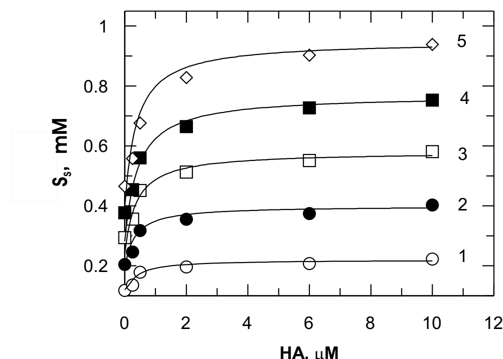

Fig. 3. The fitting of experimental data by the model. $E_s = 1.6\,\text{nmol/cm}^2$, other values as in Fig. 2. Bulk concentration of substrate: 0.2 (1), 0.4 (2), 0.6 (3), 0.8 (4) and 1.0 (5) μM.

4 Conclusions

Macrokinetics modeling shows that the maximal inhibitory effect of enzyme electrode is achieved at diffusion limiting regime. During the inhibitor action the electrode response is determined by enzyme reaction. The fitting of experimental data demonstrated that the enzyme electrode had 70% of maximal sensitivity.

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