



## Inhibition of mediated electron transfer

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### ABSTRACT

A model of electro-catalytic reaction inhibited by the product is developed. It is assumed that the stability constant of inhibiting complex depends on the electrode potential directly or indirectly. The resulting steady-state responses are characterised by maxima.

### 1. Introduction

Redox enzymes that are attached to electrode may be used for mediated electron transfer to the dissolved substrate [1–3]. This can be used for biosensors and immunosensors [4–11]. Some examples include the immobilization of glucose oxidase [4,8,11], lactate oxidase [10] and horseradish peroxidase [6]. The sensors usually work under theoretically well described steady-state conditions [12–17]. However, there are experimental systems in which these conditions cannot be established [18–20]. The present paper demonstrates that the variation of limiting current can be explained by the potential dependent inhibition. Many enzymes may be inhibited by the products of the reaction they catalyse. In the case of redox enzymes the kinetics of product inhibition can be analysed by cyclic voltammetry [21]. On the rotating disk electrode the limiting current decreases if the electrode surface is inactivated by the product adsorption [22]. Here we investigate the consequences of two particular hypotheses that may be relevant for the protein film voltammetry.

### 2. The model

A reversible electrode reaction of adsorbed enzyme ( $E_{\text{red,ads}}$ ,  $E_{\text{ox,ads}}$ ) that is coupled to totally irreversible oxidation of dissolved substrate (S) is considered. It is assumed that the product of catalytic reaction (P) forms the adsorbed complex with the oxidized form of the enzyme [21].



During the experiment, the surface concentration of enzyme does

not change. Furthermore, the substrate cannot be electro-oxidized on the bare electrode surface due to very slow electron transfer. Under steady-state conditions on the surface of the rotating disk electrode, the current is defined by the following equations [23]:

$$\Gamma_{E_{\text{ox}}} = \Gamma_{E_{\text{red}}} \exp(\varphi) \quad (4)$$

$$\varphi = F(E - E^0)/RT \quad (5)$$

$$\Gamma_{E_{\text{red}}} + \Gamma_{E_{\text{ox}}} + \Gamma_{E_{\text{ox}}P} = \Gamma_{E_{\text{tot}}} \quad (6)$$

$$K_{EP} = \Gamma_{E_{\text{ox}}P} \Gamma_{E_{\text{ox}}}^{-1} c_{P,x=0}^{-1} \quad (7)$$

$$D(c_S^* - c_{S,x=0})/\delta = k_{\text{cat}} \Gamma_{E_{\text{ox}}} c_{S,x=0} \quad (8)$$

$$Dc_{P,x=0}/\delta = k_{\text{cat}} \Gamma_{E_{\text{ox}}} c_{S,x=0} \quad (9)$$

$$I/FA = k_{\text{cat}} \Gamma_{E_{\text{ox}}} c_{S,x=0} \quad (10)$$

The meanings of all symbols are reported in the Table 1. The solution for the dimensionless current  $\Phi = I\delta(FADc_S^*)^{-1}$  depends on the positive root of the quadratic equation for the surface concentration of the oxidized form of the enzyme.

$$\Phi = k_{\text{cat}} \delta \Gamma_{E_{\text{ox}}} / (D + k_{\text{cat}} \delta \Gamma_{E_{\text{ox}}}) \quad (11)$$

$$\Gamma_{E_{\text{ox}}}^2 [(1 + \exp(-\varphi))k_{\text{cat}} \delta + K_{EP} k_{\text{cat}} \delta c_S^*] + \Gamma_{E_{\text{ox}}} [(1 + \exp(-\varphi))D - k_{\text{cat}} \delta \Gamma_{E_{\text{tot}}}] - D\Gamma_{E_{\text{tot}}} = 0 \quad (12)$$

If there is no inhibition by the product ( $K_{EP} = 0$ ), Eqs. (11) and (12) are reduced to the known forms [3]:

$$\Gamma_{E_{\text{ox}}} = \Gamma_{E_{\text{tot}}} / [1 + \exp(-\varphi)] \quad (13)$$

$$\Phi_{E \gg E^0} = k_{\text{cat}} \delta \Gamma_{E_{\text{tot}}} / (D + k_{\text{cat}} \delta \Gamma_{E_{\text{tot}}}) \quad (14)$$

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**Table 1**

Meanings of symbols.

$A$	Area of electrode surface
$c_S, c_P$	Concentrations of the substrate S and the product P
$c_S^*$	Concentration of the substrate in the bulk of solution
$D$	Diffusion coefficient
$\delta$	Diffusion layer thickness
$E$	Electrode potential
$E^0$	Standard potential of the electrode reaction
$E_2^0$	Standard potential of the relationship between the constant $K_{EP}$ and the electrode potential
$F$	Faraday constant
$\Gamma_{E_{red}}$	Surface concentration of reduced form of adsorbed enzyme
$\Gamma_{E_{ox}}$	Surface concentration of oxidized form of adsorbed enzyme
$\Gamma_{E_{ox}P}$	Surface concentration of the complex between the product P and the oxidized form of adsorbed enzyme
$\Gamma_{E_{tot}}$	Sum of surface concentrations of all forms of adsorbed enzyme
$I$	Current
$K_{EP}$	Stability constant of the immobilized product of catalytic reaction
$k_{cat}$	Rate constant of catalytic reaction
$R$	Gas constant
$T$	Temperature

### 3. Results and discussion

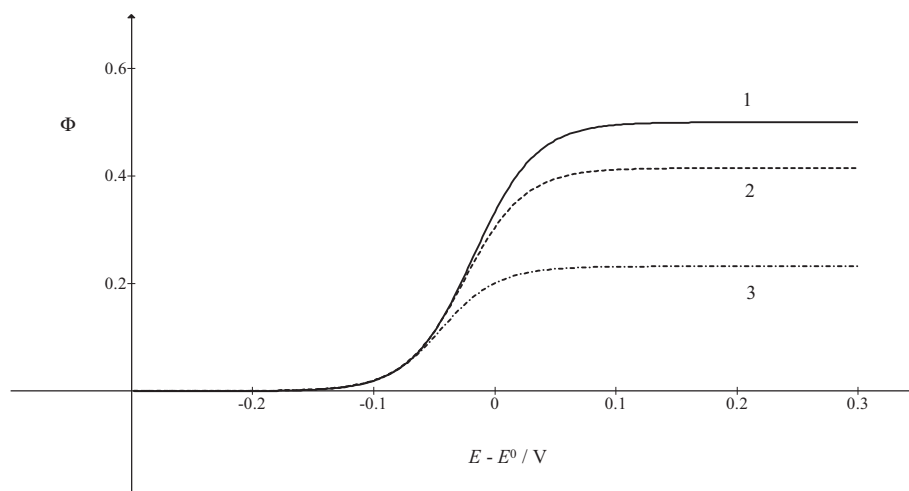
Dimensionless current of electro-catalytic reaction that is inhibited by its product depends on four independent parameters: the diffusion coefficient, the initial surface concentration of adsorbed enzyme, the product of the rate constant of catalytic reaction with the diffusion layer thickness and the product of the stability constant of the immobilized product and the bulk concentration of substrate. Three examples are shown in Fig. 1. In the absence of inhibition ( $K_{EP} = 0$ ) the current is defined by Eq. (14). For the given parameters the limiting current is equal to 0.5 and the half-wave potential is  $-0.018$  V vs.  $E^0$ . Under the influence of inhibitor, the characteristic current and potential are both diminished. Within the boundaries  $0.5 < K_{EP}c_S^* < 50$  the following linear relationships exist:  $\Phi_{lim} = -0.1825 \log K_{EP}c_S^* + 0.4142$  and  $E_{1/2} - E^0 = -0.019 \log K_{EP}c_S^* - 0.025$  V. It is important to note in Fig. 1 that the inhibition does not change the form of voltammogram if the product  $K_{EP}c_S^*$  is constant during the experiment. This can be explained by the fact that the surface concentrations of adsorbed complex  $E_{ox}P$  and the oxidized form of adsorbed enzyme depend on the electrode potential in a similar manner, as can be seen in Fig. 2.

The anomaly that we are looking for is a maximum in the current – potential relationship [18–20]. Considering the observed dependence of the limiting current on the product  $K_{EP}c_S^*$ , one can imagine that the maximum can appear if the constant  $K_{EP}$  depends on the electrode

potential. The first possibility is shown in Fig. 3. In this calculation it is assumed that the constant increases exponentially with the potential:  $K_{EP} = \exp(F(E - E_2^0)/RT)$ . For the difference  $E_2^0 - E^0 = 0.2$  V the maximum current appears at  $E_{max} - E^0 = 0.110$  V. The reason for the appearance of maximum can be seen in Fig. 4. Relative surface concentration of oxidized enzyme increases until  $E - E^0 = 0.110$  V and then decreases, while the concentration of adsorbed complex  $E_{ox}P$  increases at potentials higher than 0.1 V vs.  $E^0$ . This is because the product  $K_{EP}c_S^*$  is smaller than 0.02 if  $E - E^0$  is lower than 0.1 V. Furthermore, the product is equal to one if  $E - E^0 = 0.2$  V and increases to 49 for  $E - E^0 = 0.3$  V. Consequently, there is no inhibition of catalytic reaction below 0.1 V vs.  $E^0$ , but at the potential  $E - E^0 = 0.2$  V the limiting current decreases to 0.4142 in agreement with curve 2 in Fig. 1.

Fig. 5 shows the results of calculation performed under assumption that the stability constant of the complex  $E_{ox}P$  increases with the increasing relative surface concentration of immobilized product:  $K_{EP} = \exp(p\Gamma_{E_{ox}P}/\Gamma_{E_{tot}})$ . The parameter  $p$  could depend on interactions within the monolayer. If  $p = 1$  the response is a wave characterised by  $\Phi_{lim} = 0.3907$  and  $E_{1/2} - E^0 = -0.028$  V. The relationship between the ratio  $\Gamma_{E_{ox}P}/\Gamma_{E_{tot}}$  and the potential is shown in Fig. 6. For  $p = 1$ , this ratio is equal to 0.1465, for  $E = E^0$ , and 0.3587 for  $E - E^0 = 0.3$  V. So, the constant  $K_{EP}$  increases from 1.00 to 1.16, for  $E = E^0$ , and to 1.43 for  $E - E^0 = 0.3$  V. Comparing to curve 2 in Fig. 1, the limiting current and half-wave potential are little lower because the average product  $K_{EP}c_S^*$  is little higher. If  $p = 3$ , the response exhibits maximum:  $\Phi_{max} = 0.3010$ ,  $E_{max} - E^0 = 0.016$  V and  $\Phi_{lim} = 0.2622$ . The ratio  $\Gamma_{E_{ox}P}/\Gamma_{E_{tot}}$  rises from zero to 0.1988, at  $E = E^0$ , and to 0.6333, at  $E - E^0 = 0.1$  V, and finally to 0.6445 for  $E - E^0 = 0.3$  V. Hence, the product  $K_{EP}c_S^*$  is equal to 1.49, at  $E = E^0$ , and rises to 3.55, at  $E - E^0 = 0.1$  V, and to 3.63 at  $E - E^0 = 0.3$  V. The current decreases after the maximum because the product  $K_{EP}c_S^*$  is increasing, but the current stagnates if  $E - E^0 > 0.1$  V because  $\Gamma_{E_{ox}P}/\Gamma_{E_{tot}}$  and  $K_{EP}c_S^*$  also stagnate in this potential range. If  $p = 5$ , the ratio  $\Gamma_{E_{ox}P}/\Gamma_{E_{tot}}$  steeply increases from 0.0144 to 0.8705 within the range  $-0.050 \leq E - E^0 \leq 0.050$  V. Consequently, the product  $K_{EP}c_S^*$  increases from 1.04 to 13.62 in the same potential range. The current exhibits sharp maximum  $\Phi_{max} = 0.2515$  at  $E_{max} - E^0 = -0.006$  V and decreases to the limiting value  $\Phi_{lim} = 0.0963$ . At potentials higher than 0.050 V vs.  $E^0$  the ratio  $\Gamma_{E_{ox}P}/\Gamma_{E_{tot}}$  and the product  $K_{EP}c_S^*$  increase moderately to 0.8935 and 14.59, respectively.

Voltammograms with maxima were observed in electro-catalytic voltammetry of succinate dehydrogenase [18] and in electro-catalytic oxidation of hydrogen [19,20]. They were explained by the



**Fig. 1.** Theoretical steady-state voltammograms of electro-catalytic reactions (1)–(3) on the rotating disk electrode.  $D = 10^{-5}$  cm<sup>2</sup> s<sup>-1</sup>,  $\Gamma_{E_{tot}} = 10^{-5}$  mol cm<sup>-2</sup>,  $k_{cat}\delta = 1$  cm<sup>4</sup> mol<sup>-1</sup> s<sup>-1</sup> and  $K_{EP}c_S^* = 0$  (1), 1 (2) and 10 (3).

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