CHAPTER II

DERIVATION OF NONSTEADY-STATE ANALYTICAL SOLUTION FOR SURFACE ENZYME KINETICS

2.1. INTRODUCTION

Surface enzyme chemistry and bioaffinity interactions on biopolymer microarrays is an invaluable surface bioengineering tool [1]. In surface-based biotechnologies and bioaffinity sensor application, the parallel enzymatic processing of biopolymer microarrays is rapidly becoming an integral component. Enzymes are attractive tools for surface bioengineering for a number of reasons: surface enzyme reactions are highly specific and result in selective surface site modifications, work under biocompatible conditions often with high efficiency and in some cases are reversible. Lee et.al. [1] analysed real-time surface plasmon resonance (SPR) imaging measurements of surface enzymatic reactions on DNA microarrays using a kinetic model that couples the contributions of both enzyme adsorption and surface enzyme reaction kinetics.

Various analytical approaches have been used to study the surface approach enzymatic reaction rates quantitatively. While most research approach have been focused on the fluorescent-based technique [2-7] and SPR-based technique [8-15]. The SPR technique can be used to study the Langmuir adsorption kinetics and enzyme reaction kinetics on surfaces. Takashi Kakiuchi and co-workers [16] introduced a kinetic model to characterize the hydrolysis of phosphatidylcholine monolayers by phospholipase D. In these models, the diffusion of enzyme from the bulk to the interface in a kinetic model of the hydrolysis at the interface is incorporated. Recently Lee and co-workers [1] proposed
a new approach to the quantitative analysis of enzyme-catalyzed surface reaction that couples both adsorption kinetics and enzyme kinetics to quantitatively describe the reaction of an enzyme in solution with a surface-immobilized substrate. In this chapter, a simple and closed analytical expression of surface coverage of the intermediate $ES (\theta_{ES})$, the product $S^*(\theta_{S^*})$, the substrate $S (\theta_S)$ and the fraction of unreacted surface sites ($\lambda_{ES}$) in absence of catalytic activity ($k_{cat} = 0$) is derived. Using these analytical results, the surface enzyme kinetics for biopolymer microarrays is analyzed. In the presence of catalytic activity, the numerical solution of the problem is also reported using Matlab program.

2.2. MATHEMATICAL FORMULATION OF THE PROBLEM

Enzymes are catalysts and increase the speed of a chemical reaction without themselves undergoing any permanent chemical change. They are neither used up in the reaction nor do they appear as reaction products. The enzyme ($E$) first adsorbs from solution onto the surface-bound substrate ($S$) to create the surface complex ($ES$) then it reacts to form the surface-bound product ($S^*$). This enzymatic reaction [1] can be represented as follows:

$$S + E \xleftrightarrow{k_a}{k_d} ES$$

$$ES \xrightarrow{k_{cat}} S^* + E$$

(2.1)

(2.2)

With the assumption of Langmuir kinetics model, the reaction rates for the production of $ES$ and $S^*$ can be written as follows:

$$\frac{d \Gamma_{ES}}{dt} = k_a \Gamma_S [E] - k_d \Gamma_{ES} - k_{cat} \Gamma_{ES}$$

(2.3)
where \( \Gamma \) denotes a surface coverage. The reaction rates can also be written in terms of the relative surface coverages,

\[
\theta_s = \frac{\Gamma_s}{\Gamma_{tot}}
\]  

(2.5)

where \( x = S, ES \) or \( S^* \) and \( \Gamma_{tot} \) is the total number of surface sites. Also

\[
\theta_s + \theta_{ES} + \theta_{S^*} = 1
\]

(2.6)

Using Eq. (2.5), Eqs. (2.3) and (2.4) become

\[
\frac{d\theta_{ES}}{dt} = k_a \theta_s [E] - k_d \theta_{ES} - k_{cat} \theta_{ES}
\]

(2.7)

\[
\frac{d\theta_{S^*}}{dt} = k_{cat} \theta_{ES}
\]

(2.8)

The initial conditions of the problem is

\[
\theta_s = 1, \quad \theta_{ES} = 0, \quad \theta_{S^*} = 0 \quad \text{at} \quad t = 0.
\]

(2.9)

The solution of the above linear differential equations (Eqs. (2.7) and (2.8)) depends upon the relative values of the rate constants \( k_a, k_d \) and \( k_{cat} \). The steady-state solution of the Eq. (2.7) is given by [1]

\[
\theta_{ES} = \frac{k_a \theta_s [E]}{k_d + k_{cat}} \frac{\theta_s [E]}{K'_M}
\]

(2.10)

where

\[
K'_M = \frac{(k_d + k_{cat})}{k_a}
\]

(2.11)
Initially, the product $S^*$ is very small so that it can be neglected in Eq. (2.6). Then Eq. (2.10) becomes
\[ \theta_{ES} = \frac{[E]}{K_M' + [E]} \]
which is the steady-state surface coverage of the intermediate ES. If $k_{cat}$ is very small compared with $k_d$, then this steady-state value of $\theta_{ES}$ becomes
\[ \theta_{ES} = \frac{K_{ads}[E]}{1 + K_{ads}[E]} \tag{2.12} \]
where $K_{ads} = k_a/k_d$ is the Langmuir adsorption coefficient.

2.3. GENERAL SOLUTION

The general solution of Eqs. (2.7) and (2.8) are
\[ \theta_{ES} = k_a[E]c_1(e^{m_2 t} - e^{m_1 t}) \tag{2.13} \]
\[ \theta_s = 1 + c_1[(m_1 + K)e^{m_1 t} - (m_2 + K)e^{m_2 t}] \tag{2.14} \]
where
\[ K = k_a[E] + k_d + k_{cat} \tag{2.15} \]
\[ m_1 = \frac{-K + \sqrt{K^2 - 4k_a[E]k_{cat}}}{2} \tag{2.16} \]
\[ m_2 = \frac{-K - \sqrt{K^2 - 4k_a[E]k_{cat}}}{2} \tag{2.17} \]
Using the initial condition (2.9), we can determine the constant $c_1$,
\[ c_1 = 1/(m_2 - m_1) \tag{2.18} \]
Also from Eq. (2.6), we obtain
\[ \theta_s = 1 - \theta_{ES} - \theta_s \tag{2.19} \]
Eqs. (2.13), (2.14) and (2.19) represent simple analytical expression of \( \theta_{ES}, \theta_{S^*}, \) and \( \theta_S \) for all values of rate constants \( k_a, k_d, \) and \( k_{cat} \) respectively. Recently, Lee and coworkers [1] have used Euler integration method to find the values of \( \theta_{ES}, \theta_{S^*}, \) and \( \theta_S \) numerically. Let \( \lambda_{ES} \) denote the fraction of unreacted surface sites that are occupied by the enzyme and it is given as follows [1]:

\[
\lambda_{ES} = \frac{\theta_{ES}}{1 - \theta_{S*}}
\]  

(2.20)

Using Eq. (2.13) and (2.14), the Eq. (2.20) becomes

\[
\lambda_{ES} = \frac{k_a[E](e^{m_2t} - e^{m_1t})}{(m_2 + K)e^{m_2t} - (m_1 + K)e^{m_1t}}
\]  

(2.21)

Lee and Coworkers [1] obtained the steady-state value of \( \lambda_{ES} \) as

\[
\lambda_{ES} = -\frac{k_a[E]}{k_{cat}\lambda_{ES}} + \frac{K}{k_{cat}}
\]  

(2.22)

Eq. (2.22) is a quadratic equation which can be solved using quadratic formula. But our Eq. (2.21) is the simple and closed analytical expression of the fractional surface coverage of an unreacted sites \( ES(\lambda_{ES}) \) in terms of rate constants \( k_a, k_d, \) and \( k_{cat}. \) The surface coverage of an unreacted site \( \lambda_{ES} \) versus time \( t \) for various values of parameters is also plotted in Fig. 2.1.1-2.1.4.
Fig. 2.1. Plot of surface coverage of substrate S ($\theta_S$), product $S^*$ ($\theta_{S^*}$), intermediate ES ($\theta_{ES}$) and fractional surface coverage ($\lambda_{ES}$) versus time for various values of rate constants. Here $+$ denotes the simulation results and solid curve represents the Eqs. (2.13), (2.14), (2.19) and (2.20).

(2.1.1) $k_d[E] = k_{cat} = 0.25 \, s^{-1}$, $k_d = 0.025 \, s^{-1}$
(2.1.2) $k_a[E] = k_{cat} = 0.25 \, s^{-1}$, $k_d = 1 \, s^{-1}$
(2.1.3) $k_a[E] = k_{cat} = 5 \, s^{-1}$, $k_d = 0.025 \, s^{-1}$
(2.1.4) $k_a[E] = k_{cat} = 5 \, s^{-1}$, $k_d = 1 \, s^{-1}$. 

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2.4. DIFFUSION CONTRIBUTION TO THE SURFACE ENZYMATIC REACTION

Case (i): THE ABSENCE OF CATALYTIC ACTIVITY \( k_{cat} = 0 \).

In the absence of catalytic activity \( k_{cat} = 0 \), diffusion contributions to the rate of enzyme adsorption can be included using the following differential equation [1]:

\[
\frac{d\theta_{ES}}{dt} = \frac{k_a[E](1 - \theta_{ES}) - k_d\theta_{ES}}{1 + \beta(1 - \theta_{ES})}
\] (2.23)

The dimensionless diffusion parameter \( \beta \) \( = \frac{k_a \Gamma_{oo} \delta}{D} = \frac{k_a \Gamma_{oo}}{k_m} \) compares the rate of adsorption to the rate of diffusion. Here \( k_m = D / \delta \), where \( D \) is the diffusion constant and \( \delta \) is a diffusion layer of thickness. Solving Eq. (2.23), we get

\[
\theta_{ES} = \frac{(W - \ln(k_a)) (k_a[E] + k_d(1 + \beta)) + (k_a[E] + k_d)^2 t}{\beta (k_a[E] + k_d)}
\] (2.24)

where

\[
W = \ln \left( \frac{p \text{ Lambert } w \left( \frac{\beta \psi}{p} \right)}{\beta} \right)
\] (2.25)

\[
\psi = \exp \left( \frac{\beta k_a[E] - (k_a[E] + k_d)^2 t + \ln(k_a[E]) p}{p} \right)
\] (2.26)

and

\[
p = k_a[E] + k_d (1 + \beta)
\] (2.27)

Here the Lambert w-function \( z = w(z) \exp(w(z)) \) is also called omega function.
Case (ii): THE PRESENCE OF CATALYTIC ACTIVITY ($k_{\text{cat}} \neq 0$).

When the catalytic activity is included ($k_{\text{cat}} \neq 0$) Eq. (2.23) becomes [1]

$$
\frac{d\theta_{ES}}{dt} = \frac{k_a[E](1-\theta_{ES} - \theta_{S^*}) - (k_d + k_{\text{cat}})\theta_{ES}}{1 + \beta(1-\theta_{ES} - \theta_{S^*})}
$$

(2.28)

This above equation can be used as a direct replacement of Eq. (2.7). This equation cannot be solved analytically by using standard methods. The numerical solution of the problem can be obtained using the simple Matlab program. Lee and Coworkers [1] solved the above equation (Eq. (2.28)) using Euler integration method. An excellent agreement with this two Simulation results is noticed.

2.5. DISCUSSION

Eqs. (2.13), (2.14) and (2.19) represent the new analytical expressions for the surface coverage $\theta_{ES}$, $\theta_{S^*}$ and $\theta_{S}$ for all values of rate constants $k_a$, $k_d$ and $k_{\text{cat}}$. The Eq. (2.21) is a closed analytical expression of fraction of unreacted surface sites $\lambda_{ES}$. Fig. 2.1.1- 2.1.4 shows the profiles of relative surface coverage $\theta_{ES}$, $\theta_{S^*}$ and $\theta_{S}$ for various values parameters. In this figures our results are also compared with the simulation results. A satisfactory agreement is noted. From these figures, it is inferred that the surface coverage $\theta_{ES}$ increases abruptly when $0 \leq t \leq 5$ and decreases slowly and reaches its steady-state value at $t = 25$. $\theta_{ES}$ attains the maximum value at $t = 5$ when $k_d \leq 0.1$. Also the surface coverage $\theta_{S^*}$ is monotonically increasing when $0 \leq t \leq 20$ and for small value of $k_d$ and reaches the steady-state value ($\theta_{S^*} = 1$) when $t \geq 20$. The surface coverage $\theta_{S}$ is monotonically decreasing when $t < 20$ and reaches the steady-
state value ($\theta_S = 0$) when $t = 20$. Fig. 2.2.1-2.2.4 represents the plot of the relative surface coverage of $\theta_{ES}$, $\theta_{S^*}$ and $\theta_S$ when $k_{\text{cat}} = k_d = 0.025, 0.01, 0.001$ and $0.0001$. From these figures, it is known that $\theta_{ES}$ and $\theta_{S^*}$ reaches the steady-state value when $k_{\text{cat}} = k_d$ and $t = 15$.

Eq. (2.24) represents the new simple analytical expression of surface coverage $\theta_{ES}$ for all values of parameters. Fig. 2.3.1-2.3.4 shows the surface coverage $\theta_{ES}$ calculated using Eq. (2.24) for various values of parameters. From these figures, it is confirmed that $\theta_{ES}$ decreases when $t$ increases and reaches the steady-state value at $t = 5$ and $k_d \geq 1$. In the presence of catalytic activity ($k_{\text{cat}} \neq 0$), the relative surface coverage $\theta_{ES}$, $\theta_{S^*}$ and $\theta_S$ are plotted in the Fig. 2.4.1 - 2.4.4. The relative surface coverage of $\theta_{ES}$ reaches its maximum value when $t = 20$ and $\beta \leq 0.01$. When $\beta$ increases, the maximum value of $\theta_{ES}$ decreases. The relative surface coverage $\theta_S$ decreases from the value 1 whereas the relative surface coverage of $\theta_{S^*}$ increases from initial value zero. The maximum value of fractional surface of unreacted sites $\lambda_{ES}$ increases and attains its steady-state value 0.7 for all values of parameters.
Fig. 2.2. Plot of surface coverage of substrate $S$ ($\theta_S$), product $S^*$ ($\theta_{S^*}$), intermediate $ES$ ($\theta_{ES}$) and fractional surface coverage ($\lambda_{ES}$) versus time for various values of rate constants. Here + denotes the simulation results and solid curve represents the Eqs. (2.13), (2.14), (2.19) and (2.20).

(2.2.1) $k_a[E] = 0.25 \text{ s}^{-1}$, $k_{cat} = k_d = 0.025 \text{ s}^{-1}$ (2.2.2) $k_a[E] = 0.25 \text{ s}^{-1}$, $k_{cat} = k_d = 1 \text{ s}^{-1}$

(2.2.3) $k_a[E] = 5 \text{ s}^{-1}$, $k_{cat} = k_d = 0.025 \text{ s}^{-1}$ (2.2.4) $k_a[E] = 5 \text{ s}^{-1}$, $k_{cat} = k_d = 1 \text{ s}^{-1}$.
Fig. 2.3. Plot of surface coverage of intermediate $ES$ ($\theta_{ES}$) versus time using Eq. (2.24) when $\beta = 0.01$, $\beta = 1$, $\beta = 5$, $\beta = 10$, $\beta = 15$ for some fixed values of $k_a[E]$ and $k_d$

- **(2.3.1)** $k_a[E] = 0.1$ and $k_d = 0.01$
- **(2.3.2)** $k_a[E] = 1$ and $k_d = 0.1$
- **(2.3.3)** $k_a[E] = 1$ and $k_d = 2$
- **(2.3.4)** $k_a[E] = 5$ and $k_d = 1$
Fig.2.4.1: Plot of numerical simulation of surface coverage of substrate $S$ ($\theta_S$) and intermediate $ES$ ($\theta_{ES}$) versus time using Eqs. (2.8) and (2.23) when $k_s[E] = k_{cat} = 0.1 \text{ s}^{-1}$, $k_d = 0.01 \text{ s}^{-1}$ and various values of $\beta$.

(2.4.1) $\beta = 0.01$

(2.4.2) $\beta = 1$

(2.4.3) $\beta = 10$

(2.4.4) $\beta = 50$. 

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2.6. CONCLUSION

Simple closed analytical expressions of $\theta_{ES}$, $\theta_{S^*}$ and $\theta_S$ for all values of rate constants $k_a$, $k_d$ and $k_{cat}$ are derived. The analytical solution of the surface coverage for the three surface species $S$, $ES$ and $S^*$ are also derived in the absence of catalytic activity. In the presence of catalytic activity, the numerical solution of the problem is also reported using Matlab software program. All the analytical results are compared with our numerical results (or Euler integration method). A good agreement with the available numerical results is thus notified.

2.7. REFERENCES


