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Modeling Enzymes Inhibition During Microparticles Formation*

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Abstract. A mathematical model of enzymes inhibition during microparticles formation has been developed. The model is based on radial substrate diffusion through diffusion layer that surrounds microparticle. The inhibition of enzymes was explained by enzyme adsorption on surface of the microparticles and substrate diffusion limitation through the diffusion layer. The diffusion module is dependent on dimensions of the microparticle. The microparticles with diameter $2\,\mu\mathrm{m}$ may ensure 50% of an apparent activity decrease if half of peroxidase has been adsorbed. Further adsorption of peroxidase and aggregation of the microparticles increases the apparent inhibition.

Keywords: peroxidase, inhibition, naphthol, diffusion.

1 Introduction

Many products of enzyme-catalyzed reactions are insoluble in water. For example, laccase and peroxidase initiated polymerization of phenol precursors the most abundant process of natural polymeric compound – lignin formation [1]. Peroxidase catalyzed waste water treatment produces insoluble polymeric sediments [2, 3]. Naphthols oxidation catalyzed by laccases and peroxidase generate insoluble polymers [4, 5]. The characteristic feature of these reactions is decrease of enzymes activity [4]–[6].

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The inhibition of the enzymes decreases a yield of product and finally the affectivity of process. Exist two prepositions concerning the mechanism of enzymes inactivation. The first one hypothesizes intermediates (radicals) formation that react with active center of enzyme [7]. Following the second hypothesis microparticles adsorb enzyme [4, 5, 8]. However, the intrinsic mechanism of the inactivation of absorbed enzyme is not understood.

The task of this work is to build a model that can explain enzymes activity decrease during the microparticles formation. The essence of the model is enzyme adsorption on the microparticles and limitation of substrate diffusion.

2 Mathematical model

If we restrict ourselves to cases in which the diffusion is radial, the diffusion equation for a constant diffusion coefficient takes the form [9]:

$$\partial S/\partial t = D(\partial^2 S/\partial r^2 + 2/r\,\partial S/\partial r), \tag{1}$$

where S – substrate concentration, D – diffusion coefficient of the substrate, r – radius of sphere.

On putting $U = S \cdot r(1)$ becomes:

$$\partial U/\partial t = D\,\partial^2 U/\partial r^2. \tag{2}$$

At steady-state conditions, when $\partial U/\partial t = 0$ and $\partial S/\partial t = 0$, the general solution of (1) is $S = A + B/r$, where constants A and B could be found from boundary conditions. For hollow sphere with radius $a \leq r \leq b$ the solution of (1) gives:

$$S_r = \big(aS_s(b-r) + bS_b(r-a)\big)/\big(r(b-a)\big), \tag{3}$$

where S_b – substrate concentration (bulk concentration) at $r = b$, S_s – substrate concentration (surface concentration) at $r = a$.

If spherical microparticle with radius a contains adsorbed enzyme (Fig. 1), it is surrounded by diffusion (Nernst) layer (Fig. 1). The boundary of the layer is at $r = b$ and thickness of diffusion layer (δ) is equal to $b - a$. At steady state condition the quantity of substrate which passes through outer boundary

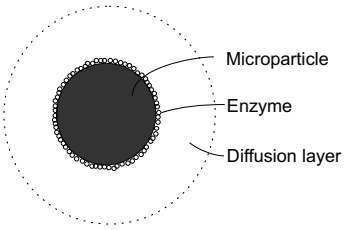

Fig. 1. Scheme of the model. Dimensions are not scaled.

layer is equal to the quantity of the substrate which is converted by adsorbed enzyme. The enzymatic rate on the surface (V) can be expressed by equation

$$V = V_{max} S_s/(K_m + S_s), \tag{4}$$

where V_{max} – maximal enzymatic rate on the surface, K_m – an apparent Michaelis constant.

At steady state conditions the equalization of the substrate flux and the enzymatic rate gives:

$$4\pi b^2 D(dS_r/dr)_{r=b} = 4\pi a^2 V_{max} S_s/(K_m + S_s). \tag{5}$$

The solution of (5) gives the dependence of the substrate concentration on the microparticle:

$$y = \big(x - (1+\rho) + \big(x^2 + 2x(1-\rho) + 1 + \rho(2+\rho)\big)^{1/2}\big)/2, \tag{6}$$

where $y = S_s/K_m,\ x = S_b/K_m,\ \rho = (V_{max}/K_m D)\big(a(b-a)/b\big)$.

The solution of (5) at $S_s \ll K_m$ is simpler:

$$y = x/(1+\rho). \tag{7}$$

The insertion of the substrate concentration (6) or (7) into (4) gives the expression of enzymatic rate on the surface of microparticle. It is possible to notice that the rate on surface changes little in comparison to the rate in the bulk solution if diffusion module ρ is less than 1 (Fig. 2). It significantly decreases at ρ larger than 1. At S_b/K_m less than 0.5 approximate solution almost fits the accurate solution.

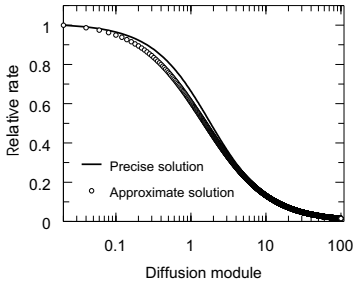

Fig. 2. The dependence of relative enzyme activity on diffusion module at $S_b/K_m = 0.5$. The solid curve – precise solution, dot curve – approximate solution.

Remarkable feature of the concentration (and the rate) expression at $a \ll b$ is it independence on b since:

$$\rho = V_{max} a / K_m D. \tag{8}$$

3 Fitting experimental data by the model

The suggested model was compared with peroxidase-catalyzed 1- and 2-napthol oxidation performed in [5]. Typically the reaction started using $1\,\mathrm{pmol\,cm^{-3}}$ of peroxidase and $25\,\mathrm{nmol\,cm^{-3}}$ of substrate. The estimated value of k_{cat}/K_m was $13.2 \cdot 10^9$ and $54.0 \cdot 10^9\,\mathrm{cm^3\,mol^{-1}\,s^{-1}}$ for 1- and 2-napthol, respectively. For calculations the diffusion coefficient of substrate $3 \cdot 10^{-6}\,\mathrm{cm^2\,s^{-1}}$ and peroxidase diameter $3 \cdot 10^{-7}\,\mathrm{cm}$ was used. V_{max} was expressed as $k_{cat} \cdot E$, where E – adsorbed enzyme concentration.

It is worth to notice that at $r = a$ the condition of $S_s < K_m$ is achieved very easy due to substrate consumption on surface of microparticle. Therefore approximate solution (7) was used. The diffusion module was calculated using (8) since thickness of diffusion (Nernst) layer that covers heterogeneous phase typically spans $(20-100) \cdot 10^{-4}\,\mathrm{cm}$ [10].

The performed experiments showed that activity of the peroxidase started to decrease when about half of initial substrate, i.e. $12.5\,\mathrm{nmol\,cm^{-3}}$ has been converted into polymer [5]. If we accept that at this moment particles with diameter of $2\,\mu\mathrm{m}$ ($a = 1\,\mu\mathrm{m}$) are formed, the number (n) of particles is $4.3 \cdot 10^5$ per $\mathrm{cm^3}$ (density of polymer is accepted $1\,\mathrm{g\,cm^{-3}}$ and molecular weight of

naphthol $144\,\mathrm{g\,mol^{-1}}$). The total surface of the microparticles calculated as $4\pi a^2 n$ is $0.054\,\mathrm{cm}^2$. The calculated concentration of adsorbed peroxidase tightly covering the surface is $23.5\,\mathrm{pmol\,cm^{-2}}$. Therefore the micro particles can adsorb $1.27\,\mathrm{pmol}$ of peroxidase, i.e. more than amount of peroxidase taken for enzymatic reaction.

The apparent activity of adsorbed peroxidase, however, decreases due to diffusion limitation (Fig. 2). If half of peroxidase ($0.5\,\mathrm{pmol}$) is adsorbed on the microparticles the diffusion module is 4.1 and 16.7 for 1- and 2-naphthol, respectively. Since ρ is more than 1, the apparent activity of adsorbed peroxidase will correspond only 19.7 and 5.7% in comparison to solution. Total activity of peroxidase calculated as a sum of activity in solution and in adsorbed state will decrease 30.3 and 44.3% respectively. Similar decrease of activity has been indicated during the naphthols oxidation [5]. Further peroxidase adsorption and the microparticles aggregation will increase diffusion module and inhibition degree.

The indirect confirmation of the suggested model follows from the experiments of inert polymers action. The inert polymers such as poly (ethylene glycol) or albumin adsorbs on microparticles and prevents adsorption and inhibition of peroxidase [5]. The direct confirmation of the model might be peroxidase activity determination on the microparticles. Preliminary experiments performed by I. Bratkovskaja show that the decrease of activity begins at the moment when sharp increase of light scattering of solution has been indicated. The increase of the scattering confirms hetero phase formation.

4 Conclusions

The inhibition of enzymes during insoluble polymer formation was explained by enzyme adsorption on surface of microparticles and substrate diffusion limitation through diffusion layer. The built model shows that diffusion module in hollow sphere limited by diffusion layer is dependent on particles dimensions. The microparticles with diameter $2\,\mu\mathrm{m}$ may ensure 30.3–$44.3\,\%$ of the apparent activity decrease if half of peroxidase is adsorbed. The mechanism discovered may be applied also to explain very recently discovered phenomena that the aggregates that form during virtual drugs high-throughput screening

are the inhibitory species [11].

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