Nonlinear Analysis: Modelling and Control, 2007, Vol. 12, No. 4, 495–501

The Effectiveness of Synergistic Enzymatic Reaction with Limited Mediator Stability*

J. Kulys, Ž. Dapkūnas

Department of Chemistry and Bioengineering
Faculty of Fundamental Sciences
Vilnius Gediminas Technical University
Saultekio ave. 11, 10223 Vilnius, Lithuania
juozas.kulys@fm.vtu.lt

Received: 25.07.2007 **Revised:** 19.08.2007 **Published online:** 12.11.2007

Abstract. Kinetics of biocatalytical synergistic reactions has been analyzed with special emphasis on stability and reactivity of mediators. The application to the model of kinetic constants taken from the experiments showed that the quasi steady state (QSS) for reduced and oxidized enzyme was achieved in 0.001 s. However, the QSS for the mediator was not established during measurable time. For this reason the kinetics of biocatalytical synergistic reactions was modeled by solving the ordinary differential equations using software package KinFitSim©.

The calculations showed the increase of an apparent life-time of the mediator in the synergistic reaction. The apparent life-time was most affected by reactivity of mediator. The change of the mediator reactivity from 1 $M^{-1}s^{-1}$ to 10^5 $M^{-1}s^{-1}$ increased the apparent life-time from 19 s to 1538 s. This mediator reactivity can be achieved even for strongly endothermic reaction when difference of redox potential of substrate and mediator is 0.35 V. The increase of mediator life-time increased product yield.

Keywords: biocatalysis, kinetics, synergy, ordinary differential equation.

1 Introduction

Many biocatalytical processes proceed in presence of *mediators* [1]. The efficiency of a process increases if a mediator has a capacity to act in *synergism* with the main substrate conversion [2–5]. The kinetics of biocatalytical synergistic reactions has been analyzed very recently [6].

In presence of oxidoreductases a mediator turns between oxidized and reduced state. The oxidized state of mediator typically has limited stability in water solution. Low stability of oxidized mediator can control the efficiency of overall process.

*The research was supported by Lithuania-Ukrainian 2007/2008 years project sponsored by Lithuanian Ministry of Education and Science.

The task of this investigation was to model kinetics of *synergistic* conversion of substrates in presence of mediator with limited stability. The special emphasis was directed to influence stability and reactivity of mediator to efficiency of synergistic process. To optimize the synergistic process the concentrations of the components were varied, and limiting values of the reaction rate constants were established.

2 Mathematical model

The scheme of the synergistic substrates conversion may be written:

$$\mathrm{E(red)} + \mathrm{Ox} \leftrightarrow \mathrm{E(ox)} + \mathrm{P}, \tag{1}$$

$$\mathrm{E(ox)} + \mathrm{S_1} \leftrightarrow \mathrm{E(red)} + \mathrm{P_1}, \tag{2}$$

$$\mathrm{E(ox)} + \mathrm{S_2} \leftrightarrow \mathrm{E(red)} + \mathrm{P_2}, \tag{3}$$

$$\mathrm{P_1} + \mathrm{S_2} \leftrightarrow \mathrm{P_2} + \mathrm{S_1}, \tag{4}$$

where $\mathrm{E(red)}$ and $\mathrm{E(ox)}$ corresponds to reduced and oxidized enzyme, Ox – oxidizer, $\mathrm{S_1}, \mathrm{S_2}$ – substrates, $\mathrm{P}, \mathrm{P_1}, \mathrm{P_2}$ – products of the reactions. The constants of the corresponding direct and reverse reaction rates are $k_1, k_{-1}, k_2, k_{-2}, k_3, k_{-3}, k_4, k_{-4}$. The $\mathrm{S_1}$ and $\mathrm{P_1}$ are reduced and oxidized *mediator*, and the constants k_4 and k_{-4} correspond to the *cross reaction*.

In water solutions the most unstable is $\mathrm{P_1}$, i.e. oxidized mediator. To analyze the role of oxidized mediator to the efficiency of synergistic process the first order reaction of $\mathrm{P_1}$ decay was added:

$$\mathrm{P_1} \rightarrow \mathrm{P_{in}}. \tag{5}$$

The constant of this process is k_{in}.

The change of the concentration of components taking part in the process was described by a system of ordinary differential equations (ODE):

$$\begin{aligned} de_{red}/dt = &-k_1 e_{red} ox + k_{-1} e_{ox} p + k_2 e_{ox} s_1 + k_3 e_{ox} s_2 \\ &- k_{-2} e_{red} p_1 - k_{-3} e_{red} p_2, \end{aligned} \tag{6}$$

$$\begin{aligned} de_{ox}/dt = &\, k_1 e_{red} ox - k_{-1} e_{ox} p - k_2 e_{ox} s_1 - k_3 e_{ox} s_2 \\ &+ k_{-2} e_{red} p_1 + k_{-3} e_{red} p_2, \end{aligned} \tag{7}$$

$$dp/dt = k_1 e_{red} ox - k_{-1} e_{ox} p, \tag{8}$$

$$ds_1/dt = -k_2 e_{ox} s_1 + k_{-2} e_{red} p_1 + k_4 p_1 s_2 - k_{-4} p_2 s_1, \tag{9}$$

$$ds_2/dt = -k_3 e_{ox} s_2 + k_{-3} e_{red} p_2 - k_4 p_1 s_2 + k_{-4} p_2 s_1, \tag{10}$$

$$dp_1/dt = k_2 e_{ox} s_1 - k_{-2} e_{red} p_1 - k_4 p_1 s_2 + k_{-4} p_2 s_1 - k_{in} p_1, \tag{11}$$

$$dp_2/dt = k_3 e_{ox} s_2 - k_{-3} e_{red} p_2 + k_4 p_1 s_2 - k_{-4} p_2 s_1, \tag{12}$$

$$dp_{in}/dt = k_{in} p_1, \tag{13}$$

where t is time, e, ox, s_1, s_2 and p, p_1, p_2, p_{in} correspond to concentrations of enzyme, oxidizer, substrates and products respectively.

The system of ODE was solved with KinFitSim software package version 2.1 [7].

3 Results and discussion

3.1 The function of synergistic process at limited mediator stability

To simplify the analysis the reactions (1)–(3) were assumed to be irreversible. This means that k_{-1}, k_{-2} and k_{-3} are equal to zero. The kinetic constant k_1 of enzymatic (peroxidase) reaction was taken from [8], k_2 value – from [9] and k_3 value – from [10] (Table 1). At the beginning the modelling of synergistic scheme was performed with the constants $k_4 = 10^6$ $\mathrm{M}^{-1}\mathrm{s}^{-1}$ and $k_{-4} = 0$ $\mathrm{M}^{-1}\mathrm{s}^{-1}$. The constant ($k_{in}$) of oxidized mediator ($\mathrm{P}_1$) decay was changed between 0.001 s^{-1} and 1000 s^{-1}. The life-time (τ) of mediator (P_1) is calculated as $\tau = \ln 2/k_{in}$ varied between $6.9 \cdot 10^2$ and $6.9 \cdot 10^{-4}$ s.

Table 1. The values of the kinetic constants. $M \equiv \mathrm{mol} \cdot \mathrm{dm}^{-3}$

Constant	Value, $\mathrm{M}^{-1}\mathrm{s}^{-1}$	Value, s^{-1}
k_1	$7.1 \cdot 10^6$	
k_2	$1.0 \cdot 10^8$	
k_3	$7.1 \cdot 10^3$	
k_4	10^5–10^0	
k_{-4}	10^2	
k_{in}		10^{-3}–10^3

The concentrations of components, that experimentally can vary in the large interval, were chosen to demonstrate intrinsic features of the process. The concentration of Ox was kept constant in all processes. The concentration of P is also constant since for peroxidase reaction P is water.

The simulations showed that the quasi steady state (QSS) for reduced and oxidized enzyme when $de_{ox}/dt \cong de_{red}/dt \cong 0$ was formed during 0.001 s. However, QSS for mediator was established during 20 seconds (Fig. 1). For this reason the QSS state that is common for simple enzymatic kinetics can not be applied for the analysis of these synergistic schemes.

The increase of oxidized mediator decay rate (and the decrease of life-time) practically does not influence the QSS of enzyme. In contrast the QSS for the mediator had not been established during 100 s (Fig. 2).

The apparent life-time of mediator (τ_{50}) was calculated as time of twofold decrease of the concentration of the mediator ($s_1 + p_1$). It is larger than τ due to reaction (4). In the case of $k_{in} = 0.001$ s^{-1}, τ_{50} is 1222 s. In the case of $k_{in} = 0.1$ s^{-1}, $t = 6.9$ s, whereas τ_{50} increases up to 25 s.

The increase of the apparent life-time of mediator has an important consequence. Calculations showed that the synergistic process may be efficient even at low mediator stability. The τ_{50} is a function of enzymatic (k_2) and chemical reactivity of mediator (k_4, k_{-4}). Ten times decrease of the enzymatic rate increased τ_{50} up to 150 s. This method of τ_{50} increase is not interesting from biotechnological point of view since the yield of final product (P_2) decreases by about 7 % at 1000 s.

The apparent life-time is most affected by the reactivity of the mediator, i.e. k_4 value. Calculations show that the apparent life-time increases from 19 s to 1538 s if k_4 changes from 1 $M^{-1}s^{-1}$ to 10^5 $M^{-1}s^{-1}$. At these values of the constants the relative life-time calculated as τ_{50}/τ increases from 2.7 to 222 times (Fig. 3). In the same range of k_4 values the yield of product P_2 increases almost 100 times (Fig. 3).

From calculations, it follows that the apparent life-time remains almost constant (18–25 s) if k_{in} increases from 0.1 s^{-1} to 10^3 s^{-1} with simultaneous k_4 increase from 10^2 $M^{-1}s^{-1}$ to 10^6 $M^{-1}s^{-1}$. In this range of constants the relative time increases by 4 orders of magnitude, however, the product yield at 1000 s practically does not change. This is the reason for the mediator decay at short times.

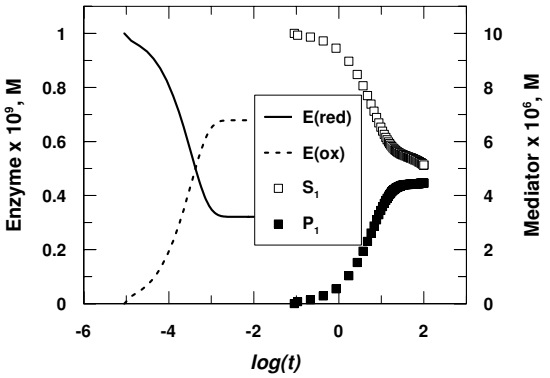

Fig. 1. Dynamics of concentration of oxidized and reduced enzyme and mediator change. Time is expressed in seconds. Concentrations: $e_{ox} + e_{red} = 10^{-9}$ M, $ox = 3 \cdot 10^{-4}$ M, $s_1 = 10^{-5}$ M, $s_2 = 10^{-3}$ M. k_4 and k_{-4} 10^2 $M^{-1}s^{-1}$, $k_{in} = 0.001$ s^{-1}, other constants were taken from Table 1.

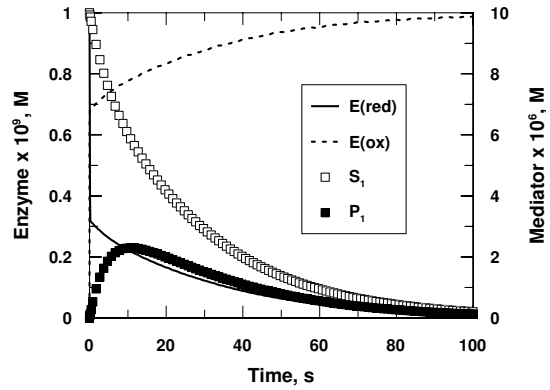

Fig. 2. Dynamics of concentration of oxidized and reduced enzyme and mediator change. $k_{in} = 0.1$ s^{-1}, other constants are the same as in Fig. 1.

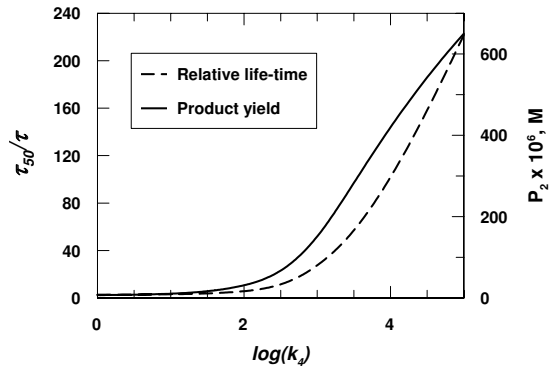

Fig. 3. The dependence of relative life-time of the mediator and product yield at 1000 s on $\log k_4$. k_4 is expressed in $\mathrm{M}^{-1}\mathrm{s}^{-1}$. Constants were the same as in Fig. 2.

In nature some oxidized mediators, for example cation radical of veratryl alcohol, that participate in the most abundant organic material (lignin) degradation, have life-times of about 59 ms [11]. It is likely that effective action of this system may be achieved by permanent synthesis of the mediator in the reaction zone or (and) stabilisation of the oxidized form of mediator by enzyme.

3.2 Estimation of mediator reactivity

Simulations show that the reactivity of the oxidized mediator is crucial for the yield of product of the synergistic scheme. It is important to establish the limiting values of this constant.

The constant of chemical reaction (4) can be estimated using the outer sphere electron transfer theory [12]. The k_4 is a function of self-exchange (k_{11} and k_{22}) and equilibrium (K) constants:

$$k_4 = (k_{11}k_{22}Kf_k)^{1/2}, \tag{14}$$

$$\log f_k = \log K^2/4 \log\left(k_{11}k_{22}Z^2\right). \tag{15}$$

For organics self-exchange constants (k_{11} and k_{22}) are about 10^8 $\mathrm{M}^{-1}\mathrm{s}^{-1}$ and $Z = 10^{11}$ $\mathrm{M}^{-1}\mathrm{s}^{-1}$ [13]. The value of the equilibrium constant determines the redox potentials of P_1 and S_2 and can be calculated using Gibbs equation:

$$\Delta G = -RT \ln K = -nF\Delta E, \tag{16}$$

where ΔG is the free energy change, R and T are the gas constant and absolute temperature, n is the of number electrons, F is the Faraday constant, ΔE is the difference of redox potentials of mediator and substrate.

Calculations show that if the redox potentials of mediator and reagent are equal ($\Delta E = 0$) k_4 for organic components may be as high as 10^8 $\mathrm{M}^{-1}\mathrm{s}^{-1}$. For endothermic

processes when $\Delta E < 0$ every 118.3 mV ($n = 1, 25\,^{\circ}$C) decrease the constant value by almost one order of magnitude. Therefore, even for strongly endothermic processes, when the mediator redox potential is significantly smaller than that of substrate the k_4 value can be as large as 10^5 $M^{-1}s^{-1}$. This explains how low potential mediator (methyl syringate) oxidizes high potential iodide [2].

It is necessary to stress out that simulations in this work were performed under the assumption of irreversible process (1)–(3). The reversibility should be taken into account for enzymes with relatively low redox potential, however, this wouldn't change significantly conclusions made.

4 Conclusions

The simulations of the biocatalytical synergistic reaction showed the increase of an apparent life-time of the mediator. The apparent life-time was the most affected by reactivity of mediator. Even for strongly endothermic reaction when difference of redox potential of substrate and mediator is 0.35 V, the apparent life-time of mediator can increase more than 80 times. The increase of mediator life-time increases product yield.

Acknowledgments

The authors thank prof. Dr. Sci. Irina Svir and dr. Oleksiy Klymenko from Kharkov National University of Radio Electronics for valuable discussions and for the supply of the software package KinFitSim v. 2.1.

References

1. J. Kulys, Exploring and modelling mediator–assisted peroxidase catalysis, *Biologija*, **4**, pp. 32–36, 1998.
2. J. Kulys, I. Bratkovskaja, R. Vidziunaite, Laccase-catalyzed iodide oxidation in presence of methyl syringate, *Biotechnol. Bioeng.*, **92**, pp. 124–128, 2005.
3. J. Kulys, L. Tetianec, Synergistic substrates determination with biosensors, *Biosens. Biolectr.*, **21**, pp. 152–158, 2005.
4. J. Kulys, R. Vidziunaite, Kinetics of laccase-catalyzed TEMPO oxidation, *J. Mol. Cat. B: Enzym.*, **37**, pp. 79–83, 2005.
5. I. Bratkovskaja, R. Ivanec, J. Kulys, Mediator-assisted laccase-catalyzed oxidation of 5-hydroxybiphenyl, *Biochemistry*, **71**, pp. 550–554, Moscow, 2006.
6. J. Kulys, Kinetics of biocatalytical synergistic reactions, *Nonlinear Analysis: Modelling and Control*, **10**, pp. 223–233, 2005.
7. I. B. Svir, O. V. Klymenko, A. I. Oleinick, M. S. Platz, KINFITSIM (version 2.1) – a powerful tool for kinetic simulation of any reaction mechanism and fitting of any number of pairs of theoretical and experimental data sets, *Radioelektronika i informatika*, **4**, pp. 22–25, 2004.

8. M. B. Andersen , Y. Hsuanyu, K. G. Welinder, P. Schneider, H. B. Dunford, Spectral and kinetic properties of oxidized intermediates of Coprinus cinereus peroxidase, *Acta Chem. Scand.*, **45**, pp. 1080–1086, 1991.

9. J. Kulys, K. Krikstopaitis, A. Ziemys, Kinetics and thermodynamics of peroxidase- and laccase-catalysed oxidation of N-substituted phenothiazines and phenoxazines. *J. Biol. Inorg. Chem.*, **5**, pp. 333–340, 2000.

10. J. Kulys, H. J. Deussen, K. Krikstopaitis, R. Lolck, P. Schneider, A. Ziemys, N-aryl hydroxamic acids as novel oxidoreductases substrates, *Eur. J. Org. Chem.*, **18**, pp. 3475–3484, 2001.

11. L. P. Candeias, P. J. Harvey, Lifetime and reactivity of the veratryl alcohol radical cation, Implications for lignin peroxidase catalysis, *J. Biol. Chem.*, **270**, pp. 16745–16748, 1995.

12. R. A. Marcus, N. Sutin, Electron transfers in chemistry and biology, *Biochim. Biophys. Acta*, **811**, pp. 265–322, 1985.

13. G. Merenyi, J. Lind, M. Jonsson, Autoxidation of closed-shell organics: an outer-sphere electron transfer, *J. Am. Chem. Soc.*, **115**, pp. 4945–4946, 1993.