Nonlinear Analysis: Modelling and Control, 2005, Vol. 10, No. 3, 223–233

Kinetics of Biocatalytical Synergistic Reactions*

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Received: 13.05.2005
Accepted: 30.06.2005

Abstract. Kinetics of biocatalytical synergistic reactions has been analyzed at non-stationary state (NSS) and at quasi steady state (QSS) conditions. The application to the model kinetic constants taken from the first type of the experiments shows that QSS can be established for the enzyme and the mediator at time less than 1 s. Therefore, the analytical solution of the initial rate (IR) may be produced at relevant to an experiment time, and the dependence of the IR on substrates concentration may be analyzed rather easy. The use of kinetic constants from the second type of reactions shows that QSS is formed for the enzyme but not for the mediator. For this reason the modeling of the synergistic process was performed by solving the ordinary differential equations (ODE). For this purpose the novel program KinFitSim© was used.

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

1 Introduction

Biological catalyzers (enzymes) show high activity and specificity. The activity of enzymes may exceed the rate of chemically catalyzed reaction by a factor 10^{10}–10^{13}. The enzymatic activity of the enzymes depends on many factors, i.e. the free energy of reaction, substrate docking in the active center, proton tunneling and other factors [1–6]. The general principles of catalytic activity of enzymes are

*The research was supported by Lithuanian State Science and Studies Foundation, project No. C-03048.

known, but particular factors that determine high enzyme activity are often not established [7].

The specificity of enzymes depends on the enzyme type [8]. There are enzymes which catalyze the conversion of just one substrate. Other enzymes show broad substrates specificity. Oxidoreductases, i.e. enzymes that catalyze electron transfer, may catalyze, for example, the oxidation or reduction of many substrates. To characterize the substrates with diverse activity a slang "good substrate" and "bad substrate" is used. Simultaneous conversion of good and bad substrate opens new possibilities of an enzyme catalysis exploitation. The kinetics of these reactions is more complex in comparison to ordinary enzyme kinetics. The modeling of these type of reactions requires solving the ordinary differential equations (ODE) that is not common for biochemists.

The task of this investigation is to model kinetics of *synergistic*, i.e. having the capacity to act in *synergism*, conversion of substrates. As an example of synergistic reaction the oxidoreductases catalyzed processes have been analyzed. To optimize the synergistic schemes the concentrations of the components were varied, and limiting values of the reaction rate constants were established.

2 Mathematical model

Two types of synergistic reactions were analyzed. The first type of reactions was realized for high sensitive biosensor building [9]. The second type of reactions were used for recalcitrants oxidation [10]. The scheme of the first type synergistic substrates conversion may be written:

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

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

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

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

where $\mathrm{E(ox)}$ and $\mathrm{E(red)}$ corresponds to oxidized and reduced enzyme, R – reducer, $\mathrm{S_1}, \mathrm{S_2}$ – 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 named as *a mediator* and the reaction with the constant k_4 – as a constant of *cross reaction*.

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

$$de_{ox}/dt = -k_1 \cdot e_{ox} \cdot r + k_{-1} \cdot e_{red} \cdot p + k_2 \cdot e_{red} \cdot s_1 + k_3 \cdot e_{red} \cdot s_2 - k_{-2} \cdot e_{ox} \cdot p_1 - k_{-3} \cdot e_{ox} \cdot p_2, \quad (5)$$

$$de_{red}/dt = k_1 \cdot e_{ox} \cdot r - k_{-1} \cdot e_{red} \cdot p - k_2 \cdot e_{red} \cdot s_1 - k_3 \cdot e_{red} \cdot s_2 + k_{-2} \cdot e_{ox} \cdot p_1 + k_{-3} \cdot e_{ox} \cdot p_2, \quad (6)$$

$$ds_1/dt = -k_2 \cdot e_{red} \cdot s_1 + k_{-2} \cdot e_{ox} \cdot p_1 + k_4 \cdot p_1 \cdot s_2 - k_{-4} \cdot p_2 \cdot s_1, \quad (7)$$

$$ds_2/dt = -k_3 \cdot e_{red} \cdot s_2 + k_{-3} \cdot e_{ox} \cdot p_2 - k_4 \cdot p_1 \cdot s_2 + k_{-4} \cdot p_2 \cdot s_1, \quad (8)$$

$$dp_1/dt = k_2 \cdot e_{red} \cdot s_1 - k_{-2} \cdot e_{ox} \cdot p_1 - k_4 \cdot p_1 \cdot s_2 + k_{-4} \cdot p_2 \cdot s_1, \quad (9)$$

$$dp_2/dt = k_3 \cdot e_{red} \cdot s_2 - k_{-3} \cdot e_{ox} \cdot p_2 + k_4 \cdot p_1 \cdot s_2 - k_{-4} \cdot p_2 \cdot s_1, \quad (10)$$

where t is time, e, r, s_1, s_2 and p, p_1, p_2 corresponds to a concentration of enzyme, reducer, substrates and products.

The scheme of the second type synergistic substrates conversion may be written:

$$\mathrm{E(red)} + \mathrm{Ox} \leftrightarrow \mathrm{E(ox)} + \mathrm{P}, \quad (11)$$

$$\mathrm{E(ox)} + \mathrm{S_1} \leftrightarrow \mathrm{E(red)} + \mathrm{P_1}, \quad (12)$$

$$\mathrm{E(ox)} + \mathrm{S_2} \leftrightarrow \mathrm{E(red)} + \mathrm{P_2}, \quad (13)$$

$$\mathrm{P_1} + \mathrm{S_2} \leftrightarrow \mathrm{P_2} + \mathrm{S_1}, \quad (14)$$

where abbreviations are the same as in equations (1)–(4), and Ox is an oxidizer. The rate constants of direct and reverse reactions are: k_{11}, k_{-11}, k_{12}, k_{-12}, k_{13}, k_{-13}, k_{14}, k_{-14}.

The solution of the system of ODE was made with KinFitSim package version 2.0 [11]. The package consists of two major parts. The first part is a kinetic simulator (KS), which allows to specify any kinetic mechanism and input the needed simulation parameters, such as initial concentrations and reaction rate constants. The program calculates the concentrations of all species taking part in the reactions as a function of time. The compiler part of the KS automatically derives the ODE system corresponding to the specified reaction mechanism. The ODEs are solved using modern numerical methods, which can handle both stiff

and non-stiff ODE systems. The calculated concentrations may be converted to other units for direct comparison with experimental data by using a wide variety of mathematical functions, which can be recognized by the program. The second part of the KinFitSim package is a fitting simulator (FS), which allows the user to load the experimental kinetic data from a file and determine the best-fit kinetic parameter values. This can be performed manually by adjusting parameter values and visual comparison of simulated and experimental curves. Another option is to use an automatic fitting procedure. In this case, the user is required to set the range for each varied parameter and the best fit is found by efficient optimization methods. The KinFitSim package has a modern user-friendly interface. The user may print out a report about the simulation and fitting results which contains the reaction scheme being simulated, the kinetic parameter values, experiment-related information and a plot of the simulated curve and experimental data (if loaded). The version 2.00 of KinFitSim contains in addition the automatic regime of numerical method selection, new sufficient method for fitting procedure (down-hill simplex method of Nelder-Mead), new possibilities of loading and editing experimental data directly in program window.

The solution of the system of kinetic equations at steady-state conditions was performed using symbolic processor of Mathcad (MathSoft Inc., Cambridge, MA).

3 Results and discussion

3.1 Non-stationary state analysis

To simplify the analysis all reactions of the first type process were assumed to be irreversible at beginning. This means that k_{-1}, k_{-2}, k_{-3} and k_{-4} is equal to zero. The kinetic constants of enzymatic and chemical reactions taken for the simulation are coming from real experimental investigations [9, 10] and are depicted in Table 1.

The simulations were performed at different concentrations of the components (Table 2). Using data Set 1, the concentration of the reducer (R) varied from 0.1 M to 0.001 M whereas the concentrations of other components were kept constant.

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

The first type process		The second type process	
Constant	Value, M^{-1}s^{-1}	Constant	Value, M^{-1}s^{-1}
k_1	$1.25 \cdot 10^4$	k_{11}	$1.0 \cdot 10^6$
k_2	$1.40 \cdot 10^6$	k_{12}	$5.0 \cdot 10^5$
k_3	$1.20 \cdot 10^2$	k_{13}	$3.8 \cdot 10^4$
k_4	$1.40 \cdot 10^6$	k_{14}	$5.5 \cdot 10^3$

Table 2. The concentrations (in M) of the components used for the simulation of the first type of the process

Set 1	Set 2	Set 3
$r = 10^{-1}; 10^{-2}; 10^{-3}$	$r = 10^{-1}$	$r = 10^{-2}$
$s_1 = 10^{-5}$	$s_1 = 10^{-5}$	$s_1 = 10^{-5}$
$s_2 = 10^{-2}$	$s_2 = 10^{-1}; 10^{-2}; 10^{-3}$	$s_2 = 5 \cdot 10^{-2}; 10^{-2}; 10^{-3}$

The simulations showed that transition process spanned almost $0.005\,$s at $r = 0.1\,$M (Fig. 1). After this period the concentration of oxidized and reduced enzyme changed slowly and $de_{ox}/dt \cong de_{red}/dt \cong 0$. For this reason the period larger than $0.005\,$s was named as a quasi steady state (QSS).

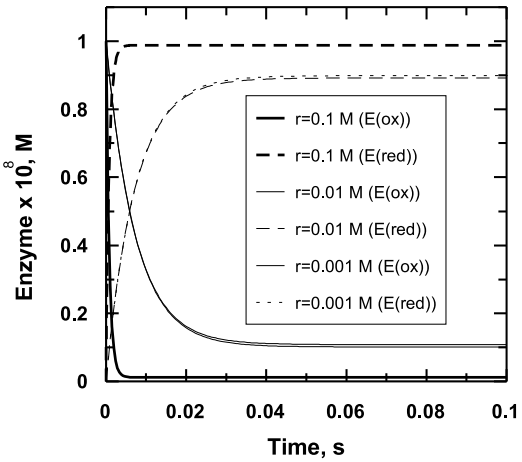

Fig. 1. Dynamics of oxidized and reduced enzyme concentration change of the first type reaction. The calculations were performed with the parameters of data Set 1 from Table 2. The constant values are taken from Table 1.

The decrease of the R concentration up to 10^{-2} M increased transition period up to $3 \cdot 10^{-2}$s (Fig. 1). Further decrease of the R little influenced the transition period.

The dependence of the QSS on the R concentration indicated limitation of the process by reaction (1). A characteristic time of the transition period expressed as $\tau = 1/k_1 \cdot r$ was $8 \cdot 10^{-4}$ s. At low R concentration the establishment of the QSS was limited by other reactions.

The transition period of the process at $r = 10^{-1}$ M and varied concentrations of S_2 (Set 2) was less than $5 \cdot 10^{-5}$ s, indicating the limitation of the process by reaction (1). The process was also limited by reaction (1) at $r = 0.01$ M and the varied concentrations of S_2 (data Set 3). At these concentrations the transition period of the process was less than $3 \cdot 10^{-2}$ s. This transition period fitted the characteristic time of $8 \cdot 10^{-3}$ s.

The analysis of S_1 concentration change showed that the QSS for this compound was established during $3 \cdot 10^{-3}$, $3 \cdot 10^{-2}$ and $1 \cdot 10^{-1}$ s at $r = 10^{-1}, 10^{-2}$ and 10^{-3} M respectively (the concentrations of other components were taken from the data Set 1) (Fig. 2). At $r = 10^{-2}$ M and $s_2 = 10^{-3}$ M (the data Set 3) the transition period was about $3.5 \cdot 10^{-2}$ s.

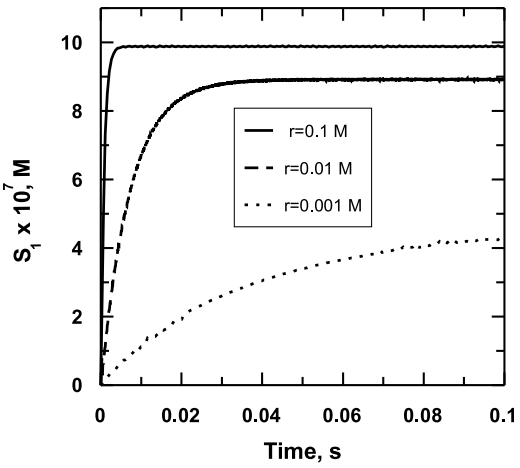

Fig. 2. The S_1 concentration change at different R concentrations of the first type process. The constant values are taken from Table 1. Initial $s_1 = 1 \cdot 10^{-5}$ M, $s_2 = 1 \cdot 10^{-2}$ M, $e_t = 2 \cdot 10^{-8}$ M.

The effect of reversibility of the reactions to the QSS establishment was analyzed at $k_{-2} = 1.4 \cdot 10^6\,\mathrm{M^{-1}s^{-1}}$. This value gives the equilibrium constant of the reaction (2) expressed as $K = k_2/k_{-2}$ equal to 1. The analysis of the transition period at all concentrations of compounds (Table 2) showed that the reversibility of reaction (2) little influenced the transition time. This indicates that equilibrium of the reaction (2) has not established. The main reason is a large value of the constant k_4 and a high concentration of S_2.

From the mathematical point of view the second type process is similar to the first type. However, the process was analyzed separately because different values of constants cause new features that are important for the enzyme kinetics.

The dynamics of the second type process was modelled at enzyme concentration $2 \cdot 10^{-8}\,\mathrm{M}$, the concentration of Ox was kept constant $2.54 \cdot 10^{-4}\,\mathrm{M}$, s_1 was $5 \cdot 10^{-6}\,\mathrm{M}$ and s_2 was $6 \cdot 10^{-6}\,\mathrm{M}$. The calculations show that QSS for the enzyme is established during $10^{-2}\,\mathrm{s}$ (Fig. 3).

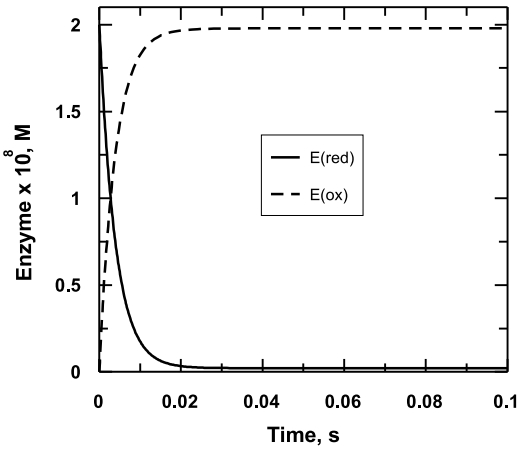

Fig. 3. The kinetics of the enzyme concentration change for the second type process. The total concentration of enzyme was $2{\cdot}10^{-8}\,\mathrm{M}$, the concentration of oxidizer was kept constant $2.54{\cdot}10^{-4}\,\mathrm{M}$, the concentration of S_1 was $5{\cdot}10^{-6}\,\mathrm{M}$ and of S_2 was $6 \cdot 10^{-6}\,\mathrm{M}$.

The calculations of the mediator concentration change of the second type process performed with the same parameters, however, revealed, that the QSS didn't established during $180\,\mathrm{s}$ (Fig. 4). Only if the initial S_1 concentration was

less than 10^{-6} M the QSS was established during a time less than 60 s.

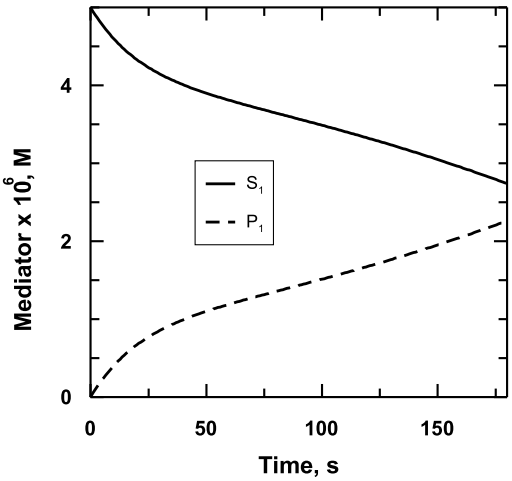

Fig. 4. The kinetics of mediator (S_1 and P_1) concentration change for the second type process. The calculations were performed with the same parameters as in Fig. 3.

3.2 Quasi steady-state analysis

The kinetic equations in enzymology is typically derived under assumption that the QSS is established for the enzyme, and that concentration of substrates do not change [12]. Usually the dependence of the calculated "initial rate" on enzyme and substrates concentration is analyzed. In the case of synergistic reactions not only enzyme but also mediator distributes between oxidized and reduced forms, i.e. between e_{ox}, e_{red}, s_1 and p_1, respectively. Therefore the QSS should be established for the enzyme and for mediator. The analysis of synergistic process of the first type process shows that the QSS for both components is established at rather short period of time ($t < 1$ s). At the QSS $de_{ox}/dt \cong de_{red}/dt \cong ds_1/dt \cong 0$. Therefore the equations (5)–(10) may be solved using computer algebra [13]. However, the expressions of the "initial steady state rate" ($V_{st} = f(e_t, m_t, r, s_2)$) of the synergistic process is rather complex even for the irreversible reactions:

$$V_{st} = (dp_2/dt)_{t\approx 0} = k_3 \cdot e_{red} \cdot s_2 + k_4 \cdot p_1 \cdot s_2, \tag{15}$$

where e_t – total enzyme concentration, m_t – total mediator concentration, s_2 is equal to initial S_2 concentration, e_{red} and p_1 are calculated from:

$$k_1 \cdot e_{ox} \cdot r - k_2 \cdot e_{red} \cdot s_1 - k_3 \cdot e_{red} \cdot s_2 = 0, \tag{16}$$

$$k_2 \cdot e_{red} \cdot s_1 - k_4 \cdot p_1 \cdot s_2 = 0, \tag{17}$$

$$e_t = e_{red} + e_{ox}, \tag{18}$$

$$m_t = s_1 + p_1. \tag{19}$$

The initial rate of the process at $s_1 = 0$, i.e. the rate of a simple enzymatic S_2 conversion is:

$$v_{st} = (dp_2/dt)_{t\approx 0} = k_3 \cdot e_{red} \cdot s_2. \tag{20}$$

A ratio (ρ) of the initial rate of the synergistic and the simple enzymatic reaction is:

$$\rho = V_{st}/v_{st}. \tag{21}$$

It is easy to notice that the ratio indicating the efficiency of synergistic reaction depends on substrates concentration (Fig. 5). At $k_4 = 1.4 \cdot 10^6\,\mathrm{M^{-1}s^{-1}}$, $r = 0.1\,\mathrm{M}$, low mediator and S_2 concentration the ratio is approaching to a value ($k_2 \cdot m_t/k_3 \cdot s_2 + 1$). For m_t and s_2 values used in the calculations $\rho_{m_t/s_2 \to 0} = 12.6$ that gives $k_2/k_3 = 1.16 \cdot 10^4$. This means that at small but equal m_t and s_2 the synergistic process is $1.16 \cdot 10^4$ times more effective in comparison to the enzymatic reaction in absence of the mediator.

At $k_4 = 1.4 \cdot 10^6\,\mathrm{M^{-1}s^{-1}}$ the ratio decreases at high mediator and S_2 concentration due to limitation of process by enzymatic reaction (1). The increase of R concentration enlarges the affectivity of the synergistic process.

The calculations show the ratio is not sensitive to the rate of chemical reaction (4). The k_4 can decrease 5 orders of magnitude without significant change of efficiency. However, significant decrease of k_4 expands time of the QSS establishment. It is worth to notice that the k_4 value can be calculated from Marcus theory [2]. For exothermic electron transfer reactions with participation of organic compounds a typical value of $1.4 \cdot 10^6\,\mathrm{M^{-1}s^{-1}}$ was measured [9].

The rate constants and concentrations of the substrates participating in the second type process does not produce the QSS in relation to the mediator. For this

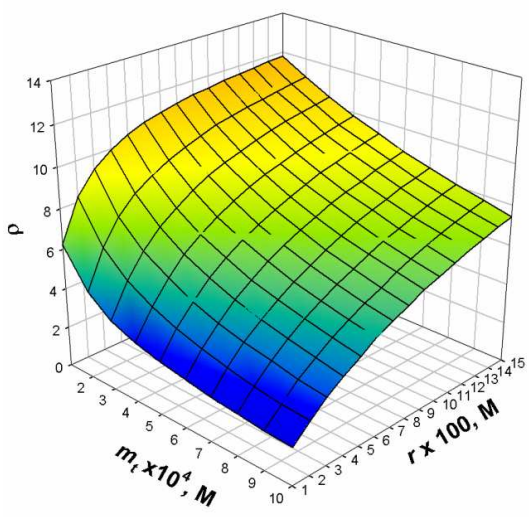

Fig. 5. The ratio of the rate of the synergistic and the simple enzymatic reaction at different mediator (m_t), substrate ($s_2 = m_t \cdot 10^3$) and reducer (r) concentration. The calculations were performed with parameters as in Fig. 1.

reason the derivation of the initial rate of process is not correct, and the analysis should be performed by using non-steady state kinetics as its was described above. However, the high ratio between substrate and mediator concentration and small mediator concentration permits aproximally to utilize expression of the initial rate like as for the first type process.

Acknowledgments

The author thanks dr. Irina Svir from Kharkov National University of Radio Electronics for the package KinFitSim v. 2.0 supply. The big thank is coming also to prof. Romas Baronas for the manuscript reading and the corrections done.

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