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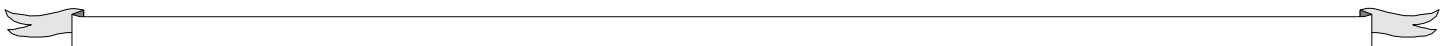
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Numerical Simulation and Cybernetic Modelling of Enzymatic Reactions with Two Intermediates

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⊕ **ABSTRACT**

Living systems are non-equilibrium dynamic structures generated under informational control, based on energy dissipation in a far-from-equilibrium regime. The control of energy input is achieved by automatic regulation systems with negative and positive reaction. The enzymes are an important component of the informational matrix. Their behavior resembles the molecular machines, taking decisions about targeting the biochemical reactions and about the metabolism ajustment to the changes in the environmental conditions. The action mechanism for the enzymes can be modelled taking into acount the basics of information theory. The first step of modelling consists in identification of signals from the information flow crossing the enzymatic reaction system: the input signals, the regulating signals for the information flow and the output signal. The initial molar concentrations of enzyme and substrate are the input signal, while the molar concentration of the enzymatic reaction product designates the output signal. The next step implies identification of the regulating circuits, consisting in identification of mathematical relation between the input and the output signals. The signal processing was performed by mathematical operations of algebraic summation, multiplication and integration using the corresponding operators.

Keywords: systems of nonlinear differential equations; numerical integration;information flow; cybernetic model; positive local reaction loop; negative local reaction loop.

INTRODUCTION

From thermodynamic point of view, living systems are spatially structured multicomponent systems in a far-from-equilibrium state. They are open systems, continuous crossed by mass, energy and information flows, leading to interesting properties different from the ones of non-living systems[1]:

- Biochemical reactions progress to near equilibrium steady states, characterized by a maximum in free enthalpy and a minimum entropy. Preservation of phisiological parameters in certain limits complies with a stable, near equilibrium steady state with low entropy.

- Oscillation of thermodynamic forces leads the system toward a far-from-equilibrium regime. By a spontaneous self-structuring process, metastable supramolecular structures are generated – the so-called dissipative structures – based on dissipation of outer energy. Thus, the macromolecules of certain proteins assemble by non-covalent interactions in the tridimensional space. The as-resulted structure complies better to its biological function than to the state with minimum potential energy.

- Mass and energy conversion occurs in individual elementary volums joined together by mass transport, diffusion. Can also be the result of the action of external forces or of the interference of special adjustment mechanisms typical for living bodies. Each elementary volum is an far-from-equilibrium open system. Moreover, energy transporting substances or other energy sources are distributed in space and interconected by mass and energy flows.

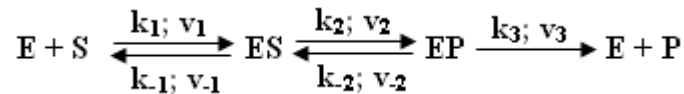[*] Corresponding author: dobri_aur@yahoo.com

Information flow between biosystems and exterior, as well as inside the biosystems, generates the information matrix. This coordinates the regulation mechanism of thermodynamic force oscillations and the synchronous irreversible processes which generate energy and entropy and processes with entropy decrease by energy consumption.

Therefore, the viability of living systems is the result of information controlled way by which they use energy and mass. Thus, the development of a new approach of living system processes based on information theory is possible.

RESULTS AND DISCUSSIONS

The enzymes are proteic macromolecules which accelerate the biochemical reactions by promoting a transition state with a lower free energy than the one corresponding to the transition state of uncatalyzed reaction. Thus, a reaction way with a lower activation energy is developed. Lowering the activation energy is due to a number of week non-covalent interactions established between the enzyme and the substrate. The substrate is bonded to the enzyme active site by at least three contacts. The active site has a tidimensional structure and act as a shield between the substrate and the solvent. The enzymatic reaction occurs by formation of a enzyme-substrate transition complex, ES. The enzyme-substrate interactions in the ES complex are mediated by the same forces which stabilize the proteine structure, such as: hydrogen bonds, hidrofobe forces, electrical forces corresponding to ionic intercations. During enzymatic reaction, a transient enzyme-product complex is also formed, its dissociation leading to the reaction product.

The general mechanism of enzyme action in the process of converting the substrate S into the product P has three steps, the fist and the second steps being reversible:

$$E + S \underset{k_{-1};\, v_{-1}}{\overset{k_1;\, v_1}{\rightleftarrows}} ES \underset{k_{-2};\, v_{-2}}{\overset{k_2;\, v_2}{\rightleftarrows}} EP \xrightarrow{k_3;\, v_3} E + P$$

Numerical simulation of the enzymatic reaction dynamic system implies in the first step the generation of kinetic model[2-7]. In this step, the nonlinear system of ordinary differential equations (contain a single independent variable, time t) is elaborated, expressing the rate of changes in concentration for all involved species (five species).

The mathematic model describing the dynamics of enzymatic reaction with two intermediates (enzyme-substrate complex – ES, enzyme-product complex – EP) is one with continuous time: the set of time moments in which the dynamic system evolves is a subset of the real number set. This model is based on the rate equations for all elementary reactions involved in the three steps:

$$\begin{cases} v_1 = k_1[\text{S}][\text{E}] \\[2mm] v_{-1} = k_{-1}[\text{ES}] \\[2mm] v_2 = k_2[\text{ES}] \\[2mm] v_{-2} = k_{-2}[\text{EP}] \\[2mm] v_3 = k_3[\text{EP}] \end{cases} \tag{1}$$

Appling the mass conservation law, the following system of five balance equation is obtained:

$$\begin{cases} \dfrac{d[S]}{dt} = v_{-1} - v_1 \\[2mm] \dfrac{d[E]}{dt} = v_{-1} + v_3 - v_1 \\[2mm] \dfrac{d[ES]}{dt} = v_1 + v_{-2} - v_{-1} - v_2 \\[2mm] \dfrac{d[EP]}{dt} = v_2 - v_{-2} - v_3 \\[2mm] \dfrac{d[P]}{dt} = v_3 \end{cases} \tag{2}$$

If the expressions of the rate for the five elementary reactions are substituted in the equation system (2), the system of ordinary nonlinear differential equations (3) is obtained, descriebing the temporary evolution of the dynamic system. Thus, the enzymatic reaction system is a dynamic system with intensive parameters.

$$\begin{cases} \dfrac{d[S]}{dt} = k_{-1}[ES] - k_1[S][E] \\[2mm] \dfrac{d[E]}{dt} = k_{-1}[ES] + k_3[EP] - k_1[S][E] \\[2mm] \dfrac{d[ES]}{dt} = k_1[S][E] + k_{-2}[EP] - k_{-1}[ES] - k_2[ES] \\[2mm] \dfrac{d[EP]}{dt} = k_2[ES] - k_{-2}[EP] - k_3[EP] \\[2mm] \dfrac{d[P]}{dt} = k_3[EP] \end{cases} \tag{3}$$

The analysis of the five ordinary differential equations of the nonlinear system (3) denotes that only the molar concentrations of substrate, enzyme, enzyme-substrate complex and enzyme-product complex are independent, i.e. only the first four equations are coupled: integrating the fifth equation of the system, the product P concentration can be determined if the system formed by the first four equations is solved:

$$[P]_t = k_3 \int [EP]_u \, dt' \tag{4}$$

The differential model also allows the determination of invariance in enzyme total concentration in time, as they act as a catalyst in the reaction system:

$$\frac{d[E]}{dt} + \frac{d[ES]}{dt} + \frac{d[EP]}{dt} = \left(k_{-1}[ES] + k_3[EP] - k_1[S][E] \right) + \left(k_1[S][E] + k_{-2}[EP] - k_{-1}[ES] - k_2[ES] \right)$$
$$+ \left(k_2[ES] - k_{-2}[EP] - k_3[EP] \right)$$

$$\Rightarrow \frac{d}{dt}\big([E]+[ES]+[EP]\big)=0 \Rightarrow [E]+[ES]+[EP]=[E]_0 \tag{5}$$

The nonlinear differential equation system (3) can be solved either by an analytical method which leads to an approximate analytic solution (closed solution), or by a monopas numerical method which leads to an approximate numerical solution (open solution).

With the aim to solve the differential system (3) by a numerical method, it is convenient to express the ordinary differential equations by a common variable x representing the variable vector depending on time. This is a column vector with the number of components equal to the number of variables dependent on time and can be represented as a 5x1 type matrix.

$$x = \big(x_1(t) \quad x_2(t) \quad x_3(t) \quad x_4(t) \quad x_5(t)\big)^T = \begin{pmatrix} x_1(t) \\ x_2(t) \\ x_3(t) \\ x_4(t) \\ x_5(t) \end{pmatrix}$$

Therefore, with the notations:

$[S]=x_1(t)$

$[E]=x_2(t)$

$[ES]=x_3(t)$

$[EP]=x_4(t)$

$[P]=x_5(t)$

the system (3) can be rewritten as:

$$\begin{cases} \dfrac{dx_1}{dt} = k_{-1}x_3 - k_1 x_1 x_2 \\[2mm] \dfrac{dx_2}{dt} = k_{-1}x_3 + k_3 x_4 - k_1 x_1 x_2 \\[2mm] \dfrac{dx_3}{dt} = k_1 x_1 x_2 + k_{-2}x_4 - k_{-1}x_3 - k_2 x_3 \\[2mm] \dfrac{dx_4}{dt} = k_2 x_3 - k_{-2}x_4 - k_3 x_4 \\[2mm] \dfrac{dx_5}{dt} = k_3 x_4 \end{cases} \tag{6}$$

The ordinary differential equation system which fulfills the initial conditions is a Cauchy problem. Its resolution can be performed by numerical integration using a monopass numerical method based on Taylor series expansion of the unknown function $x(t)$ in $t=t_0$. The method with adaptive stepsize Runge-Kutta of 4 and 5 order, with Dormand-Prince adaptation has a small truncation error. This method was implemented in the solvation function **ODE45** of MATLAB.

Since the differential equations of system (6) have the form and the solution has to fulfill the initial condition $x(t_0)=x_0$, the evaluation of x vector in each step i of the integration implies the use of the following formulas:

$$\Delta x_i = \frac{h}{6} \cdot \left(K_1^i + 2K_2^i + 2K_3^i + K_4^i \right)$$

$$x_{i+1} = x_i + \Delta x_i$$

(7)

in which:

$$K_1^i = f\left(t_i, x_i\right);$$

$$K_2^i = f\left(t_i + \frac{h}{2}, x_i + \frac{K_1^i}{2}\right);$$

$$K_3^i = f\left(t_i + \frac{h}{2}, x_i + \frac{K_2^i}{2}\right);$$

$$K_4^i = f\left(t_i + h, x_i + K_3^i\right);$$

$h=t_{n+1}-t_n$ is the elementary increase of independent variable t.

For the system with five differential equations, the evaluation of dependent variable vector in each step i of the integration consist in the use of the following formulas:

$$\Delta x_i = \frac{h}{6} \cdot \left(F_1^j + 2F_2^j + 2F_3^j + F_4^j \right), \quad j = 1,2,3,4,5$$

(8)

$$x_{i+1} = x_i + \Delta x_i$$

in which:

$$F_1^j = f_j\left(t_i, x_i^j\right);$$

$$F_2^j = f_j\left(t_i + \frac{h}{2}, x_i^j + \frac{h}{2} \cdot F_1^j\right);$$

$$F_3^j = f_j\left(t_i + \frac{h}{2}, x_i^j + \frac{h}{2} \cdot F_2^j\right);$$

$$F_4^j = f_j\left(t_i + h, x_i + h \cdot F_3^j\right)$$

Numerical simulation with interactive MATLAB implies to rewrite the differential system (6) under the matrix form $\dot{x}(t) = f(t, x)$. The standard form represents a vectorial equation having as left member a column vector and as right member a vectorial function which generates the differential functions:

$$\begin{pmatrix} \dot{x}_1 \\ \dot{x}_2 \\ \dot{x}_3 \\ \dot{x}_4 \\ \dot{x}_5 \end{pmatrix} = \begin{pmatrix} k_{-1}x_3 - k_1 x_1 x_2 \\ k_{-1}x_3 + k_3 x_4 - k_1 x_1 x_2 \\ k_1 x_1 x_2 + k_{-2}x_4 - k_{-1}x_3 - k_2 x_3 \\ k_2 x_3 - k_{-2}x_4 - k_3 x_4 \\ k_3 x_4 \end{pmatrix} \quad (9)$$

For the numerical simulation with the result shown in Fig. 1, the code lines written in MATLAB language have been used. They have been embedded in two text files with the extension .m. One of them is of type function with input arguments and returns output values. The MATLAB code lines in this file are:

```
function dx=simreactenz(t,x,k1,k_1,k2,k_2,k3)

dx=zeros(5,1);

dx(1)=k_1*x(3)-k1*x(1)*x(2);

dx(2)=k_1*x(3)+k3*x(4)-k1*x(1)*x(2);

dx(3)=k1*x(1)*x(2)+k_2*x(4)-k_1*x(3)-k2*x(3);

dx(4)=k2*x(3)-k_2*x(4)-k3*x(4);

dx(5)=k3*x(4);

end
```

The second file is of type script and its code lines call the function file:

```
clear all;

close all;

[t,x]=ode45(@simreactenz,[0,100],[8;0.5;0;0;0],[],5,0.1,2,0.1,1);

plot(t,x)

title('General solution of the first order differential equation')
```