

MATHEMATICAL MODEL OF BISTABILITY IN THE PEROXIDASE-OXIDASE
REACTION UPON ACTION OF FREE-RADICAL TRAPS

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Peroxidase-oxidation of a number of substrates, including heteroauxin (indolyl-3-acetic acid, IAA) and NADH, at the present time is the subject of both experimental and theoretical research [1-6]. The interest of researchers is due especially to the possibility of observation in these reactions of a broad spectrum of complex dynamic regimes: trigger [2,3], self-oscillatory with the existence of two limit cycles (large and small) [2, 4, 5] and chaos [2]. Study of the different regimes in turn is a research tool: it allows us to construct a model for the peroxidase-oxidase reaction, determining its different branches. Experiments of interest from this viewpoint are those in which bistability has been observed with respect to the inhibitor concentration in the reaction of enzymatic oxidation of IAA upon inhibition by a natural inhibitor of the reaction: caffeic acid [7]. The results of these experiments allow us to take a fresh look at the inhibition mechanism, hypothesizing that the substituted phenols act as free-radical traps in the given reaction and not as competing inhibitors of the enzymatic reaction.

The goal of this paper is to construct a mathematical model describing the bistability in peroxidase-oxidase reactions upon action of the inhibitor, a radical trap. Ideas about the peroxidase-oxidase process as a free-radical branched chain reaction allow us to write the elementary steps in the formation of free radicals and their reaction with the inhibitor in the following form:



where S is the substrate to be oxidized in the peroxidase-oxidase reaction, R' is the free radical participating in the chain reaction, I and I' are the inhibitor and its radical respectively, k_i are the rate constants of the corresponding reactions. The equation for reaction (1) describes formation of free radicals R' by a nonenzymatic route; (2) describes formation of radicals in an enzymatic branched chain reaction; (3) describes bimolecular reaction of radicals with each other, with formation for example of the dimer R-R; (4) describes reaction of the radical R' with the inhibitor leading to formation of the product P and the inhibitor radical. The equations for reactions (5) and (6) describe respectively reduction of the inhibitor radical (without specifying the electron donor) and

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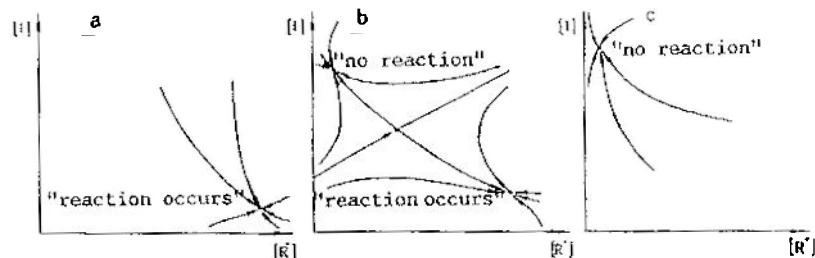


Fig. 1. Phase portrait of the dynamic system describing the reaction of free radicals formed during the peroxidase-oxidase reaction with their traps (see text for explanation).

bimolecular reaction of the inhibitor radicals with formation, for example, of the dimer of the inhibitor I-I.

System (1)-(6) corresponds to the following system of differential equations:

$$d[S]/dt = -k_1[S] - k_2[S][R], \quad (7)$$

$$d[R]/dt = k_1[S] + k_2[S][R] - k_3[R]^2 - k_4[R][I], \quad (8)$$

$$d[I]/dt = -k_4[R][I] + k_5[I'], \quad (9)$$

$$d[I']/dt = k_4[R][I] - k_5[I'] - k_6[I']^2. \quad (10)$$

$$[S]_{t=0} = [S]_0, [R]_{t=0} = [R]_0, [I]_{t=0} = [I]_0, [I']_{t=0} = 0.$$

The square brackets indicate the concentration of the corresponding substances.

Further study of the dynamics of the system is reduced to study of the system of four differential equations (7)-(10) and construction of the phase portrait corresponding to this system. In construction of the phase portrait, we first of all will be interested in answering the question: Does a region of the system parameters (rate constants of the chemical reactions and initial conditions) exist within which, for a change in the initial inhibitor concentration $[I]_0$, in the dynamic system there is a bifurcation of the equilibrium state with generation of three states (two of which are stable) from one equilibrium state [8]. Or in other words, the problem involves determining if it is possible to describe the experimentally obtained bistability [7] by only systems (1)-(6), without drawing on additional chemical reaction.

Under the experimental conditions in [7], the substrate concentration did not change over time within the accuracy determined by the sensitivity of the methods used, while the concentrations of the radical and the inhibitor varied by an order of magnitude. This fact allows us in the following to restrict ourselves to the case which is quasisteady-state with respect to $[S]$. Furthermore, considering Eq. (10), describing the kinetics of the variation in the inhibitor radical concentration, we note that in the case of a low concentration $[I']$, the term $k_6[I']^2$ can be neglected as being of the next order of smallness compared with the term $k_5[I']$ describing reduction of the radical to the inhibitor molecule. For high concentrations $[I']$, we also can hypothesize that the rate of reduction of I' is higher than the rate of their bimolecular reaction, since the inhibitor radical (like any radical) is an active particle, tending to be reduced to the molecule. Then system (7)-(10) can be reduced to a second-order system of the following form:

$$d[R']/dt = k_1[S]_0 + k_2[S]_0[R'] - k_3[R']^2 - k_4[R'] [I], \quad (11)$$

$$d[I]/dt = -k_4[R'] [I] + k_5([I]_0 - [I]). \quad (12)$$

Further study of the system of differential equations (11), (12) was done using the standard scheme: determination of the stationary points and determination of the nature of these points, that is, investigation of them for stability. The phase trajectories of system (11), (12) satisfy a differential equation of the form

$$\frac{d[R']}{d[I]} = \frac{k_1[S]_0 + k_2[S]_0[R'] - k_3[R']^2 - k_4[R'] [I]}{-k_4[R'] [I] + k_5([I]_0 - [I])} \quad (13)$$

Thus the position of the stationary points on the phase plane $[I]; [R']$ is described by the coordinates of the points of interaction of the isoclines of Eq. (13), corresponding to the vertical and horizontal tangents to the phase trajectories. The coordinates $[R']$ of the intersection points of the isoclines are a solution to the equation

$$k_1[S]_0 + k_2[S]_0[R'] - k_3[R']^2 = k_4 k_5 [R'] [I]_0 / k_4 [R'] + k_5. \quad (14)$$

Equation (14) as a function of the values of the parameters may have one, two, or three positive roots. In order for bifurcation of the equilibrium state to be observed in the dynamic system, it is necessary that three stationary states appear with a change in the initial conditions in the system. The conditions for the existence of three roots to Eq. (14) (of which two possibly coincide) are defined by the relations:

$$k_1[S]_0 < k_5[I]_0. \quad (15)$$

$$k_1[S]_0 + (k_2[S]_0)^2 / 4k_3 > k_5[I]_0. \quad (16)$$

$$k_2[S]_0 < \frac{k_4}{k_5} (\sqrt{k_5[I]_0} - \sqrt{k_1[S]_0})^2. \quad (17)$$

The next step in solution of the problem involves studying the stationary points of the phase portrait for stability. The stability of the stationary points of system (11), (12) is determined by the stability of the given system, linearized close to the stationary points. Investigation of the linearized system as a function of the parameters shows that of the three stationary points, two are stable nodes while one is a saddle point.

The phase portrait describing system (11), (12) has the form presented in Fig. 1. If there is one stationary point in the system, then it is a stable node. If there are three stationary points in the system, then the middle point is a saddle point while the two extremal points are stable nodes.

Let us consider how, using the phase portrait obtained, we can describe the bistability in the peroxidase-oxidase reaction upon inhibition by radical traps. For a low initial inhibitor concentration $[I]_0$, conditions (15)-(17) are not satisfied. Then there is one stationary stable point in the system with a low value of $[I]^*$ and a high value of $[R']^*$, corresponding to the "no reaction" regime (Fig. 1a), since the rate of the enzymatic reaction is proportional to $[R']$.

With an increase in the initial inhibitor concentration $[I]_0$, the steady-state value of $[I]^*$ increases while $[R']^*$ decreases, and for some value $[I]_0$ three stationary points appear in the system (if the parameters of the system satisfy the conditions (15)-(17)). One of the stationary states in this case is unstable while the other two correspond respectively to the "reaction occurs" regime and the "no reaction" regime (Fig. 1b).

Upon further increase in the initial concentration of the inhibitor, the point corresponding to the initial conditions goes through the separatrix and proves to be in the region of attraction of a stable state of the "no reaction" type. A change in the stable state occurs in the system. Upon further increase in $[I]_0$ one stable stationary state remains in the system, corresponding to the "no reaction" regime (Fig. 1c).

Let us then consider what the numerical values should be for the parameters of a specific chemical reaction in order for conditions (15)-(17) to be satisfied; these conditions are necessary for the existence of bistability in the system. In this case, let us take as the numerical values of the initial concentrations of the substances the values of the concentrations used in experiments on peroxidase-oxidase decomposition of IAA in the presence of the inhibitor caffeic acid [7]: $[S]_0 = 10^{-3}$ M, $[I]_0 = 10^{-6}$ M. The estimates obtained for the chemical reaction rates are: $k_1 < 10^{-8}$ sec $^{-1}$, $k_2 = 2 \cdot 10^3$ sec $^{-1}$, $k_3 < 10^9$ M $^{-1}$ ·sec $^{-1}$, $k_4 = 10^7$ M $^{-1}$ ·sec $^{-1}$, $k_5 < 10^6$ ·sec $^{-1}$. In this case, an upper bound for the constant k_1 was estimated from measurement of the rate of decomposition of the inhibitor in a solution of IAA in the absence of the enzyme, with analysis of the results obtained using a reduced system, including only equations for reactions (1) and (4). The constant k_2 was determined from measurement of the rate of enzymatic decomposition of IAA. The upper limit for k_3 corresponds to the highest possible rate for diffusion-controlled processes. For k_4 , we took the characteristic value of the rate constant for the radical transfer reaction [9]. An upper bound was estimated for the constant k_5 assuming that transfer from an electron donor to

I[•] occurs with diffusion-controlled rates, while the electron donor concentration is equal to the concentration of the substance present in the reaction mixture in the maximum amount, i.e., the concentration of the substrate (10^{-3} M). Conditions (15)-(17) for the existence of two stationary stable points in the system are satisfied if we have $k_5 < 10^{-3} \cdot \text{sec}^{-1}$. Via a comparison of the results obtained with the initial estimate for ($k_5 < 10^6 \cdot \text{sec}^{-1}$), we can see that the model allows us not only to describe the phenomenon of bistability but also to estimate more exactly the rate constant for reduction of the inhibitor radical to the inhibitor molecule, which could not be done by the familiar methods.

Thus in this paper we have constructed a mathematical model describing the experimentally observed phenomenon of bistability in the peroxidase-oxidase reaction upon its inhibition by radical traps. We have determined the region of the parameters of the problem for which the bistability phenomenon occurs within this model.

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