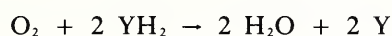


Sensitivity Studies of a Computer Model for the Peroxidase-oxidase Oscillating Reaction

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Introduction

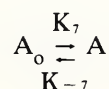
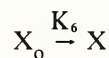
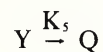
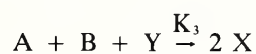
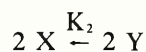
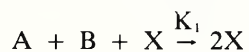
The peroxidase-oxidase enzyme catalyzed reaction is considered to have the general form



where YH_2 is a hydrogen donor such as nicotinamide adenine dinucleotide (NADH). In an experiment performed by Olsen and Degn (3) a continuous flow of NADH was pumped into a reaction mixture containing the peroxidase-oxidase enzyme. A continuous flow of O_2 was supplied by blowing a mixture of nitrogen and oxygen over the surface. When the reaction mixture was stirred, oscillations in the concentrations of NAD (the oxidized product) and O_2 were observed.

Method

A model for the peroxidase-oxidase reaction has been proposed by Olsen and Degn (3). The essential steps in the mechanism are:



where A is $[O_2]$, B is $[NADH]$, and X and Y are intermediate free radicals. The rate constants K_1 - K_8 and initial concentrations A_0 and B_0 were chosen by comparing computer simulations to experiment. The computer simulations involve the numerical solution of

$$\begin{aligned}\frac{dA}{dt} &= -K_1ABX - K_3ABY + K_7A_0 - K_{-7}A \\ \frac{dB}{dt} &= -K_1ABX - K_3ABY + K_8B_0 \\ \frac{dX}{dt} &= -K_1ABX - 2K_2X^2 + 2K_3ABY - K_4X + K_6 \\ \frac{dY}{dt} &= 2K_2X^2 - K_3ABY - K_5Y\end{aligned}\quad [2]$$

When the constants K_1 - K_8 , A_0 and B_0 are chosen appropriately,¹ the solutions to [2] are found to be oscillating functions of time, t .

The validity of this model was tested using sensitivity analysis. Sensitivity analysis provides several different kinds of information about the changes in the solution of a set of differential equations due to changes in the values of its parameters. For this model, it will allow us to determine which rate parameters affect the oscillation's characteristics, such as its period. The sensitivity analysis yields sensitivity coefficients which are gradients of the limit cycle in parameter space. The general form of equation [2] is:

$$dC_i/dt = R_i(C_1, \dots, C_N, \alpha_1, \dots, \alpha_m) \quad i = 1, \dots, N \quad [3]$$

where C_i is the concentration of the species i , R_i is an algebraic function describing the rate of change of C_i due to chemical reactions, and $\alpha_1, \dots, \alpha_m$ are parameters such as rate constants. The solution to equation [3] for an oscillatory mechanism may be written as

$$C_i(t) = \sum_{n=0}^{\infty} \left[a_n^i \cos \frac{2n\pi t}{\tau} + b_n^i \sin \frac{2n\pi t}{\tau} \right] \quad [4]$$

where τ is the period of the oscillation, and τ , a_n^i and b_n^i are all functions of the system parameters.

Differentiating equation [4] with respect to a parameter α_j gives the following expression for the sensitivity coefficients:

$$\begin{aligned}\frac{\partial C_i}{\partial \alpha_j}(t) &= \frac{2n\pi t}{\tau^2} \frac{\partial \tau}{\partial \alpha_j} \sum_{n=0}^{\infty} \left[na_n^i \sin \frac{2n\pi t}{\tau} - nb_n^i \cos \frac{2n\pi t}{\tau} \right] \\ &+ \sum_{n=0}^{\infty} \left[\frac{\partial a_n^i}{\partial \alpha_j} \cos \frac{2n\pi t}{\tau} + \frac{\partial b_n^i}{\partial \alpha_j} \sin \frac{2n\pi t}{\tau} \right]\end{aligned}\quad [5]$$

Larter, Rabitz and Kramer (2) have shown that [5] reduces to:

$$\frac{\partial C_i}{\partial \alpha_j} = \frac{-t}{\tau} \frac{\partial \tau}{\partial \alpha_j} \frac{\partial C_i}{\partial t} + \left(\frac{\partial C_i}{\partial \alpha_j} \right)_{\tau} (t) \quad [6]$$

where the subscript τ on the second term indicates that the period, τ , is constant for that term. The first term consists of a linear function of time [$t/\tau(\partial\tau/\partial\alpha_j)$] multiplied by a periodic function ($\partial C_i/\partial t$). This equation has also been derived by Tomovic and Vukobratovic (5). Unless $\partial\tau/\partial\alpha_j = 0$, $\partial C_i/\partial\alpha_j$ will grow as an undamped oscillation as t get large. This means that $\partial C_i/\partial\alpha_j$ gives us physically meaningful result since it becomes infinitely large as t progresses.

In contrast, the modified sensitivity coefficient, $(\partial C_i/\partial\alpha_j)\tau(t)$, does give physically meaningful results since it is well-defined and periodic for a differential equation system that is structurally stable to perturbation of α_j . Since the second term is purely periodic, the values will be consistent as t progresses. In order to solve for this second term, the period sensitivity, $\partial\tau/\partial\alpha_j$ must be determined. First, the sensitivity coefficients in equation [6] are integrated from t to $t + \tau$ and evaluated at two different times, t , i.e., $t = t_1$ and $t = t_2$, and then these results are subtracted giving,

$$\frac{\partial \tau / \partial \alpha_j}{C_i(t_1) - C_i(t_2)} = \frac{\int_{t_2}^{t_2 + \tau} dt' \frac{\partial C_i}{\partial \alpha_j} - \int_{t_1}^{t_1 + \tau} dt' \frac{\partial C_i}{\partial \alpha_j}}{C_i(t_1) - C_i(t_2)} \quad [7]$$

Numerical instabilities encountered by Edelson and Thomas (1) with a similar calculation were avoided by eliminating choices where the concentration at t_1 and t_2 were approximately equivalent. Now the modified sensitivity coefficient can be found because $\partial C_i/\partial t$ have already been calculated, and t/τ can be found by measuring the period, τ , with t already known.

Discussion

The peroxidase-oxidase reaction is an oscillatory reaction with a limit cycle solution, so we used the above equations to obtain a modified sensitivity coefficient. In order to oscillate, a reaction must operate far from equilibrium; this system satisfies this requirement because substrates, A and B, are being continually added. Also, some product of at step in the reaction sequence must exert an influence on its own rate of formation; the intermediate free radicals, X and Y, are produced autocatalytically and thus do influence their own formation.

These two facts seem to indicate the importance of the rate constants for the addition of the substrates and the autocatalytic steps. We have found this to be true for the rate constant, K_8 , which is the rate constant for the addition of NADH. For most values of K_8 , the solution is not a true periodic function, and the sensitivity coefficients do not show unbounded growth with time. These quasi-periodic regions give some unusual results which appear to include some chaotic regions; Olsen (4) has also noted these chaotic regions. We intend to investigate this further at a later date. The solution is truly periodic when K_8 is equal to 0.4939; for the periodic solution, the sensitivity coefficients had the expected time behavior given by equation [6].

Note

1. The values used were $K_1 = 8.5 \times 10^{-2}$, $K_2 = 1.25 \times 10^3$, $K_3 = 4.8675 \times 10^{-2}$, $K_4 = 20.0$, $K_5 = 2.0$, $K_6 = 1.0 \times 10^{-3}$, $K_7 = .94$, $K_{-7} = .1175$, $K_8 = .4939$, $A_0 = 3.84$, $B_0 = 33.73$, $X_0 = 1.1 \times 10^{-4}$, $Y_0 = 3.62 \times 10^{-6}$.

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