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THEORY OF OSCILLATIONS IN PEROXIDASE CATALYZED OXIDATION REACTIONS IN OPEN SYSTEM

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SUMMARY

1. The applicability of the (1910) LOTKA[6] model to the horseradish peroxidase (donor: H_2O_2 oxidoreductase, EC 1.11.1.7) catalyzed oxidation reactions in an open system is investigated.

2. The input of reactant by diffusion in stead of a constant input flux does not prevent oscillations of low damping. The gain of 3 in the branched chain reaction in the peroxidase system in stead of 2 in the Lotka model causes an increased damping, but the low damping observed in the experiments is still possible.

3. The combination of linear branching and quadratic termination, which is commonly believed to prevail in the peroxidase system, leads to a damping which is much higher than observed in the experiments.

4. A critical lower concentration of donor is known to exist below which the peroxidase catalyzed oxidation does not take place. This phenomenon is predicted by models of the Lotka type where the branching and the termination are both linear or both quadratic. It does not occur if the branching is linear and the termination is quadratic.

5. Compound III is not assumed to play any significant role in relation to the origin of the damped oscillation.

INTRODUCTION

YAMAZAKI *et al.*[1] and YAMAZAKI AND YOKOTA[2] have reported damped oscillations in the rate of the horseradish peroxidase (donor: H_2O_2 oxidoreductase, EC 1.11.1.7) catalyzed aerobic oxidation of NADH and NADPH in a system open to O_2. Later it was found by DEGN[3,4] that the horseradish peroxidase catalyzed oxidation of dihydroxyfumaric acid and indole-3-acetic acid also exhibit damped oscillations in the open system. The mechanism of the oscillation in the NADH oxidation was investigated by YAMAZAKI AND YOKOTA[2] who emphasized the conversion of the enzyme to Compound III. Having found that a high degree of conversion to Compound III was associated with a high rate of NADH oxidation they postulated that Compound III is a regulator of the enzyme activity and as such responsible for the oscillations.

The view held by YAMAZAKI *et al.*[1] on the importance of Compound III in the

mechanism of the damped oscillation is not supported by the experimental findings obtained by DEGN[4]. Firstly, the conversion of the enzyme to Compound III was found to result in a decrease of the rate of oxidation of NADH (ref. 5) and not an increase as claimed by YAMAZAKI *et al.*[1]. Secondly, the Compound III kinetics in the oxidation of NADH, dihydroxyfumaric acid and indole-3-acetic acid was found to be widely different[4].

One common property of the three horseradish peroxidase catalyzed oxidation reactions mentioned above is the autocatalytic character of the reaction[4]. It is known from LOTKA's[6] early theoretical investigations that damped oscillations may occur in an open system where an autocatalytic reaction takes place. We suggest that the observed damped oscillations in the peroxidase system are examples of the type of oscillations predicted by Lotka. It is the purpose of the present work to elucidate the applicability of the Lotka model to the peroxidase system.

THEORETICAL

Description of the Lotka model

An autocatalytic reaction

$$B + X \xrightarrow{k_1} 2X \qquad (1)$$

is assumed to take place in a reaction chamber where the reactant, B, is fed in at a constant rate and the reaction product is formed from the autocatalytic intermediate, X, in a first order reaction

$$X \xrightarrow{k_2} \text{product} \qquad (2)$$

Lotka found that for certain values of the parameters the concentrations of B and X will exhibit damped oscillations in their approach to stationary values. An example of a digital computer solution of the Lotka model was given previously[7].

In the language of chain reaction kinetics, Reaction 1 is a chain propagating as well as a chain branching reaction. Because the rate of chain branching is proportional to the concentration of the chain propagating species, X, the reaction is called a linearly branched chain reaction. Reaction 2 is the chain terminating reaction. The rate of chain termination is also a linear function of the concentration of X.

Recently different modified forms of the Lotka model, where the order of the chain branching or chain termination was changed, have been investigated. It was found that if the chain branching is of an order higher than one and the chain termination is of the order one (*i.e.* linear) a sustained oscillation may occur[7,8]. If the chain branching is of the order one and the chain termination is of an order lower than one, a sustained oscillation may also occur[8,9]. The two different modified forms of the Lotka model found to give sustained oscillations were proposed to explain the sustained oscillations observed in the H_2O_2–KIO_3 reaction in dilute H_2SO_4 (ref. 10) and in the glycolytic reactions[11], respectively.

Comparison of the peroxidase system with the Lotka model

1. *Input of reactant.* The reaction which was studied experimentally is a two substrate enzyme reaction. One substrate, the donor, is added initially. Its concen-

tration is, therefore, decreasing during the reaction. This effect can be disregarded when a large excess of donor is used. The other substrate, O_2, enters the reaction solution by diffusion from the gas phase above the solution. Therefore, the rate of transport of O_2 into the solution is not constant as required by the Lotka model but depends on the O_2 concentrations in the gas and the solution according to the equation

$$v_t = K([O_2]_e - [O_2]) \quad (3)$$

where v_t is the rate of transport of O_2, $[O_2]_e$ is the O_2 concentration in the solution when it is in equilibrium with the gas phase, and K is a diffusional conductivity constant.

2. Chain branching. The horseradish peroxidase catalyzed aerobic oxidation reactions are branched chain reactions whose general reaction mechanism is given in Fig. 1. The rate of consumption of O_2 is a linear function of the concentration of the chain propagating species $YH^{\cdot}$ and there is no positive interaction between chains as discussed by SEMENOV (ref. 12, p. 83). The reaction is, therefore, a linearly branched chain reaction. In the Lotka model 2 molecules of X are formed at the expense of 1 X. In the peroxidase reaction according to the mechanism in Fig. 1 3 molecules of $YH^{\cdot}$ are formed at the expense of 1 $YH^{\cdot}$.

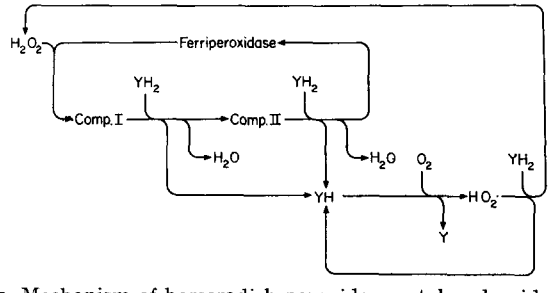

Fig. 1. Mechanism of horseradish peroxidase catalyzed oxidation of donor (YH_2) (ref. 4).

3. Chain termination. Chain termination in the peroxidase reaction is believed to be caused by the dismutation of $YH^{\cdot}$ according to the reaction

$$2YH^{\cdot} \rightarrow Y + YH_2 \quad (4)$$

The dismutation of $HO_2^{\cdot}$

$$2HO_2^{\cdot} \rightarrow H_2O_2 + O_2 \quad (5)$$

is not a chain termination reaction because H_2O_2 is equivalent to 2 free radicals. Another possible chain termination reaction is

$$\text{Ferriperoxidase} + HO_2^{\cdot} \rightarrow \text{Compound III} \quad (6)$$

This reaction would cause a linear termination at the same time as the enzyme is converted to Compound III. In the oxidation of NADH a stationary concentration of Compound III may occur as a result of Reaction 6 and the decomposition of Compound III by the reaction

$$\text{Compound III} + NAD^{\cdot} + H^+ \rightarrow \text{Ferriperoxidase} + H_2O_2 + NAD^+ \quad (7)$$

Because 1 H_2O_2 is equivalent to 2 free radicals the formation and decomposition of Compound III according to Reactions 6 and 7 does not result in chain termination.

Mathematical analysis

The complete reaction system described above is a many-body problem which cannot be solved analytically. The present mathematical treatment is based on the assumption that valid conclusions about the reaction system can be drawn from the analysis of a two-body model which has the same general properties as the reaction system. In other words we assume that the reaction

$$B + X \rightarrow 3X \tag{8}$$

where $B = O_2$ and $X = YH^*$, describes the essential kinetic properties of the reaction scheme in Fig. 1. Even at this degree of simplification a model containing all the aforementioned deviations from the Lotka model is too difficult to analyse. We shall, therefore, examine the effect of these deviations on the Lotka model one by one. This procedure is believed to give a reasonably good indication whether or not the complete reaction mechanism can produce damped oscillations. However, a final conclusion on this question can only be obtained by analysis or computer solution of the complete system.

The time evolution of a general two dimensional system is determined by the equations

$$\frac{dx}{dt} = P(x,y) \tag{9}$$

$$\frac{dy}{dt} = Q(x,y) \tag{10}$$

The singular points, *i.e.* stationary states, (x_i,y_i) of the system are determined by the intersection of the curves

$$P(x,y) = 0 \tag{11}$$

$$Q(x,y) = 0 \tag{12}$$

The Taylor expansions of Eqn. 9 and Eqn. 10 at a singular point are of the form

$$\frac{dx}{dt} = P_x x + P_y y + \text{higher order terms} \tag{13}$$

$$\frac{dy}{dt} = Q_x x + Q_y y + \text{higher order terms} \tag{14}$$

where

$$P_x = \frac{\delta P(x_i,y_i)}{\delta x} \quad etc.$$

BENDIXSON[13] has shown that the nature of the phase trajectories near a singular point is determined by the linear terms of Eqn. 13 and Eqn. 14, provided that the coefficients are not all zero and the determinant of the coefficient matrix

$$J = P_x Q_y - Q_x P_y \tag{15}$$

is also different from zero. The roots λ_1 and λ_2 of the characteristic equation[14]

$$\begin{vmatrix} P_x - \lambda & P_y \\ Q_x & Q_y - \lambda \end{vmatrix} = 0 \tag{16}$$

may be written

$$\lambda_{1,2} = \frac{1}{2}(R \pm \sqrt{\Delta}) \tag{17}$$

where

$$R = P_x + Q_y \tag{18}$$

and

$$\Delta = R^2 - 4J \tag{19}$$

With the help of J, R and Δ as defined by Eqns. 15, 18 and 19 the character of all singular points satisfying the conditions mentioned above can be determined[13]. A summary of the possible cases is given in Table I. If the singular point is a node or a saddle point, oscillations do not occur. If the singular point is a stable focus, the phase trajectories are spiralling towards the singular point which means that a damped oscillation occurs. If the singular point is an unstable focus, the phase trajectories are spiralling away from the singular point. In this case a limit cycle may exist and the system is capable of sustained oscillations. However, the existence of a limit cycle is not a necessary consequence of the existence of an unstable focus. If the singular point is a center it is surrounded by an infinite number of closed trajectories[15]. The system

TABLE I

$J > 0$	$R > 0$	$\Delta \geqq 0$	Unstable node
$J > 0$	$R > 0$	$\Delta < 0$	Unstable focus
$J > 0$	$R = 0$	$\Delta < 0$	Center
$J > 0$	$R < 0$	$\Delta \geqq 0$	Stable node
$J > 0$	$R < 0$	$\Delta < 0$	Stable focus
$J < 0$	$R \gtreqless 0$	$\Delta > 0$	Saddle point

exhibits sustained oscillations whose amplitude depends on the initial conditions.

As an example let us first analyse Lotka's original mechanism.

$$\begin{aligned} &\xrightarrow{\gamma} \mathrm{B} \\ \mathrm{B} + \mathrm{X} &\xrightarrow{k_1} 2\mathrm{X} \\ \mathrm{X} &\xrightarrow{k_2} \text{product} \end{aligned} \tag{20}$$

where γ is the constant rate of input of B. The corresponding differential equations are

$$\frac{\mathrm{d}b}{\mathrm{d}t} = \gamma - k_1 bx \tag{21}$$

$$\frac{\mathrm{d}x}{\mathrm{d}t} = k_1 bx - k_2 x \tag{22}$$

There is one singular point located at

$$x_1 = \frac{\gamma}{k_2}; \qquad b_1 = \frac{k_2}{k_1} \tag{23}$$

Evaluating the partial derivatives and Eqns. 15, 18 and 19 we find

$$J = k_1\gamma \tag{24}$$

$$R = -\frac{\gamma k_1}{k_2} \tag{25}$$

$$\Delta = \frac{k_1^2\gamma}{k_2^2}\left(\gamma - \frac{4k_2^2}{k_1}\right) \tag{26}$$

Eqns. 24 and 25 determine the singular point as a stable node or focus. From Eqn. 26 is found that the singular point is a stable focus, *i.e.* damped oscillations may occur for

$$\gamma < \frac{4k_2^2}{k_1} \tag{27}$$

We shall now analyse models which are derived from the Lotka model by the introduction of the modifications discussed in the previous section.

1. Input of reactant. Replacement of the constant input flux in the Lotka model by the diffusion mechanism given by Eqn. 3 gives rise to the following set of differential equations

$$\frac{db}{dt} = K(a - b) - k_1bx \tag{28}$$

$$\frac{dx}{dt} = k_1bx - k_2x \tag{29}$$

where b represents the O_2 concentration in the solution, $[O_2]$, and a represents the equilibrium concentration, $[O_2]_e$. For $a < k_2/k_1$ the system has one singular point located at

$$x_1 = 0; \qquad b_1 = a \tag{30}$$

Obviously, oscillations around this point cannot take place because the concentration of X cannot pass through negative values.

For $a > k_2/k_1$ there are two singular points one of which has coordinates given by Eqn. 30. The second singular point is located at

$$x_2 = \frac{K}{k_2}\left(a - \frac{k_2}{k_1}\right); \qquad b_2 = \frac{k_2}{k_1} \tag{31}$$

Evaluation of the functions in Eqns. 15, 18 and 19 yields

$$J = Kk_1\left(a - \frac{k_2}{k_1}\right) \tag{32}$$

$$R = -a\frac{Kk_1}{k_2} \tag{33}$$

$$\Delta = a^2 - 4a\frac{k_2^2}{Kk_1} + 4\frac{k_2^3}{Kk_1^2} \tag{34}$$

By comparison with Table I it is found that the singular point is a stable focus, *i.e.* damped oscillations occur for

$$\frac{2k_2^2}{Kk_1}\left(1-\sqrt{1-\frac{K}{k_2}}\right)<a<\frac{2k_2^2}{Kk_1}\left(1+\sqrt{1-\frac{K}{k_2}}\right) \tag{35}$$

For values of a outside these limits the singular point is a stable node, *i.e.* oscillations do not occur.

2. Chain branching. If 3 in stead of 2 molecules of X are produced at the expense of 1 molecule of X we obtain the following differential equations

$$\frac{db}{dt}=\gamma-k_1bx \tag{36}$$

$$\frac{dx}{dt}=2k_1bx-k_2x \tag{37}$$

As before γ denotes the constant rate of input of B. This system has one singular point located at

$$x_1=\frac{2\gamma}{k_2};\qquad b_1=\frac{k_2}{2k_1} \tag{38}$$

Evaluating the functions given by Eqns. 15, 18 and 19 yields

$$J=2\gamma k_1 \tag{39}$$

$$R=-\frac{2\gamma k_1}{k_2} \tag{40}$$

$$\Delta=\frac{4\gamma k_1^2}{k_2^2}\left(\gamma-\frac{2k_2^2}{k_1}\right) \tag{41}$$

By comparison with Table I it is found that for

$$\gamma<\frac{2k_2^2}{k_1} \tag{42}$$

the singular point is a stable focus and damped oscillations exist.

3. Chain termination. Quadratic in stead of linear termination in the Lotka model gives the following differential equations

$$\frac{db}{dt}=\gamma-k_1bx \tag{43}$$

$$\frac{dx}{dt}=k_1bx-2k_2x^2 \tag{44}$$

For this system there is one singular point located at

$$x_1=\sqrt{\frac{\gamma}{2k_2}};\qquad b_1=\sqrt{\frac{2\gamma k_2}{k_1}} \tag{45}$$

Evaluation of Eqns. 15, 18 and 19 yields

$$J=2\gamma k_1 \tag{46}$$

$$R=-\frac{\gamma(k_1+2k_2)}{\sqrt{2\gamma k_2}} \tag{47}$$

$$A = \gamma \frac{k_1^2 + 4k_2^2 - 12k_1k_2}{2k_2} \tag{48}$$

By comparing with Table I we find that the singular point is a stable focus, *i.e.* damped oscillations may occur for

$$\frac{3 - \sqrt{8}}{2} < \frac{k_2}{k_1} < \frac{3 + \sqrt{8}}{2} \tag{49}$$

For k_2/k_1 outside these limits the singular point is a stable node and oscillations do not occur.

Damping

Neither of the modified forms of the Lotka model treated above has lost the ability to produce damped oscillations for certain values of the parameters. However, it remains to be found out whether the damping of the oscillations in the modified models is compatible with the damping observed experimentally. The damping is defined as the ratio between the amplitudes, b' and b'', of two consecutive periods. Under favorable conditions the damping observed in the oscillations in the peroxidase system is about 3.

In the neighborhood of the singular point the damping is

$$\frac{b'}{b''} = \exp \frac{2\pi R}{\omega} \tag{50}$$

where R is the function defined by Eqn. 18 and ω is the angular velocity[16]. The latter can be expressed in terms of the earlier defined functions as

$$\omega = \sqrt{R^2 - 4J} \tag{51}$$

Substituting Eqn. 51 into Eqn. 50 yields

$$\frac{b'}{b''} = \exp \frac{2\pi}{\sqrt{\frac{4J}{R^2} - 1}} \tag{52}$$

The damping in the original Lotka model is found by substituting Eqns. 24 and 25 into Eqn. 52. We obtain

$$\frac{b'}{b''} = \exp \frac{2\pi}{\sqrt{\frac{4k_2^2}{\gamma k_1} - 1}} \tag{53}$$

By inspection of Eqn. 53 it is seen that the damping goes monotonously from 1 to ∞ as γ goes through the range of values where oscillations exist, namely from 0 to $4k_2^2/k_1$.

In the modified Lotka model where the reactant enters by diffusion the damping is

$$\frac{b'}{b''} = \exp \frac{2\pi}{\sqrt{\frac{4k_2^2(a - k_2/k_1)}{a^2 K k_1} - 1}} \tag{54}$$

In the experiments where the damped oscillation was observed the equilibrium concen-

tration of O_2 in the gas was much larger than the final stationary concentration of O_2 in the reaction solution. This experimental situation corresponds to $a \gg k_2/k_1$ which reduces Eqn. 54 to

$$\frac{b'}{b''} = \exp \frac{2\pi}{\sqrt{\frac{4k_2^2}{aKk_1} - 1}} \tag{55}$$

Because aK is the rate of input of reactant when $a \gg k_2/k_1$ it follows that Eqn. 55 is identical with Eqn. 53. It is concluded that for $a \gg k_2/k_1$ the damping in the model where the reactant enters by diffusion does not differ significantly from the damping in the original Lotka model.

In the modified Lotka model where 3 in stead of 2 X are formed at the expense of 1 X the damping is

$$\frac{b'}{b''} = \exp \frac{2\pi}{\sqrt{\frac{2k_2^2}{\gamma k_1} - 1}} \tag{56}$$

It is seen from Eqn. 56 that, as in the original Lotka model, the damping goes from 1 to ∞ as γ goes through the values where oscillations exist, namely from 0 to $2k_2^2/k_1$. For one particular set of values of the parameters the damping is higher in this modified form of the Lotka model than in the original model.

In the modified Lotka model where quadratic termination is introduced in stead of linear termination the damping is

$$\frac{b'}{b''} = \exp \frac{2\pi}{\sqrt{\frac{16k_2/k_1}{(1 + 2k_2/k_1)^2} - 1}} \tag{57}$$

This function has a minimum at $k_2/k_1 = 1/2$ which is inside the range of values where oscillations occur. At the minimum the damping is

$$\frac{b'}{b''} = \exp 2\pi \approx 536 \tag{58}$$

It is observed that the minimal damping in the quadratic termination model is much higher than the damping observed in the experimental oscillations.

DISCUSSION

The horseradish peroxidase catalyzed aerobic oxidation in a system open to O_2 has three main features which are also found in the Lotka model, namely a continuous input of reactant, an autocatalytic reaction converting the reactant to an intermediate and, finally, a reaction converting the autocatalytic intermediate to an inert product. However, some differences in details exist between the peroxidase system and the Lotka model. The present work was undertaken in order to establish whether these deviations are of significance with regard to the damped oscillations. The analysis of different modified forms of the Lotka model, where the presumably most significant deviations of the experimental system from the original model were

introduced, revealed that in all cases the modified model was capable of damped oscillations. From an evaluation of the damping of the different models it was found that two of the deviations, namely the diffusion input of reactant and the gain of 3 instead of 2 molecules of active intermediate, both allow a low damping as observed in the experimental oscillations. However, the model having linear branching and quadratic termination was found to be incompatible with the experimental results because the minimal damping in this model is much higher than the damping observed in the experiments.

This finding may indicate that the termination in the peroxidase catalyzed oxidation reaction is linear and not, as it is commonly assumed, quadratic according to Reaction 4. An alternative, although less likely possibility is that both the branching and the termination are quadratic. As can be shown by analysis in the same way as before, this combination will also allow a damped oscillation whose damping can have any value between 1 and ∞.

From the work of YOKOTA AND YAMAZAKI[17] it is known that the horseradish peroxidase catalyzed oxidation of NADH does not take place to a measurable degree when the NADH concentration is below a certain limit. The same phenomenon is known from many other branched chain reactions and it is predicted by the Lotka model. The differential Eqn. 22 can be written

$$\frac{dx}{dt} = (k_1 b - k_2)x \tag{59}$$

The coefficient of x is the branching factor. When this is positive the reaction accelerates and when it is negative the reaction stops. It follows that no measurable reaction takes place if $b < k_2/k_1$ (ref. 12, p. 21). A similar result is obtained if both the branching and the termination are quadratic. However, if the branching is linear and the termination is quadratic there does not exist any critical lower limit of b below which the reaction does not occur. The existence of a critical lower limit of NADH concentration in the horseradish peroxidase catalyzed oxidation of NADH, therefore, indicates that the combination of linear branching and quadratic termination does not prevail in this reaction. This conclusion was also obtained from the evaluation of the damping.

In their treatment of the mechanism of the oscillation YAMAZAKI AND YOKOTA[2] discuss at some length the gain in the chain reaction. Unfortunately they do not define the word gain, which makes their argument difficult to follow. The gain in a branched chain reaction, not to be confused with the branching factor, may be defined as the number of molecules of active intermediate which are formed at the expense of one such molecule in one cycle of the reaction mechanism. The gain according to this definition is 2 in the Lotka mechanism and it is 3 in the horseradish peroxidase catalyzed oxidation reaction according to Fig. 1. Intuitively it would be expected that a high gain favors the oscillation. However, the analysis shows that there is no such effect. An increase of the gain from 2 to 3 reduces to one half the range of values of the input flux where oscillations occur, and at any particular set of values of the rate constants an increase of the gain from 2 to 3 causes an increased damping of the oscillation.

Conclusion

The damped oscillations observed in the horseradish peroxidase catalyzed aerobic oxidation reactions in open system can be explained by a modified Lotka

model having diffusion input of the reactant and an autocatalytic reaction with linear branching and termination and a gain of 3. The common assumption of linear branching and quadratic termination in the peroxidase reaction is incompatible with the low damping observed in the experiments as well as with the existence of a lower critical limit of donor concentration below which the reaction does not take place. No significance is ascribed to Compound III in relation to the origin of the damped oscillations.

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