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COMPUTER SIMULATION OF SUSTAINED OSCILLATIONS IN PEROXIDASE-OXIDASE REACTION

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A system of differential equations of second order exhibiting transitional behaviour and sustained oscillations has been obtained for a complete scheme of the peroxidase-oxidase reaction. The concentrations of hydrogen peroxide and of hydrogen donor radicals are slow variables of the system. The most essential reactions responsible for oscillations have been selected. Analysis of the system in phase plane and in parameter space has been carried out. The dependence of oscillation period and amplitude on the parameter values has been investigated.

1. Introduction

Oscillations in the peroxidase-oxidase reaction were first observed in 1965 by Yamazaki et al. [1] in a system open to oxygen. Later this reaction was investigated by Yamazaki and Yokota [2,3] and Degn [4]. Degn and Mayer [5] were the first to simulate these oscillations and further these studies were continued in refs. 6 and 7. These mathematical models were created by modification of models of already known oscillatory reactions whose dynamic behaviour is similar to that observed in the peroxidase-oxidase reaction [5–7]. A simulation of the peroxidase-oxidase reaction based on its detailed scheme was carried out in refs. 8 and 11. These models involved many (8–9) kinetic equations. A system of differential equations of such high order cannot be investigated analytically. However, to describe all types of kinetic behaviour observed experimentally, a system of two or three dynamic equations is sufficient, i.e., the number of essential variables in the system of high order can be much smaller than that of all variables. Hence, the model may be reduced to an equivalent system of second or third order which has the same general properties as the reaction system and for which a complete qualitative analysis can be carried out. In the present work we have reduced the eighth order system, based on a scheme of the peroxidase-oxidase reaction containing nine elementary steps, to a model of two differential equations. This simple model describes the transitional behaviour and sustained oscillations in the reaction. The oscillatory regions for the model were found in the parameter planes: (rate of oxygen infusion, peroxidase concentration), (rate of oxygen infusion, hydrogen donor concentration). Analysis of the dependence of the oscillation period on the parameters of the system has shown that the period is mainly determined by the reactions with the participation of coIII.

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2. The model

The following scheme was used for the peroxidase-oxidase reaction [11]:

$$
\begin{aligned}
&\mathrm{Per}^{3+} + \mathrm{H_2O_2} && \xrightarrow{k_1'} && \mathrm{coI} \\
&\mathrm{coI} + \mathrm{YH} && \xrightarrow{k_2'} && \mathrm{coII} + \mathrm{Y}^{\cdot} \\
&\mathrm{coII} + \mathrm{YH} && \xrightarrow{k_3'} && \mathrm{Per}^{3+} + \mathrm{Y} \\
&\mathrm{O_2} + \mathrm{Y}^{\cdot} && \xrightarrow{k_4'} && \mathrm{O_2^-} + \mathrm{Y} \\
&\mathrm{O_2^-} + \mathrm{YH} && \xrightarrow{k_5'} && \mathrm{Y} + \mathrm{H_2O_2} \\
&\mathrm{Per}^{3+} + \mathrm{O_2^-} && \xrightarrow{k_7'} && \mathrm{coIII} \\
&\mathrm{coIII} + \mathrm{Y} && \xrightarrow{k_8'} && \mathrm{coI} + \mathrm{Y} \\
&\xrightarrow{G'} \mathrm{O_2} && && \mathrm{Y}^{\cdot} \xrightarrow{k_6'}
\end{aligned}
$$

where coI–coIII are compounds I–III of peroxidase. respectively. Per^{3+} the initial form of peroxidase. $\mathrm{Y}^{\cdot}$ the substrate free radical, YH the hydrogen donor and. $\mathrm{O_2^-}$ the superoxide anion radical.

The interrelationships of these reactions are well illustrated in fig. 1. In the present scheme (as compared to that of Yamazaki) the following reductions are made: coIII formation occurs via a single pathway; the reactions of coIII monomolecular decomposition, ferroperoxidase (Per^{2+}) formation and superoxide anion radical termination are omitted; quadratic termination affected by dismutation of $\mathrm{Y}^{\cdot}$ is replaced by a linear one. We also assume the concentration of hydrogen donor to be constant in the course of reaction. Let us introduce dimensionless time and variables:

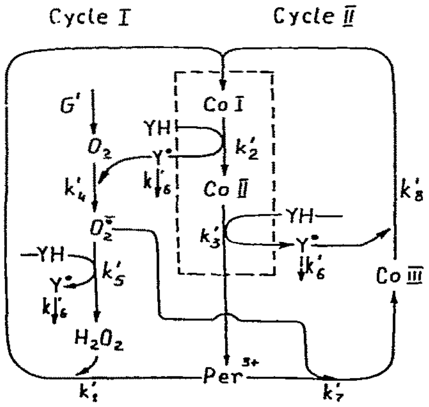

Fig. 1. The scheme of the peroxidase-oxidase reaction. The two connected cycles are marked. Reactions common to both cycles are enclosed by the dashed lines.

$$\tau = k_1' E_0 t; \quad z = \frac{[\mathrm{Per}^{3+}]}{E_0}; \quad x_1 = \frac{[\mathrm{coI}]}{E_0}; \quad \dot{x}_2 = \frac{[\mathrm{coII}]}{E_0};$$

$$x_3 = \frac{[\mathrm{coIII}]}{E_0}$$

$$W = [\mathrm{O_2}]_0 + [\mathrm{H_2O_2}]_0; \quad x_4 = \frac{[\mathrm{H_2O_2}]}{W}; \quad x_5 = \frac{[\mathrm{O_2}]}{W};$$

$$x_6 = \frac{[\mathrm{Y}^{\cdot}]}{W}; \quad x_7 = \frac{[\mathrm{O_2^-}]}{W}$$

$$\epsilon_1 = \frac{E_0}{[\mathrm{YH}]_0}; \quad \epsilon_2 = \frac{W}{[\mathrm{YH}]_0}; \quad k_i = \frac{k_i'}{k_1'}; \quad S = \frac{[\mathrm{O_2}]_0}{W};$$

$$k_6 = \frac{k_6'}{k_1' E_0}; \quad G = \frac{G'}{k_1' E_0}$$

The kinetic equations in dimensionless form are:

$$
\begin{aligned}
z &= 1 - x_1 - x_2 - x_3 \\
\dot{x}_1 &= \frac{1}{\epsilon_1}(\epsilon_2 z x_4 - k_2 x_1 + k_8 \epsilon_2 x_3 x_6) \\
\dot{x}_2 &= \frac{1}{\epsilon_1}(k_2 x_1 - k_3 x_2) \\
\dot{x}_3 &= \frac{\epsilon_2}{\epsilon_1}(k_7 z x_7 - k_8 x_3 x_6) \\
\dot{x}_4 &= -z x_4 + \frac{k_5}{\epsilon_1} x_7 \\
\dot{x}_5 &= G(S - x_5) - \frac{\epsilon_2}{\epsilon_1} k_4 x_5 x_6 \\
\dot{x}_6 &= \frac{1}{\epsilon_2}(k_2 x_1 + k_3 x_2) - \frac{\epsilon_2}{\epsilon_1} k_4 x_5 x_6 \\
&\quad + \frac{k_5}{\epsilon_1} x_7 - k_8 x_3 x_6 - k_6 x_6 \\
\dot{x}_7 &= \frac{1}{\epsilon_1}(k_4 \epsilon_2 x_5 x_6 - k_5 x_7) - k_7 z x_7
\end{aligned}
\tag{1}
$$

In our case $\epsilon_1 = 10^{-2} \ll 1$, so x_1, x_2 and x_3 are the fast variables. Supposing that $k_7 \epsilon_1 \approx 1$, x_7 is also a fast variable. We can replace differential equations for fast variables by algebraic ones obtained from corresponding differential equations by equating their right-hand sides to zero. Then slow motions in the system are described by the system of third order:

$$x_4 = -zx_4 + \frac{k_5}{\epsilon_1}x_7$$

$$x_5 = G(S - x_5) - \frac{\epsilon_2 k_4}{\epsilon_1}x_5 x_6 \tag{2}$$

$$x_6 = 2zx_4 - k_6 x_6$$

To simplify further analysis we have considered system 2 under the condition $G(S - x_5) = (\epsilon_2 k_4/\epsilon_1)x_5 x_6$. This means that the oxygen concentration is supposed to change in a quasi-stationary manner at the characteristic times of the 'slow subsystem'. The validity of this assumption can be verified by comparison of the calculated and experimental results. Finally, we obtain the planar system:

$$\dot{x}_4 = -zx_4 + \frac{k_5}{\epsilon_1}x_7$$

$$\dot{x}_6 = 2zx_4 - k_6 x_6 \tag{3}$$

where x_7 is the positive root of the quadratic equation:

$$Ax_7^2 + Bx_7 + C = 0$$

$$A = \frac{k_5}{\epsilon_1}V; \quad B = \frac{k_5}{\epsilon_1}U + k_7 - qx_5x_6V; \quad C = -qx_5x_6U;$$

$$q = \frac{\epsilon_2 k_4}{\epsilon_1}$$

$$V = \epsilon_2 k_7\left(\frac{1}{k_2} + \frac{1}{k_3}\right) + \frac{k_7}{k_8 x_6}; \quad U = 1 + \epsilon_2 x_4\left(\frac{1}{k_2} + \frac{1}{k_3}\right);$$

$$z = \frac{1}{U + x_7 V}$$

If $k_5U/k_7\epsilon_1 \ll 1$ and assuming that $\epsilon_2 x_4(1/k_2 + 1/k_3) \ll 1$, we may substitute x_7 by an approximate expression:

$$x_7 = m\frac{x_6}{x_6 + g_1}\cdot\left(\frac{(h - x_6)(f_1 - f_2)}{x_6 + g_2} + f_3\right)$$

$$m = \begin{cases} 1 \text{ if } x_6 \leqslant h \\ 0 \text{ if } x_6 > h \end{cases}; \quad f_1 > f_2$$

$$h = \frac{G\left(\frac{S}{k_8} - \frac{\epsilon_1}{\epsilon_2 k_4}\right)}{1 - GS\epsilon_2\left(\frac{1}{k_2} + \frac{1}{k_3}\right)}; \quad f_1 = \frac{1}{k_5\epsilon_2\left(\frac{1}{k_2} + \frac{1}{k_3}\right)};$$

$$f_2 = \frac{GS\epsilon_1}{k_5}; \quad g_1 = \frac{G\epsilon_1}{\epsilon_2 k_4}$$

$$g_2 = \frac{1}{k_8\epsilon_2\left(\frac{1}{k_2} + \frac{1}{k_3}\right)}; \quad f_3 = \frac{GS}{k_7\left(1 - GS\epsilon_2\left(\frac{1}{k_2} + \frac{1}{k_3}\right)\right)}$$

Supposing that $\epsilon_2 x_4(1/k_2 + 1/k_3) \ll 1$, it is easy to obtain expressions for the null-clines of system 3:

$$\dot{x}_4 = 0, \quad x_4 = \frac{k_5 x_7}{\epsilon_1 z}$$

$$\dot{x}_6 = 0, \quad x_4 = \frac{k_6 x_6}{2z}$$

System 3 may have one or two singular points. When the only singular point is ($x_4 = 0$, $x_6 = 0$) the system is always stable. More interesting is the case when the system has two singular points. One ($x_4 = 0$, $x_6 = 0$) is always unstable (saddle), and the second (focus or node) may be stable or not depending upon parameter values. When the second steady state is unstable the system has a limit cycle. Null-clines and the limit cycle of system 3 are plotted in fig. 2. The wave form of the oscillations is presented in fig. 3.

Thus, the model can describe two possible types of reaction behaviour: transitional process (including damped oscillations) and sustained oscillations. Bistability is not described by the model. Transition from one type of dynamic behaviour of the system to another can be performed by varying

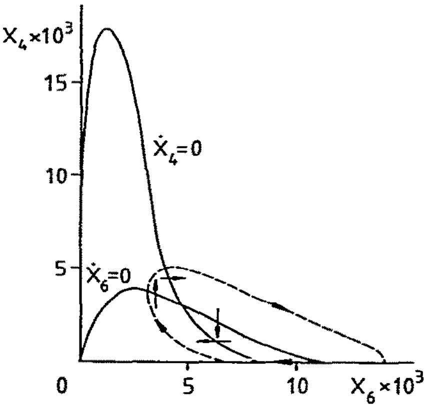

Fig. 2. Phase plane for eqs. 3. The plots are of the two null-clines $\dot{x}_4 = 0$ and $\dot{x}_6 = 0$ for $\epsilon_1 = 10^{-2}$, $\epsilon_2 = 0.1$, $k_3 = 10^{-3}$, $k_4 = 10^{-3}$, $k_5 = 10^{-5}$, $k_6 = 10^{-3}$, $k_7 = 2$, $G = 10^{-5}$, $S = 1$, $k_8 = 10^{-3}$ and a periodic solution (dashed line).

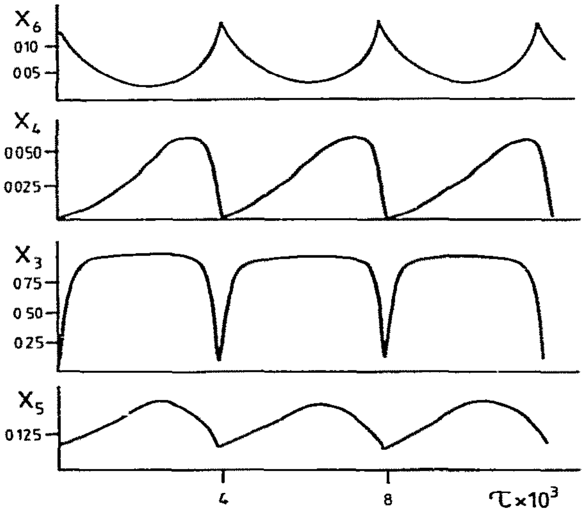

Fig. 3. The periodic solution of system 3. The parameter values are the same as those in fig. 2.

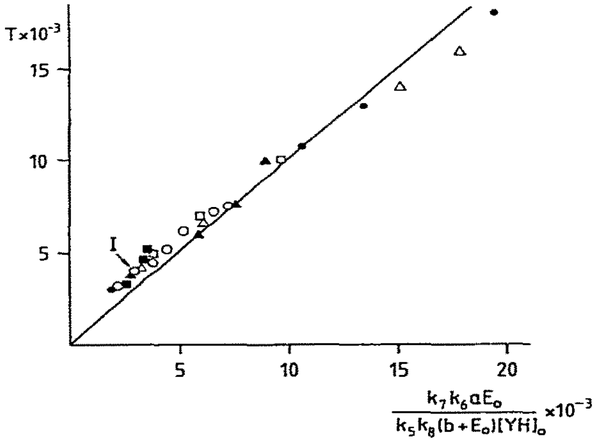

Fig 5. The dependence of oscillation period on the parameters of system 3. T is the dimensionless period obtained from numerical integration of system 3. $ak_6k_7E_o/k_5k_8[YH]_o(b+E_o)$ is the empirical expression for the period. The parameter values at point I are the same as those in fig. 2. (●, ▲, ○, △, ■, □) Points obtained by varying k_5, k_6, k_7, k_8, E_o and $[YH]_o$, respectively. The solid line is the dependence $T = ak_6k_7E_o/k_5k_8[YH]_o(b+E_o)$; $a = 2 \times 10^{-5}$ M; $b = 3 \times 10^{-6}$ M.

the system parameters. The most convenient parameters for affecting the peroxidase system experimentally are the rate of oxygen infusion (G) and the oxygen percentage in a gas mixture as well as the hydrogen donor concentration ([YH]) and peroxidase concentration (E_o). Parameter portraits of system 3 in these parameter planes are plotted in fig. 4. The dependence of the oscillation period on parameter values obtained from numerical integration of system 3 is shown in fig. 5. The oxygen infusion rate (G) and rate constants k_3 and k_4 have no influence on the period. (The fact that the oscillation period is independent of oxygen infusion rate can be attributed to the assumption made on the quasi-stationary property of the oxygen concentration.) With respect to the other parameters, their effect on the period is well described by the empirical expression:

$$T = \frac{ak_6k_7E_o}{k_5k_8[YH]_o(b+E_o)} \tag{4}$$

(in dimensionless form), where $a = 2.0 \times 10^{-5}$ M and $b = 3 \times 10^{-6}$ M. It follows from this expression that the oscillation period can be controlled by the concentration of hydrogen donor ($[YH]_o$) and of peroxidase (E_o). In our preliminary experiments the period varied inversely with NADH concentration. Therefore, the slow oscillations described in ref. 9 seem to be due to low NADH concentration.

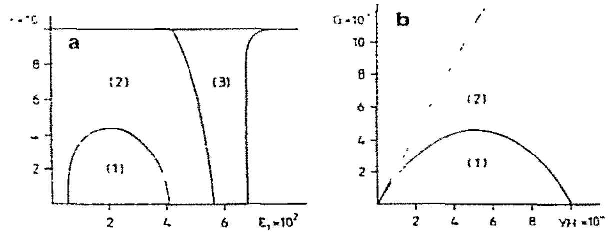

Fig. 4. Parameter portraits of system 3 in the parameter planes: (a) (G, ϵ_1), (b) (G, [YH]). (1) Oscillating state, (2) steady state of the stable focus type, (3) steady state of the stable node type; when $G \to 0$ the boundary line between regions 1 and 2 on the scale used coincides with the abscissa. The constants are the same as those in fig. 2.

The amplitude of oscillations changes directly proportional to the oxygen infusion rate and rate constants k_6 and k_7, but inversely proportional to rate constants k_5 and k_8 as well as to hydrogen donor and peroxidase concentrations.

To elucidate the role of coIII we considered the model without the coIII reactions, with the assumptions mentioned above. The analysis has shown that only damped oscillations are possible in this system. Thus, we may conclude that participation of coIII is necessary to obtain sustained oscillations.

3. Discussion

The model allows selection of the elementary reactions responsible for oscillations. These are autocatalysis, formation of coIII through the reaction with O_2^- radicals and its decay through the reaction with $Y^{\cdot}$ radicals. These reactions are shown in fig. 1, the two connected cycles being marked. The essential difference between them is that the first cycle is autocatalytic while the second is not, although the consumption of oxygen and hydrogen donor takes place in both cases. Due to autocatalysis the reaction rate of the first cycle may significantly exceed that of the second. Switching between the two cycles plays a key role in the generation of oscillations. Let us consider the process of oscillations.

The trace amounts of hydrogen peroxide available in the system initiate the reaction. Both cycles begin operating. Since the concentration of free peroxidase is high, it captures superoxide anion radicals, preventing the development of cycle I. Cycle II proceeds mainly at this stage, the rate of reaction being low. Then peroxidase converts into coIII and the decrease in free peroxidase concentration promotes development of cycle I, i.e., of the chain reaction resulting in rapid oxygen consumption and successive accumulation of hydrogen donor radicals.

As the oxygen concentration is near to zero at the end of the fast phase of the reaction, the hydrogen donor radicals can participate only in the reaction of coIII decay. In the course of its transformation to the initial peroxidase form through coI and coII, compound III produces two substrate radicals which are also involved in coIII decomposition. Therefore, the reaction is accelerated by its products. Hence, in the absence of oxygen the peroxidase enzyme cycle contributes to coIII decomposition. It should be emphasized that such a type of coIII decay does not take place if the process runs through the reaction with the substrate itself (but not with its radicals) as suggested previously [10]. The main argument in favour of this pathway is acceleration of the reaction induced by substrate addition. We suppose it to be due to the increase in radical production rate upon substrate addition.

Finally, the system appears in a state in which the concentration of free peroxidase is high, that of oxygen low and active species are present in trace amounts. These species again initiate reactions of cycle II when some oxygen accumulates due to its infusion.

Thus, the free form of the enzyme acts as the chain reaction inhibitor providing the delay necessary to reach the original state (i.e., the period during which the initial oxygen concentration is attained). This fact manifests itself in the dependence of the oscillation period on the enzyme concentration: the dimensionless period increases with peroxidase concentration according to a hyperbolic law (see eq. 4). The experimental data [11] also show the induction phase to increase with increasing enzyme concentration.

Comparing the characteristic reaction times of the systems with and without coIII indicates that introduction of coIII increases the characteristic time about 10-fold. Thus, the oscillation period is determined by the reactions of cycle II. So most of the constants of eq. 4 are those of cycle II. The larger is the contribution of cycle II ($k_7 E_0 / k_5 [\mathrm{YH}]_0$), the greater are the delay of the chain reaction and the oscillation period. On the other hand, the more rapidly coIII decays, the faster cycle II runs and hence the smaller is the oscillation period. The decomposition rate of coIII changes directly with k_8 and inversely with k_6 (because with k_6 increasing the concentration of $Y^{\cdot}$, participating in coIII decay, decreases). Thus, the oscillation period is proportional to $k_7 k_6 E_0 / k_5 k_8 [\mathrm{YH}]_0$. Exactly this type of dependence was obtained from numerical integration of system 3.

Consequently, the analysis performed has shown that in the system considered, a free radical chain reaction is responsible for autocatalysis and reactions with participation of coIII cause the delay. Interaction between these two mechanisms occurs through enzymic steps. Both autocatalysis and delay are necessary for oscillations. Since the characteristic time of the system is determined by the reactions in which coIII participates, the period of oscillations depends mainly on these reaction constants.

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