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Nonlinear oscillatory reaction of catalase induced by gradual entry of substrate

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Abstract

Characteristic oscillatory reactions were observed when hydrogen peroxide migrated through semipermeable membrane into a solution of catalase. Measurements were made with DO and an mV meter. Oscillation cleraly occurred in the range between 25°C and 37°C and between pH 6.0 and pH 7.6. It was also shown that a driving force for the permeation of H_2O_2, which was the cause of the oscillations, was a deviation from its equilibrium concentration. We made simulations for oscillatory reactions on the basis of these findings. The result indicated that considering the evaporation of O_2 was necessary in order to interpret the oscillatory reactions of catalase in addition to the slow entry of substrate caused by deviation from the equilibrium concentration. The fact that oscillations arise by using this method may provide an important insight into the study of enzyme reactions mediated by membranes in living systems, because many enzyme reactions take place with the mediation of a biomembrane.

Keywords: Oscillation; Enzyme; Catalase; Hydrogen peroxide; Semipermeable membrane

1. Introduction

It is well known that nonlinear phenomena such as oscillations and chaos appear far from equilibrium. We have already reported that the gradual entry of ethanol caused an oscillatory reaction of alcohol dehydrogenase in an oil/water system [1]. In addition, we have corroborated the oscillation by reaction simulations [2].

We devised a new method using semipermeable membrane instead of the oil/water system to provide for the gradual entry of substrate, in order to apply this to a substrate which is water-soluble but oil-insoluble. In addition, this system provides important insights into enzyme reactions in vivo, as many enzyme reactions in living systems occur with mediation of a biomembrane. The enzyme used in this study was catalase and a detailed analysis of the oscillatory reaction and its simulation has been made.

2. Materials and procedure

2.1. Materials

The enzyme and substrate used for the oscillatory reaction in this study were catalase and hydrogen peroxide. Catalase derived from beef liver was purchased from Bioenzyme Laboratories, and hydrogen peroxide was from Wako Pure Chemical Industries. They were used without further purification. The pH of all solutions was adjusted to 7.0 or 7.6 with Tris-HCl, and to 6.0 with phosphate buffer.

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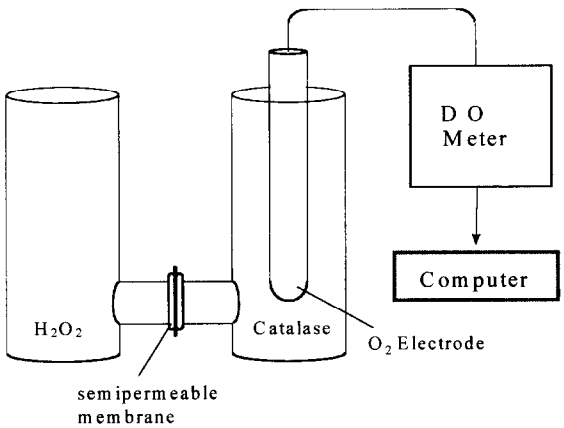

Fig. 1. Apparatus for measurement of oscillatory reaction. The volumes of the solutions of catalase and hydrogen peroxide are 20 and 10 ml, respectively.

2.2. Procedure

The measurements were made using two cells, which were made of glass, as shown in Fig. 1. Dialysis membrane (from Vikase Sales Corp., 24/32 tube) was put between the two cells; 10 ml of each solution of enzyme and H_2O_2 were put into each cell. The area of dialysis membrane for permeation of the substrate (H_2O_2) was 1.13 cm^2. An oxygen electrode or combination O.R.P electrode (TOA PTS-5011c) was dipped into the enzyme solution 1 cm from the surface and the concentration of O_2 was measured by a Horiba DO Meter OM-14; the potential was measured by a TOA ion meter IM 40S. These meters were connected to a microcomputer to record measurements at intervals of 10 or 15 s. The measurement cells were immersed in a thermally controlled water bath and all the experiments were carried out without stirring.

3. Results

3.1. Observation of oscillation

Fig. 2 shows an example of the time courses of the reactions measured by the DO meter. An oscillation of oxygen concentration with sharp peaks was clearly obtained. Its period was 10–15 min on the onset of oscillation. In this system, the oscillation

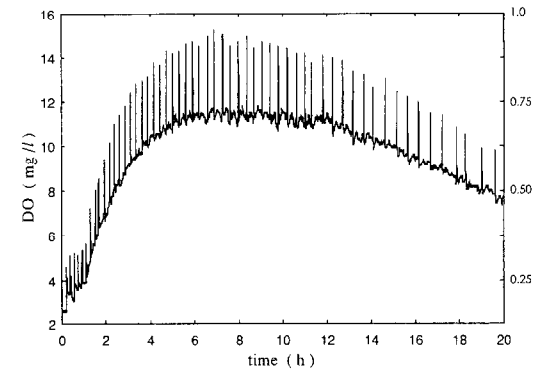

Fig. 2. Time course of oxygen concentration. The temperature is 25°C. The pH is 7.6. Initial concentrations of catalase and hydrogen peroxide are 8.17×10^{-8} M and 0.128 M, respectively.

continued for a long time, at least more than 20 h, although its period became longer with time. Similar results were also obtained in the measurement of electrical potential, as shown in Fig. 3.

3.2. Concentration dependence of oscillation

A dependency of the enzyme concentration for oscillations was barely discernible. Even when a 100 times increase in the initial concentration of the enzyme was made, the period of oscillation did not change. In contrast, an increase in the initial concentration of hydrogen peroxide shortened the period of oscillation (Fig. 4). The increase in the initial concentration of H_2O_2 implies incremental change in

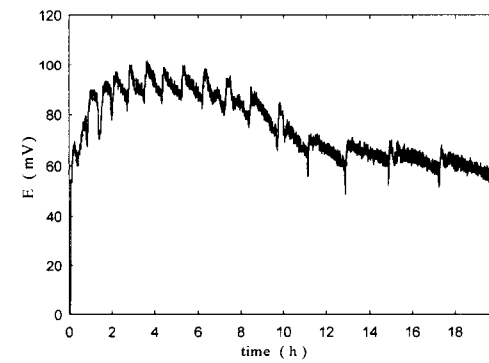

Fig. 3. Time course of potential. The volumes of solutions of catalase and hydrogen peroxide are 10 ml and 10 ml, respectively, i.e. different from that of DO meter. The temperature is 37°C. The pH is 7.0. Initial concentrations of catalase and hydrogen peroxide are 4.17×10^{-8} M and 0.128 M, respectively.

the permeation rate constants across the membrane, as shown below.

3.3. Temperature dependence of oscillation

Even when the temperature was lowered to less than 37°C, the oscillation was clearly observed. Moreover, the periods were almost the same at 37°C, 30°C and 25°C in higher concentrations of catalase. In lower concentrations of enzyme, the decrease in temperature yielded random oscillations.

3.4. pH Dependence of oscillation

In order to investigate the effect of pH on the oscillatory reaction, the pH was varied in the range between 6.0 and 7.6. No appreciable change was observed in this pH range.

3.5. Measurement of the permeation rate of H_2O_2

The gradual entry of H_2O_2 into the enzyme solution seems to be essential to the oscillatory reaction. Therefore, the permeation rate of H_2O_2 at a distance of 5–7 mm from the membrane was measured by masking part of the quartz cuvette as shown in Fig. 5. The concentrations of H_2O_2 were determined by measuring the absorbance at 210 nm. These results indicated that the concentration of substrate at a time t obeyed the equation

$$\ln([S_e] - [S]) = \ln([S_e] - [S_0]) - k_S t$$

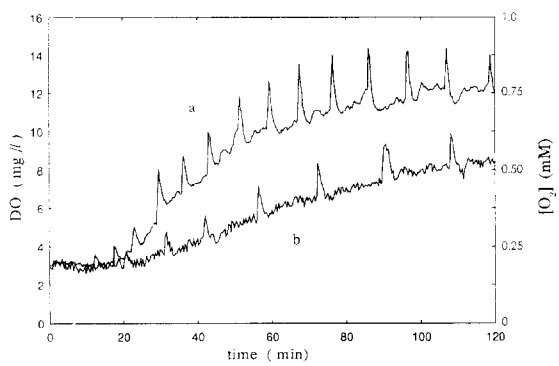

Fig. 4. Concentration dependence of the time course of the reaction. Initial concentrations of hydrogen peroxide are (a) 0.384 M and (b) 0.256 M, respectively, and that of catalase is 8.33×10^{-8} M. The temperature is 37°C. The pH is 7.0.

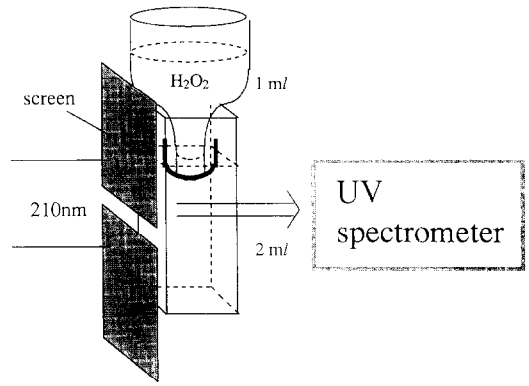

Fig. 5. Concentration dependence of the time course of the reaction. Initial concentrations of hydrogen peroxide are (a) 0.384 M and (b) 0.256 M, respectively, and that of catalase is 8.33×10^{-8} M. The temperature is 37°C. The pH is 7.0.

where $[S_0]$, $[S]$ and $[S_e]$ are the concentrations of H_2O_2 at time zero, time t and infinity, respectively, and k_S is the permeation rate constant of H_2O_2. This fact implies that the driving force for the permeation of substrate is the deviation of H_2O_2 concentration from the equilibrium value. Consequently, the derivative of [S] with respect to time becomes

$$\frac{d[S]}{dt} = k_S([S_e] - [S])$$

This equation is consistent with that of permeation through the oil/water interface [3,4]. As shown in Table 1, the rate constant, k_S, becomes large with increase in the initial concentration of H_2O_2. Increase in temperature also increased the k_S value. However, no remarkable difference was observed with respect to the pH. By considering this equation, the next simulation was made.

3.6. Simulation of oscillatory reaction

The mechanism for this oscillatory reaction was assumed as shown in Fig. 6, where E, S, P_1 and P_2 denote catalase, H_2O_2, O_2 and H_2O, respectively.

The rate equation of each species is probably written considering both the equation of the permeation of the substrate, as described above, and a term representing product efflux, as follows

$$\frac{d[E]}{dt} = -k_1[E][S] + (k_2 + k_3)[ES] - k_4[E][P_1]^{1/2}[P_2]$$

Table 1
The values of k_S

(a) Initial concentration dependence [a]	
$[S]_i$	k_S/s^{-1}
0.032 M	1.73×10^{-5}
0.064 M	2.03×10^{-5}
0.128 M	3.31×10^{-5}
(b) Temperature dependence [b]	
Temperature	k_S/s^{-1}
25°C	1.40×10^{-5}
30°C	$1.46\times^{-5}$
37°C	$2.03\times^{-5}$
(c) pH dependence [c]	
pH	k_S/s^{-1}
6.0	2.32×10^{-5}
6.0	2.03×10^{-5}
7.6	2.06×10^{-5}

[a] The temperature and pH are 37°C and 7.0, respectively.[b] The initial concentrations of H_2O_2 and pH are 0.064 M and 7.0, respectively.[c] The initial concentrations of H_2O_2 and temperature are 0.064 M and 37°C, respectively.

$$\frac{d[S]}{dt} = k_S([S_e] - [S]) - k_1[E][S] + k_2[ES]$$

$$\frac{d[ES]}{dt} = k_1[E][S] - (k_2 + k_3)[ES] + k_4[E][P_1]^{1/2}[P_2]$$

$$\frac{d[P_1]}{dt} = k_3[ES] - k_4[E][P_1]^{1/2}[P_2] - k_P[P_1]$$

where [E], [S], [ES], $[P_1]$ and $[P_2]$ are the concentrations of enzyme, substrate, intermediate, product (O_2) and product (H_2O), respectively; k_1, k_2, k_3 and k_4 are rate constants for each reaction and k_P is the rate of outflow of O_2.

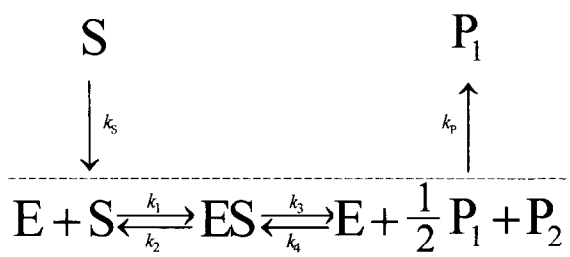

Fig. 6. Apparatus for the measurement of permeation of hydrogen peroxide.

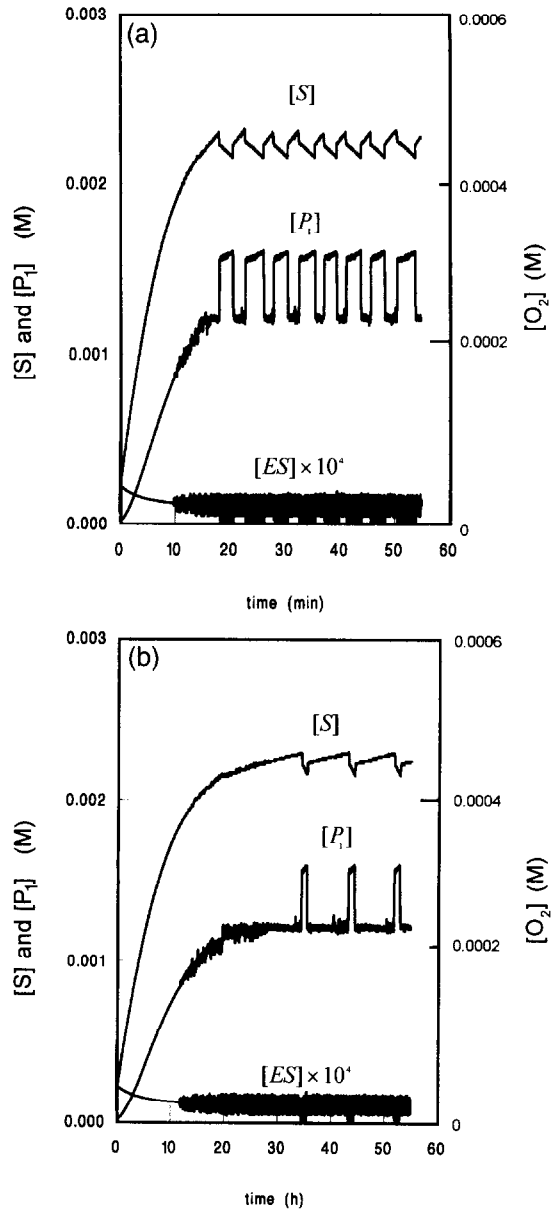

Fig. 7. Scheme of the reaction.

Fig. 7 shows one of the results of calculation using the Gear method. In this calculation, as values of the rate constants k_1, k_2, k_3 and k_4, we used the values estimated from the simulation for the result of measurement of the absorbance at 210 nm in the reaction of catalase and H_2O_2 which were dissolved

Table 2
Conditions which gave the same period as the experimental result

(a) Temperature change $[E] = 2.5\times10^{-8}$ M, 37°C			
pH	6.0	7.0	7.6
k_S /s^{-1}	3.26×10^{-5}	2.9×10^{-5}	2.86×10^{-5}
k_1 /M^{-1}s^{-1}	8.0×10^{6}	9.0×10^{6}	9.0×10^{6}
k_2 /s^{-1}	1.8×10^{4}	1.0×10^{4}	1.0×10^{4}
k_3 /s^{-1}	2.0×10^{4}	2.0×10^{4}	2.0×10^{4}
k_4 /M$^{3/2}$ s^{-1}	3.0×10^{4}	3.0×10^{4}	5.5×10^{4}
k_P /s^{-1}	0.015	0.015	0.015
(b) pH change $[E] = 2.5\times10^{-8}$ M, 37°C			
pH	6.0	7.0	7.6
k_S /s^{-1}	3.26×10^{-5}	2.9×10^{-5}	2.86×10^{-5}
k_1 /M^{-1} s^{-1}	8.0×10^{6}	9.0×10^{6}	9.0×10^{6}
k_2 /s^{-1}	1.8×10^{4}	1.0×10^{4}	1.0×10^{4}
k_3 /s^{-1}	2.0×10^{4}	2.0×10^{4}	2.0×10^{4}
k_4 /M$^{3/2}$ s^{-1}	3.0×10^{4}	3.0×10^{4}	5.5×10^{4}
k_P /s^{-1}	0.015	0.015	0.015
(c) Change of enzyme concentration pH 7, 37°C			
[E]	2.5×10^{-8} (M)	3.0×10^{-8} (M)	5.0×10^{-8} (M)
k_S /s^{-1}	2.90×10^{-5}	3.04×10^{-5}	2.98×10^{-5}
k_1 /M^{-1} s^{-1}	9.0×10^{6}	9.0×10^{6}	9.0×10^{6}
k_2 /s^{-1}	1.0×10^{4}	1.0×10^{4}	1.0×10^{4}
k_3 /s^{-1}	2.0×10^{4}	2.0×10^{4}	2.0×10^{4}
k_4 /M$^{3/2}$ s^{-1}	3.0×10^{4}	3.0×10^{4}	3.0×10^{4}
k_P /s^{-1}	0.015	0.015	0.015

in a cuvette without a semipermeable membrane. The interval of the calculation was 0.02 s. When the value of k_P, probably due to the evaporation of product, was increased to the order of $10-2$ s, the oscillation appeared. It was also shown that bursting or very short period vibrations of the concentrations of the enzyme, E, and intermediate, ES, occurred during the oscillations of the substrate and product. Decrease in the permeation rate of substrate at a fixed value of k_S led to an increase in the period. The other conditions with the same periods as those of the experimental results are shown in Table 2. In cases of changes in both the temperature and pH, the change in k_S retained the same period of oscillation. With the change in enzyme concentration, the periods of oscillation were constant within small variations of k_S.

4. Discussion

Catalase is an enzyme that catalyzes the decomposition reaction of hydrogen peroxide and has the highest reaction rate among enzymes. However, it is known that catalase also works as a peroxidase. As with the nonlinear oscillation of other peroxidases, many studies have been carried out, both experimental and theoretical [5,6]. In this study, we examined only the decomposition reaction of hydrogen peroxide.

CSTR is well known as a method with which to examine chemical oscillation under well-stirred conditions [7–11]. However, in this study, the measurements were all made without stirring. Fig. 8 shows the time course of the absorbance at 210 nm during the reaction of catalase and H_2O_2 in the same apparatus as that of Fig. 5. The result demonstrated that the concentration of H_2O_2 increased with time accompanying the oscillation and a concentration gradient of H_2O_2 with respect to the distance was observed. However, it was found that the oscillation clearly took place far from the membrane, although the amplitude became small.

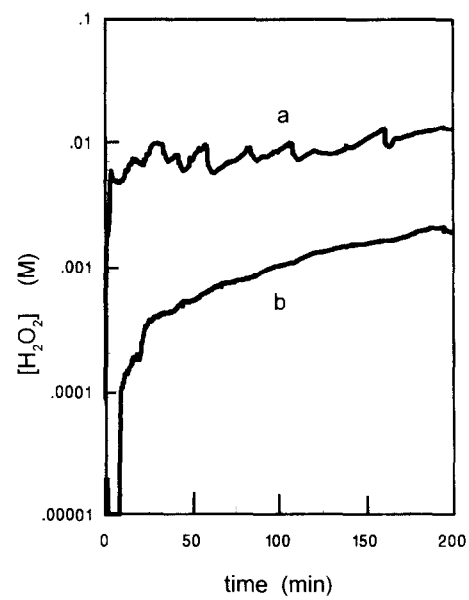

Fig. 8. Simulation of reaction. The concentration of enzyme is 8.33×10^{-8} M; $k_1 = 6\times10^{6}$ M^{-1}s^{-1}, $k_2 = 1\times10^{4}$ s^{-1}, $k_3 = 2\times10^{4}$ s^{-1}, $k_4 = 3\times10^{4}$ M$^{3/2}$ s^{-1}, $k_P = 1.5\times10^{-2}$ s^{-1} and $[S]_t = 0.384$ M. (a) $k_S = 2.1\times10^{-5}$ s^{-1}. (b) $k_S = 2.02\times10^{-5}$.

From the measurements of the time course of substrate concentration in the absence of enzyme, it was found that the driving force for the permeation of H_2O_2 across the semipermeable membrane was the deviation from the equilibrium concentration. This is consistent with the result for permeation of a material in the oil/water system [3,4]. Bearing this in mind, we carried out simulations for the oscillatory reactions in the absence of efflux of the product. These results showed that nonlinear phenomena could occur, but the values obtained were very different from the experimental results. In particular, the concentration of oxygen obtained was too large. However, this discrepancy was solved by considering a term for the efflux of oxygen. The large value of K_P seems to be due to the vaporization rate of O_2.

In this study, an exact estimation of the K_P value could not be made. In order to simulate this precisely, accurate measurement of K_P is necessary. Nevertheless, it is evident that the primary cause of the oscillations is the gradual entry of substrate due to the driving force to attain equilibrium. The equilibrium concentration of substrate changes constantly because either the forward reaction of H_2O_2 to $O_2 + H_2O$ or the reverse reaction of O_2 to H_2O_2 occurs during the oscillatory reaction. Consequently, the driving force for a flow of H_2O_2, $k_S([S_e] - [S])$, does not reduce monotonously with time, but always fluctuates. The stationary flow of substrate did not cause the oscillation in this simulation.

As shown in Fig. 7, a decrease in the permeation rate of H_2O_2 led to an increase in the period of oscillation. This is consistent with the experimental observation. In the measurement of $[O_2]$, the frequency of the oscillation did not change with respect to the change in temperature. It was found that this could be interpreted in terms of the change in k_S as shown in Table 2. Likewise, with respect to the pH dependence of the oscillations, the change in k_S also accounted for the experimental results. However, it was found that the periods of the oscillation were almost independent of the enzyme concentration.

Many enzyme reactions occur with the mediation of biomembranes. It is likely that oscillations take place ubiquitously in living systems. Application of this method will provide significant information not only on catalase, but also on other enzyme reactions in vivo, since the reaction process in this system involves the outflow of product across the membrane as well as the inflow of substrate.

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