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Microcalorimetric studies on catalase reaction and inhibition of catalase by cyanide ion

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Abstract

As Chance et al. [1–3] proposed, the decomposition of hydrogen peroxide catalyzed by catalase is an overall first-order reaction. In this paper, we have studied this enzyme-catalyzed reaction with a thermokinetic method. The rate constant and the molar reaction enthalpy of this reaction have been measured. At 310.15 K and pH=8.2, $k_{cat}=1.75\times10^{6}\,l\,mol^{-1}\,s^{-1}$, $\Delta_r H_m=88.99\ kJ\ mol^{-1}$. Furthermore, we have studied the competitive inhibition of catalase by cyanide ion and reported some correlated parameters.

Keywords: Catalase; Microcalorimetry; Thermokinetics; Kinetics; Competitive inhibition

1. Introduction

Catalase is an important enzyme of biological defense against oxygen toxicity. It has been detected in a wide range of aerobe organisms. Catalase catalyzes the decomposition of hydrogen peroxide (H_2O_2), which is harmful to the organism. The kinetics and mechanism of this reaction have been extensively researched, but the mechanism is not completely determined yet. It is generally believed that catalase and H_2O_2 form a complex (I) during the first step. Chance et al. [1–3] proposed the mechanism that (I) directly reacts with another H_2O_2 molecule, so the decay of H_2O_2 can be expressed as first-order reaction. On the other hand, Ogura et al. [4–6] suggested a mechanism involving a ternary complex (ESS), and the H_2O_2 decay rate conform Michaelis–Menten equation.

The thermokinetic method can provide in situ, online, quasi-continuous, non-invasive and accurate measurements of not only thermodynamic data but also the kinetic data of the reaction under investigation. Consequently, microcalorimetry has become a significant tool for the study of biothermochemistry and the kinetics of biochemical processes. It has been extensively used in the study of enzyme-catalyzed reactions [6–9].

In this paper, using Chance's model, we studied the decomposition of H_2O_2 catalyzed by catalase and the competitive inhibition of catalase by cyanide ion.

2. Experimental

2.1. Reagents

Beef liver catalase was purchased from Sigma Corporation. Other reagents were of analytical grade. All solutions were prepared with double distilled water.

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2.2. Method

The calorimetric curves were obtained at 310.15 K (37.00°C) using an LKB-2107 batch microcalorimeter system, which is the combination of a micro-batch reactor (BR) with a conduction calorimeter. Before each calorimetric experiment, all reagent solutions were diluted to the required concentrations with buffer solution (0.025 M Tris–HCl, pH=8.2). In the decomposition experiment, 2.00 ml catalase solution was injected into reaction cell I, and 4.00 ml H_2O_2 solution was injected into reaction cell II. In the inhibition experiment, 2.00 ml of a mixed solution of catalase and sodium cyanide was injected into reaction cell I, and 4.00 ml H_2O_2 solution was injected into reaction cell II. In order to avoid the influence of the heat effect of diluting and mixing, etc., the same sample was added to the reference cell except in the case of catalase addition. When the microcalorimetry system had been in thermal equilibrium and a steady baseline obtained on the recorder, the reaction run was initiated by rotating the calorimeter 360° clockwise and counterclockwise, respectively, so as to fully mix the enzymes and substrate solutions, etc. The heat generated in the reaction process was detected in the form of thermal potential by thermoelectric piles, and the amplified output signal was recorded as the calorimetric curve by LKB-2210 dual-pen integrating recorder.

3. Results

3.1. Measurements of first-order reaction rate constant by analyzing calorimetric curves

When a thermal change of a chemical reaction takes place in a batch conduction calorimeter, the relation between input function Q and output function Δ should obey Tian's equation, so we can obtain the following equations [10]:

$$Q = KA_t + c\Delta \tag{1}$$

$$Q_\infty = KA \tag{2}$$

where c is the heat capacity constant and K the proportionality constant, Q the heat liberated before time t, Q_∞ the total heat effect. As Fig. 1 shows, Δ is the peak height at time t, A_t the peak area up to time t, and A the total area under the thermogram.

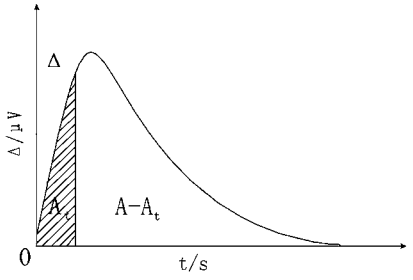

Fig. 1. Microcalorimetric curves of LKB-2107.

For a chemical reaction:

$$Q_\infty = n\,\Delta_r H_m \tag{3}$$

where n is the number of moles of initial substrate. Then,

$$\Delta_r H_m = \frac{KA}{n} \tag{4}$$

K can be obtained from the calibration experiment.

Defining the substrate conversion ratio $\Phi_t=([S_0]-[S_t])/[S_0]$, where $[S_0]$, $[S_t]$ stand for the substrate concentration at the beginning and time t, then Φ_t can be described as

$$\Phi_t = \frac{[S_0]-[S_t]}{[S_0]} = \frac{Q}{Q_\infty} = \frac{KA_t + c\Delta}{KA} = \frac{\tau\Delta + A_t}{A} \tag{5}$$

where $\tau=c/K$ is a constant with time dimension. To determine this parameter, we can run an electric calibration after each experiment. From the calorimetric curve of the calibration, we choose a series of points after the peak. For these points, the system is under a natural cooling procedure, so they should obey the equation: $\ln \Delta=\ln \Delta_0-t/\tau$. As we plot $\ln \Delta$ against t, we can obtain the parameter τ from the slope of the linear curve. At the same time by dividing the heat of calibration by the area under the calibration curve, the parameter K mentioned above can be determined.

For a first-order reaction, it is easy to prove that

$$-\ln(1-\Phi_t) = k_0 t \tag{6}$$

where k_0 stands for the rate constant of first-order reaction.

Thus, choosing a series of points from microcalorimetric curves, plot $-\ln(1-\Phi_t)$ against t, from the slope of the linear curve, we can obtain the rate constant of

Table 1
An example of analyzing a microcalorimetric curve[a]

t (s)	Δ (μ V)	a (mV s)	Φ_t	$-\ln(1-\Phi_t)$
270	435.4	84.2	0.5020	0.6972
310	415.9	101.4	0.5571	0.8143
360	384	121.3	0.6167	0.9588
420	340.5	143.0	0.6778	1.1326
480	297.5	162.2	0.7300	1.3092
540	256.4	178.8	0.7733	1.4842
600	220.7	193.1	0.8106	1.6636
720	158.7	215.7	0.8669	2.0169
840	114.1	232.0	0.9077	2.3822

[a] Result: $k_1=2.94\times10^{-3}$ s^{-1}, correlation coefficient $R=0.9999$, molar reaction enthalpy $\Delta_r H_m=89.931$ kJ mol^{-1}. Other parameters: $K=3.052\times10^{-3}$ J mV^{-1} s^{-1}, $\tau=114.9$ s, $A=270.5$ mV s, $[S_0]=1.53\times10^{-3}$ mol l^{-1}.

first-order reaction. Table 1 and Fig. 2 show an example of analyzing a microcalorimetric curve.

3.2. The kinetics of catalase-catalyzed reaction

Chance et al. [1–3] proposed that the decomposition of H_2O_2 catalyzed by catalase obeys such mechanism:

$$\mathrm{E} + \mathrm{S} \overset{k_1}{\rightarrow} \mathrm{ES}$$

$$\mathrm{ES} + \mathrm{S} \overset{k_4}{\rightarrow} \mathrm{E} + \mathrm{P} \qquad (7)$$

where E, S, ES, and P stand for enzyme (catalase), substrate (H_2O_2), enzyme–substrate complex, and product, respectively. According to this mechanism, the H_2O_2 decay rate (v) is expressed as follows:

$$v = -\frac{\mathrm{dS}}{\mathrm{d}t} = k_0 \mathrm{S} \qquad (8)$$

where

$$k_0 = \frac{2k_1k_4}{k_1 + k_4}[\mathrm{E_t}] \qquad (9)$$

k_0 stands for the overall first-order rate constant of catalase-catalyzed reaction, and $[E_t]$ is the total catalase heme concentration.

From the above, we know the rate constant is directly proportional to the total catalase heme concentration, while also being independent of substrate concentration. Furthermore, we can define a constant $k_{cat}=k_0/[E_t]$, which is independent of catalase heme concentration. In this paper, we present the results of a series of reactions under different substrate concentrations and enzyme concentrations. The results are listed in Tables 2 and 3.

From Table 2, we see the results of low substrate concentration show good linear dependence relations. Among this group the molar reaction enthalpy and rate constant are close to each other. But the results of high substrate concentration show poor linear dependence

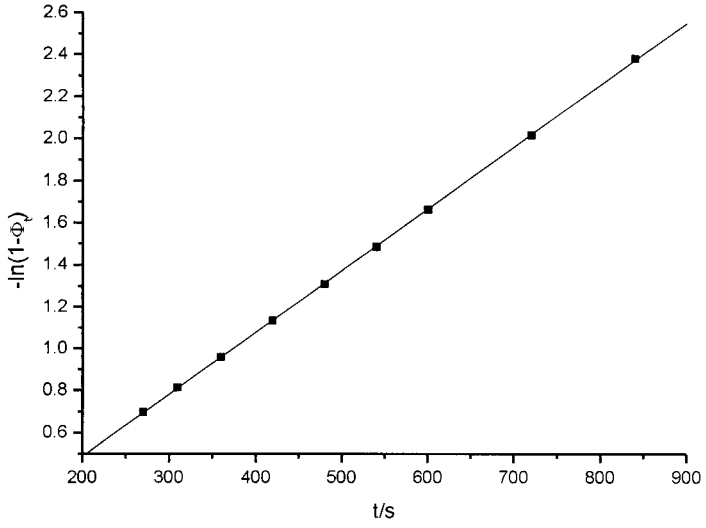

Fig. 2. An example of thermogram analysis.

Table 2
Result under different substrate concentrations for $[E_t]=1.72\times10^{-9}$ mol l^{-1}

$[S_0]$ (mol l^{-1})	$k_0\times10^{-3}$ (s^{-1})	R	Δ_rH_m (kJ mol^{-1})
1.53×10^{-3}	2.94	0.9999	89.93
2.70×10^{-3}	2.99	0.9998	90.00
5.46×10^{-3}	3.09	0.9998	87.05
1.09×10^{-2}	2.46	0.9992	71.90
2.18×10^{-2}	2.96	0.9989	40.67
5.46×10^{-2}	3.82	0.9808	18.57

relations and discrete rate constant values. Furthermore, the value of molar enthalpy is much smaller than expected. This indicates that under high substrate concentration, the reaction is incomplete, possibly because high-concentration H_2O_2 can irreversibly inhibit catalase.

From Table 2, using results obtained under low substrate concentration, we determined that at 310.15 K, pH=8.2, molar reaction enthalpy $\Delta_rH_m=88.99$ kJ mol^{-1} and rate constant $k_{cat}=1.75\times10^6$ l mol^{-1} s^{-1}.

From Table 3 and Fig. 3, k_0 shows a significant linear relation with $[E_t]$. It proves the conclusion above. For the slope of the linear $k_{cat}=1.45\times10^6$ l mol^{-1} s^{-1}, this value is close to the result from Table 2.

Table 3
Result under different catalase concentrations[a] for $[S_0]=2.70\times10^{-3}$ mol l^{-1}

$[E_t]\times10^{-9}$ (mol l^{-1})	k_0 (s^{-1})
0.86	1.32×10^{-3}
1.12	2.64×10^{-3}
1.72	3.40×10^{-3}
3.44	6.40×10^{-3}
5.16	7.59×10^{-3}
6.88	1.12×10^{-2}
8.60	1.30×10^{-2}

[a] Linear fitting result: $k_0=7.35\times10^{-4}+1.45\times10^6$ $[E_t]$, $R=0.993$.

3.3. Inhibition of catalase by cyanide ion

The inhibition of catalase by cyanide ion is competitive reversible inhibition [2,11], which can be shown as follows:

$$E + S \overset{k_1}{\rightarrow} ES \overset{k_4}{\rightarrow} E + P$$

$$E + I \overset{K_i}{\leftrightarrow} EI \tag{10}$$

where, I stands for the inhibitor (cyanide ion).

From the above discussions, we find that the enzyme exists in three forms: [E], [ES] and [EI], then

$$[E_t] = [E] + [ES] + [EI] \tag{11}$$

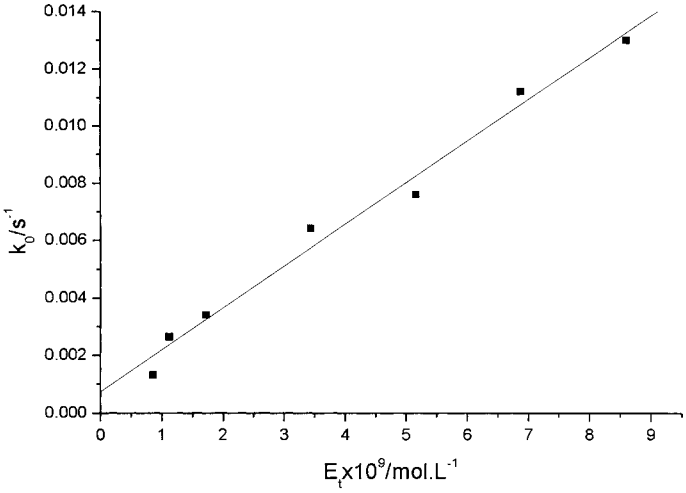

Fig. 3. Rate of catalase reaction against catalase heme concentration.

The enzyme can reach chemical equilibrium with inhibitor quickly, so

$$\frac{[EI]}{[E][I]} = K_i \tag{12}$$

Substituting Eq. (12) in Eq. (11), we obtain

$$[E] = \frac{1}{1 + K_i[I]}([E_t] - [ES]) \tag{13}$$

As the hypothesis of steady state shows:

$$\frac{d[ES]}{dt} = k_1[E][S] - k_4[ES][S] = 0 \tag{14}$$

Substituting Eq. (13) in Eq. (14), we obtain

$$\frac{d[ES]}{dt} = k_1 \frac{1}{1 + K_i[I]}([E_t] - [ES])[S] - k_4[ES][S] = 0 \tag{15}$$

then

$$[ES] = \frac{k_1[E_t]}{k_1 + k_4 + k_4K_i[I]} \tag{16}$$

So, the rate of reaction (v) can be shown as in the following equation:

$$v = 2k_4[ES][S] = \frac{2k_1k_4}{k_1 + k_4 + k_4K_i[I]}[E_t][S] \tag{17}$$

From Eq. (17), we conclude that when there exist an inhibition by cyanide ion, the catalase reaction still has the form of first-order reaction rate equation, the rate constant is

$$k_0' = \frac{2k_1k_4[E_t]}{k_1 + k_4 + k_4K_i[I]} \tag{18}$$

On inversion we obtain

$$\frac{1}{k_0'} = \frac{1}{k_0} + \frac{B}{[E_t]}[I] \tag{19}$$

where $k_0 = [2k_1k_4/(k_1+k_4)]\ [E_t]$ is the rate constant when cyanide ion does not exist; and $B = K_i/2k_1$, a constant for this reaction.

As Eq. (19) shows the reciprocal value of rate constant k_0', which has linear relation with concentration of cyanide ion. The results of experiment listed in Table 4 and Fig. 4 proved the conclusion. Two

Table 4
Result of inhibition by cyanide ion[a] for $[E_t] = 1.721\times10^{-9}$ mol l^{-1}

$[CN^-]$ (mol l^{-1})	k_0' (s^{-1})	$1/k_0'$ (s)
7.76×10^{-4}	2.57×10^{-3}	389.1
3.10×10^{-3}	1.21×10^{-3}	826.4
6.21×10^{-3}	7.98×10^{-4}	1253.1
9.31×10^{-3}	6.31×10^{-4}	1584.8
1.24×10^{-2}	4.50×10^{-4}	2222.2

[a] Linear fitting result: $1/k_0' = 1.50\times10^5$ $[CN^-] + 301.6$, correlation coefficient: $R = 0.9946$.

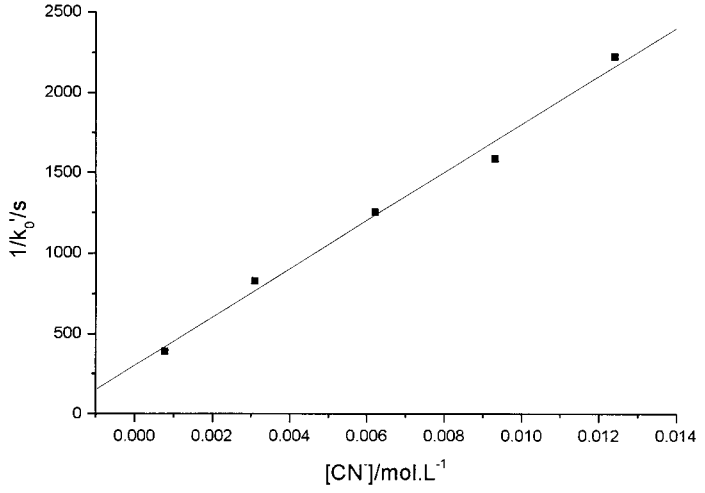

Fig. 4. Reciprocal of rate of catalase reaction inhibited by CN^- against CN^- concentration.

parameters in the linear relation are: $1/k_0=301.6$ s, $B=2.49\times10^{-4}$ s, then $k_0=3.32\times10^{-3}$ s^{-1}. This value is close to the value obtained before.

4. Discussion

Catalase-catalyzed reaction is a bi-substrate enzyme-catalyzed reaction. Chance [1] proposed the mechanism as follows:

$$E + S \overset{k_1}{\rightarrow} ES + S \overset{k_4}{\rightarrow} E + P \tag{20}$$

So the H_2O_2 decay rate (v) can be expressed as follows:

$$v = \frac{2k_1k_4}{k_1 + k_4}[E_t][S] \tag{21}$$

On the other hand, Ogura [4] proposed a mechanism involving an ESS.

$$E + S \overset{k_1}{\rightarrow} ES$$

$$ES + S \overset{k_4}{\rightarrow} ESS \overset{k_6}{\rightarrow} E + P \tag{22}$$

So the H_2O_2 decay rate (v) is expressed as follows:

$$v = \frac{2k_6[E_t][S]}{(1/k_1 + 1/k_4)k_6 + [S]} \tag{23}$$

In Eq. (21), the rate equation has the form of first-order reaction rate equation, while in Eq. (23), it has the form of Michaelis–Menten equation, hence:

$$K_m = \left(\frac{1}{k_1} + \frac{1}{k_4}\right)k_6 \tag{24}$$

In this paper, we deal with catalase-catalyzed reaction as first-order reaction. However, this does not mean the ternary complex does not exist, as Eq. (23) reduces to Eq. (21) when $K_m \gg S$. In the literature, the value of K_m is very different from 1.1 M [4] to 0.025 M [5]. Compared with the concentration of substrate used in experiment, it is consistent with $K_m \gg S$. But from the results shown previously, when the substrate concentration $[S_0]>0.01$ M, the catalase is inhibited by high concentration substrate, hence the reaction is incomplete.

Therefore, when we study the catalase reaction under low substrate concentration, we just use Chance's model. When we study the catalase reaction under high substrate concentration, the inhibition of substrate must be considered.

Acknowledgements

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