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Thermokinetic models of enzyme-catalyzed reactions in batch and plug-flow reactors

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Abstract

This paper reports the thermokinetic models of single-substrate, enzyme-catalyzed reactions occurring in batch and plug-flow reactors, respectively. By analyzing the calorimetric curves of these reactions, these models can be used to produce not only the thermodynamic data ($\Delta_r H_m$) but also the kinetic data (K_m and k_2). Using a LKB-2107 batch microcalorimeter and an LKB-2277 Bioactivity Monitor, the catalase-catalyzed decomposition of hydrogen peroxide was studied and its molar reaction enthalpy ($\Delta_r H_m$) measured as -88.88 ± 0.6 kJ mol^{-1}. The Michaelis constant (K_m) for H_2O_2 was determined by the batch and plug-flow thermokinetic models to be $5.03\pm0.18\times10^{-3}$ mol dm^{-3} and $(5.27\pm0.11)\times10^{-3}$ mol dm^{-3}, respectively. The reliability of these models for determination of the thermokinetics of single-substrate, enzyme-catalyzed reactions occurring in these two types of reactors was verified by the experimental results. © 1997 Elsevier Science B.V.

Keywords: Batch reactor; Enzyme-catalyzed reaction; Microcalorimetry; Plug-flow reactor; Thermokinetic model

1. Introduction

One of the major objectives of enzyme engineering is to study the kinetic models of enzyme-catalyzed reactions occurring in certain types of reactors [1,2]. The thermokinetic method, which started at the beginning of this century [3], stands out as one of the most powerful tools for kinetic studies of chemical processes because it provides in-situ, on-line, quasi-continuous, non-invasive and accurate measurements of not only thermodynamic data but also the kinetic data of the reaction under investigation and these data can help shed light on the reaction mechanisms [4–6]. There is also no constraint on both the solvent and the spectral, electrochmical, or other properties of the reaction systems involved. Owing to these advantages, the thermokinetic method has recently received increasing attention from researchers in many fields [4–18]. Since the absorption or production of heat is an intrinsic property of virtually all enzyme-catalyzed reactions, it seems reasonable to believe that investigation of the kinetics of enzymatic reactions occurring in certain types of reactors by the thermokinetic method can incorporate the generality of this method with the specificity of enzymes and furnish important information which is applicable to the design and performance of these types of bioreactors.

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The intrinsic kinetic models of single-substrate, enzyme-catalyzed reactions in batch reactors (BRs), continuously stirred tank reactors (CSTRs) and plug-flow tubular reactors (PFRs) have been reported [1,2]. Based on these kinetic models and the Tian equation [19,20], the thermokinetic models of single-substrate, enzyme-catalyzed reactions occurring in BRs and PFRs, respectively, are proposed in this paper. By analyzing the calorimetric curves of these reactions, these thermokinetic models can be used to calculate both molar reaction enthalpy ($\Delta_r H_m$) and kinetic parameters (K_m and k_2). A well-studied single-substrate enzymatic reaction, the decomposition of hydrogen peroxide catalyzed by catalase [21,22], was employed to test the validity of these thermokinetic models, and the thermokinetic parameters of this reaction were determined using a LKB-2107 batch microcalorimeter and an LKB-2277 Boiactivity Monitor (BAM).

2. Thermokinetic models

For a simple single-substrate, single-intermediate, enzyme-catalyzed reaction occurring in a BR or PFR with negligible mass-transfer limitations, according to Michaelis–Menten kinetics, it is easy to prove that [1,2]

$$x - (K_m/[S]_0)\ln(1-x) = (k_2[E]_0/[S]_0)t \quad (1)$$

where K_m is the Michaelis constant, $[S]_0$ and $[E]_0$ the initial concentrations of substrate and enzyme in the reacting systems, respectively, and k_2, also known as the turnover number of the enzyme [23], is the rate constant of breakdown of the enzyme-substrate complex to product.

If the heat-transfer process in a BR or PFR obeys the Tian equation, the substrate conversion x at time t in a BR and a PFR may be written, respectively, as

$$x = (\Delta_t + ka_t/(kA) \quad (2)$$

$$x = P/(2F[S]_0\Delta_r H_m) \quad (3)$$

here, Δ_t is the peak height at time t, a_t the peak area before time t, A the total area under the batch calorimetric curve and k the cooling constant of the batch microcalorimeter, F the flow rates of substrate (S) and enzyme (E) pumped into the measuring cell of BAM and $\Delta_r H_m$ the molar reaction enthalpy which can be determined by

$$\Delta_r H_m = Q_\infty/(V[S]_0) \quad (4)$$

where Q_∞ is the total heat effect of reaction, V the total volume of the reacting system in the batch microcalorimeter (in this paper, V=6.00 cm^3); P, the real steady-state power output of reaction in a flow calorimetric experiment, can be calculated by the equation below:

$$P = \mu(P_a - P_d) \quad (5)$$

where P_a is the apparent steady-state power output of reaction, P_d corresponds to the steady-state power output of dilution of substrate at the same flow rate, and μ a correction factor of power output. Because part of the power output can be taken away by the flow system and the larger the flow rate, the more the power output losses, the measured power output must be corrected for μ, which was calibrated by the introduction of electric energy at the same flow rate.

Substituting Eqs. (2) and (3) in Eq. (1), respectively, we obtain

$$(1/t)\ln[kA/(ka_t^* - \Delta_t)] = (k_2[E]_0)/K_m - ([S]_0/K_m)(\Delta_t + ka_t)/(kAt) \quad (6)$$

and

$$(1/t)\ln[2F[S]_0\Delta_r H_m/(2F[S]_0\Delta_r H_m - P)] = (k_2[E]_0)/K_m - (1/K_m)P/(2Ft\Delta_r H_m) \quad (7)$$

where a_t^* is the peak area after time t ($a_t^* = A - a_t$), t the reaction time for BRs and the residence time for PFRs ($t = V_R/2F$; here, V_R is the effective volume of measuring cell, in this paper, V_R =0.6 cm^3). Eqs. (6) and (7) are called the thermokinetic models of single-substrate, enzyme-catalyzed reactions occurring in batch and plug-flow reactors, respectively. Thus, the plots of $(1/t)\ln[kA/(ka_t^* - \Delta_t)]$ against $(\Delta_t + ka_t)/(kAt)$ and of $(1/t)\ln[2F[S]_0\Delta_r H_m/(2F[S]_0\Delta_r H_m - P)]$ against $P/(2Ft\Delta_r H_m)$ are linear with y-axis intercept (z) of V_{max}/K_m and slopes (y) of $-[S]_0/K_m$ and $-1/K_m$, respectively. Then, the values of K_m and k_2 can be calculated from the slope and intercept, respectively.

3. Experimental

3.1. Reagents

Doubly distilled water was used throughout. Analytical grade sodium orthophosphate (dimetallic) and sodium orthophosphate were used for the preparation of the buffer solution. The pH of the solution was adjusted to 7.0 by means of a pH meter, mixing slowly two solutions of concentrations 6.7×10^{-2} mol dm^{-3}.

The solid catalase with a molecular weight of 232000, which was extracted from beef liver was obtained from the Shanghai Institute of Biochemistry of Academia Sinica and purified. Two catalase solutions of 4.14×10^{-2} mg cm^{-3} (or 0.450 mg cm^{-3}) and 2.25×10^{-2} mg cm^{-3} were prepared for the batch and flow calorimetric experiments, respectively, by dissolving the solid catalase in the buffer solution. The stock solutions were stored in a refrigerator.

The substrate solution was made by dissolution of analytical grade hydrogen peroxide in buffer solution and its concentration was measured by the iodimetric method to be 7.795×10^{-3} mol dm^{-3} (or 1.042×10^{-2} mol dm^{-3}) for the batch calorimetric experiment and 9.694×10^{-4} mol dm^{-3} (or 3.878×10^{-3} mol dm^{-3}) for the flow calorimetric experiment, respectively.

All solutions were freshly prepared before each set of experiments.

3.2. Instrumentation

Two microcalorimeters, a LKB-2107 batch microcalorimeter and an LKB-2277 Bioactivity Monitor, which are combination of a conduction calorimeter with a micro-BR and micro-PFR, respectively, were used to obtain the calorimetric curves of the decomposition of H_2O_2 catalyzed by catalase. The microcalorimeters were thermostated at 298.15 K (25.00°C). The BAM was operated in the flow-mix mode [24,25], and the method of the sample adding to the batch microcalorimeter was similar to that already described [18]. The performance of these instruments and the details of their constructions had been previously represented [17,18,26].

4. Results

The catalase-catalyzed decomposition of hydrogen peroxide (the substrate) in the presence of phosphate buffer at pH 7.0 in a BR and PFR was studied to test the validity of these thermokinetic methods. The catalase reaction obeyed typical Michaelis–Menten kinetics and Ogura mechanism [22]. Therefore, the models (6) and (7) can be used to analyze the calorimetric curves of the reaction. The heat capacity of LKB-2107 batch microcalorimeter system was calibrated by electrical energy and $k=(8.4633\pm0.0917)\times10^{-3}$ s^{-1}. The molar reaction enthalpy of catalase reaction was determined by batch microcalorimetry to be -88.88 ± 0.6 kJ mol^{-1} at the condition of 298.15 K and pH 7.0 (see Table 1). The kinetic parameters (K_m, k_2) calculated by these models from the calorimetric data are listed in Tables 2–5. Here, the mean standard relative error of these calorimetric data (Δ_t, a_t, a_t^*, P_a and P_d) is about 2%.

From these tables, it can be seen that the values of measurements of K_m and k_2 have better repeatability and self-consistency. Furthermore, the kinetic parameters (K_m, k_2) calculated by model (6) are in agreement with those calculated by model (7), and the correlation coefficients (r) of these models ≈-1. Therefore, the correctness and validity of these thermokinetic models applied to the studies of kinetics of single-substrate, enzyme-catalyzed reactions occurring in batch and plug-flow reactors should be considered as proved by the experimental results.

Table 1
Molar reaction enthalpy, Δ_rH_m, of catalase-catalyzed decomposition of H_2O_2 at 298.15 K and pH 7.0 with catalase concentration of 1.875×10^{-3} mg cm^{-3} in cell

$10^3[S]_0$/(mol dm^{-3})	$-Q_\infty$/J	$-\Delta_rH_m$/(kJ mol^{-1})
0.6947	0.3728	89.44
0.8683	0.4631	88.89
1.476	0.7768	87.71
1.737	0.9294	89.19
2.605	1.3762	88.05
3.473	1.8670	89.59
4.342	2.3260	89.28
5.210	2.7779	88.86
Average value		88.88±0.67

Table 2
Michaelis constant, K_m, and enzymatic turnover number, k_2, of catalase reactions calculated by model (6) from the batch calorimetric data at 298.15 K and pH = 7.0 with catalase concentration of 1.035×10^{-3} mg cm^{-3} in cell

No.	$10^3[S]_0$/(mol dm^{-3})	$-y$	10^3z/s^{-1}	$-r$	10^3K_m/(mol dm^{-3})	$10^{-3}k_2$/s^{-1}
1	0.6496	0.1294	3.327	0.9929	5.02	3.74
2	1.299	0.2474	3.276	0.9945	5.25	3.86
3	1.949	0.3795	3.307	0.9918	5.14	3.81
4	2.598	0.5443	3.410	0.9986	4.77	3.65
5	3.248	0.6522	3.333	0.9962	4.98	3.72
Average values				0.9948±0.0027	5.03±0.18	3.76±0.08

Table 3
An example of analysis of the batch calorimetric curve of a catalase reaction (No. 4 in Table 2, A = 80.472 mV s)

t/s	Δ_t/μV	a_t/mV s	a_t^*/mV s	$10^3(\Delta_t+ka_t)/(kAt)$/s^{-1}	$10^3(1/t)\ln[kA/(ka_t^*-\Delta_t)]$/s^{-1}
630	64.80	58.195	22.277	1.299	2.707
660	59.90	60.038	20.434	1.264	2.721
690	55.30	61.759	18.713	1.230	2.737
720	51.00	63.384	17.088	1.198	2.756
750	47.00	64.889	15.583	1.167	2.776
780	43.20	66.252	14.220	1.137	2.792
810	40.00	67.493	12.979	1.108	2.812
840	36.80	68.614	11.858	1.079	2.823
870	33.80	69.665	10.807	1.052	2.838
900	31.00	70.618	9.854	1.026	2.850

Table 4
Michaelis constant, K_m, and enzymatic turnover number, k_2, of catalase reactions calculated by model (7) from the flow calorimetric data at 298.15 K and pH 7.0 with catalase concentration of 1.125×10^{-2} mg cm^{-3} in cell

No.	10^4 $[S]_0$/ (mol dm^{-3})	$-y$/(dm^3 mol^{-1})	10^2z/s^{-1}	$-r$	10^3 K_m/ (mol dm^{-3})	$10^{-3}k_2$/s^{-1}
1	19.39	191.6	3.410	0.9912	5.22	3.67
2	9.694	194.2	3.301	0.9934	5.15	3.51
3	4.847	185.3	3.246	0.9953	5.40	3.61
4	2.424	189.0	3.270	0.9968	5.29	3.57
Average values				0.9942±0.0024	5.27±0.11	3.59±0.07

Table 5
An example of analysis of the flow calorimetric curve of a catalase reaction (No. 3 in Table 4)

10^3F/ (cm^3 s^{-1})	$-P_a$/μW	$-P_d$/μW	μ	$-P$/μW	t/s	$(1/t)\ln[2F[S]_0\Delta_rH_m/(2F[S]_0\Delta_rH_m-P)]$/s^{-1}	$P/(2Ft\Delta_rH_m)$/ (mol dm^{-3}s^{-1})
27.64	759.0	428.0	2.007	664.3	10.85	3.014×10^{-2}	1.246×10^{-5}
21.37	679.0	329.5	1.824	637.5	14.04	3.027×10^{-2}	1.195×10^{-5}
16.82	596.0	218.5	1.606	606.3	17.84	3.038×10^{-2}	1.137×10^{-5}
12.50	579.0	181.4	1.406	559.0	24.00	3.050×10^{-2}	1.048×10^{-5}
8.269	493.0	97.8	1.212	479.0	36.28	3.075×10^{-2}	8.982×10^{-6}
6.168	449.0	81.2	1.126	414.1	48.64	3.106×10^{-2}	7.765×10^{-6}

5. Discussion

From the intrinsic kinetic Eq. (1), a single-substrate, enzyme-catalyzed reaction can be regarded as a mixed-order reaction composed of zero-order and first-order reactions. Under the condition that the initial concentration of substrate $[S]_0$ is very much greater ($[S]_0 \gg K_m$), model (1) reduces to the intrinsic kinetic model of zero-order reactions occurring in BRs or PFRs:

$$x = (k_2[E]_0/[S]_0)t = k_0 t \tag{8}$$

and models (6) and (7) reduce, respectively, to the thermokinetic models of zero-order reactions in BRs and PFRs:

$$(\Delta_t + ka_t)/(kA) = k_0 t \tag{9}$$

$$P/(2F[S]_0\Delta_r H_m) = k_0 t \tag{10}$$

here, k_0 is the ratio of zero-order rate constant to $[S]_0$.

In the event that $[S]_0 \ll K_m$, Eq. (1) reduces to the form of a simple first-order equation:

$$-\ln(1 - x) = (k_2[E]_0/K_m)t = k_1 t \tag{11}$$

and Eqs. (6) and (7) simplify, respectively, to the thermokinetic models of first-order reactions in BRs and PFRs:

$$\ln[kA/(ka_t^* - \Delta_t)] = k_1 t \tag{12}$$

$$\ln[2F[S]_0\Delta_r H_m/(2F[S]_0\Delta_r H_m - P)] = k_1 t \tag{13}$$

where k_1 is the first-order rate constant.

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