Study of Activation and Inhibition of Certain Metal Ions to Amylase Catalyzed Reaction by Microcalorimetry

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With or without activation or inhibition of metal ion, the power-time curves of amylase catalyzed reaction were determined by a 2277 thermal activity monitor (Sweden). The Michaelis constant (K), apparent Michaelis constant (K_m), maximum velocity (v_m) and apparent maximum velocity (v_{am}) of amylase catalyzed reaction were obtained using thermokinetic theory and reduced extent method.

On the basis of data obtained, the following relationships between K_m and concentration of metal ion (c) were established:

for inhibitor of Ni^{2+}
$K_m = 2.9648 \times 10^{-3} - 1.3912 \times 10^{-4} c$
$R = 0.9998$
for inhibitor of Co^{2+}
$K_m = 1.0227 \times 10^{-3} + 8.2676 \times 10^{-6} c$
$R = 0.9955$
for activator of Ca^{2+}
$K_m = 1.0630 \times 10^{-7} c^2 - 1.8311 \times 10^{-6} c + 9.3058 \times 10^{-6}$
$R = 0.9999$
for activator of Li^{+}
$K_m = 5.6300 \times 10^{-8} c^2 - 1.5329 \times 10^{-6} c + 1.2662 \times 10^{-5}$
$R = 0.9999$

The K_m-c relationships show a strenuous inhibitory effect for Ni^{2+} and a strenuous active effect for Ca^{2+}.

Keywords microcalorimetry, thermokinetics, reduced extent, amylase catalyzed reaction, activation, inhibition, metal ions (Ca^{2+}, Li^{+}, Co^{2+}, Ni^{2+})

Introduction

Most complicated reactions happened in living creatures, among them enzyme catalyzed reaction is an important class. It is significant in both theory and practice to investigate enzyme catalyzed reaction. There are many experimental methods such as spectrophotometry, titrimetry, isotope method, microcalorimetry and so on, in which microcalorimetry is a new one due to its high sensitivity and accuracy. We can study the whole process of the heat effect using a microcalorimeter. Since the absorption or production of heat is an intrinsic property of enzyme catalyzed reaction, it should be possible to obtain thermodynamic and kinetic information.

The enzyme was extracted from Huanghai Sea mud, and it belongs to the typical amylase class catalyst which can hydrolytically break the glucosides bonds of the starch molecule and its derivatives. The optimum temperature of this amylase is low, and therefore it has a bright future in industrialization, for example, in medicine, food, drinking, washing, leather, and so on. But the activity of the amylase is influenced directly by metal ions. In order to satisfy the need of application, it is necessary to study the character of amylase such as its active or inhibitory type and effect of metal ions.

Thermokinetic theory of enzyme catalyzed reaction has greatly progressed these years,[1-8] but there is no report on active and inhibitory effect and relationships of K_m-c of amylase catalyzed reaction by using microcalorimetry.

Theory

Michaelis-Menten equation describing the enzyme catalyzed reaction of single substrate is

$$\frac{1}{v} = \frac{K}{v_m}\frac{1}{[S]} + \frac{1}{v_m} \qquad (1)$$

In existence of single substrate and inhibitor, enzyme catalyzed reaction has three inhibitory types. The corresponding equations are:

for competitive inhibition

$$\frac{1}{v} = \frac{K}{v_m}\left(1 + \frac{[I]}{K_i}\right)\frac{1}{[S]} + \frac{1}{v_m} \text{ or } \frac{1}{v} = \frac{K_m}{v_m}\frac{1}{[S]} + \frac{1}{v_m} \qquad (2)$$

for non-competitive inhibition

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$$\frac{1}{v}=\frac{K}{v_m}\left(1+\frac{[I]}{K_i}\right)\frac{1}{[S]}+\frac{1}{v_m}\left(1+\frac{[I]}{K_i}\right)$$

$$\text{or } \frac{1}{v}=\frac{K_m}{v_m}\frac{1}{[S]}+\frac{1}{v_{am}} \quad (3)$$

for counter competitive inhibition

$$\frac{1}{v}=\frac{K}{v_m}\frac{1}{[S]}+\frac{1}{v_m}\left(1+\frac{[I]}{K_i}\right) \text{ or } \frac{1}{v}=\frac{K}{v_m}\frac{1}{[S]}+\frac{1}{v_{am}} \quad (4)$$

$$K_m=K\left(1+\frac{[I]}{K_i}\right) \qquad v_{am}=\frac{v_m}{1+\frac{[I]}{K_i}}$$

$[I]$ is concentration of inhibitor.

Without inhibitor and existence of inhibitor, comparing Eqs. (1)—(4), data (v_m, v_{am}, K, K_m) of enzyme catalyzed reaction are different, and when the temperature and concentration of inhibitor are constant, v_{am} and K_m are constant. Eqs. (1), (2), (3) and (4) are similar.

Eq. (1) could be changed into:

$$\frac{1}{\frac{d[S]}{dt}}=\frac{K}{v_m}\frac{1}{[S]}+\frac{1}{v_m} \quad (5)$$

Integrating Eq. (5), Eq. (6) could be derived:

$$\frac{K}{[S_0]}\ln\frac{[S]}{[S_0]}+\frac{[S]-[S_0]}{[S_0]}=-\frac{k_2[E_0]}{[S_0]}t \quad (6)$$

If $\frac{[S_0]-[S]}{[S_0]}=\varphi$, then $\frac{[S]}{[S_0]}=1$, $v_m=k_2[E_0]$

where φ is reduced extent, $[E_0]$ is total enzyme concentration and the following reduced extent equation could be derived

$$\varphi-\frac{K}{[S_0]}\ln(1-\varphi)=\frac{v_m}{[S_0]}t \quad (7)$$

According to the area under the power-time curve, Q and Q_∞ could be gotten. Q represents thermal effect at the time of t and Q_∞ the total thermal effect of enzyme catalyzed reaction.

$\varphi(=Q/Q_\infty)$. Three points, φ_1, φ_2 and φ_3 were chosen on φ-t curve with identical time interval ($\Delta t=t_3-t_2=t_2-t_1$), the following equations were derived from Eq. (7).

$$K=\frac{2\varphi_2-\varphi_1-\varphi_3}{2\ln(1-\varphi_2)-\ln(1-\varphi_1)-\ln(1-\varphi_3)}[S_0] \quad (8)$$

Using the above method we treated Eqs. (2), (3) and (4), Eqs. (8) and (9) could be derived into the same type just replacing v_m with v_{am}, K with K_m. For activator, we treated Eqs. (2), (3) and (4) similarly. Same Eqs. (8) and (9) were derived.

Experimental

Reagent

The concentration of amylase (the amylase was extracted from marine filamentous fungus screened from deep Huanghai Sea mud) is 3.2×10^{-2} g·L^{-1} and the contrast activity is 2.14×10^{3} U·mg^{-1}. The concentration of soluble starch solution is 5×10^{-3} g·L^{-1}.

Instrument

All measurements were made using a titration microcalorimetry from Thermometric AB Company (Sweden). The working temperature range of which is 10—90 ℃; it was maintained constant to within $\pm2\times10^{-4}$ ℃ at the given temperature. The detection limit is 0.15 μW and the baseline stability is 0.2 μW (over a period of 24 h). Titration unit is equipped with a stirrer motor to rotate the stirrer shaft at the desired speed (between 0 and 120 r/min).

Method

In the experiment, a 4 mL titration stainless steel ampoule unit was used, and 2 mL of starch solution containing metal ion was put into the ampoule. The thin plastic tube was rolled on the rod of titration unit, and 0.05 mL of amylase solution was put into plastic tube. When the temperature was constant, the stirrer system with a speed of 120 r/min was employed and 0.05 mL of amylase solution was put into the ampoule with peristaltic pump. The monitor began to record the power-time curve, when the recording pen returned to the baseline and began to stabilize, the process of amylase catalyzed reaction was completed.

Results and discussion

Results of without activator and inhibitor

Power-time curves of amylase catalyzed reactions at 0.05 mL of amylase, 2 mL of starch solution, pH = 5.24 and 310 K were determined. The area under the curves represent thermal effect. The reduced extent could be obtained by the ratio of area at time t with the total area. K and v_m could be calculated by Eqs. (8) and (9). The

$$v_m=\frac{K}{\Delta t}\cdot\frac{(\varphi_3-\varphi_1)\ln(1-\varphi_2)-(\varphi_3-\varphi_2)\ln(1-\varphi_1)-(\varphi_2-\varphi_1)\ln(1-\varphi_3)}{2\varphi_2-\varphi_1-\varphi_3} \quad (9)$$

data are listed in Table 1. This curve is shown in Fig. 1a.

Table 1 Values of K and v_m of amylase catalyzed reaction at 310 K and pH = 5.24

φ_1	φ_2	φ_3	Δt (s)	$K \times 10^3$ ($g \cdot L^{-1}$)	$v_m \times 10^5$ ($g \cdot L^{-1} \cdot s^{-1}$)
0.1790	0.3690	0.5458	120	1.0058	1.0120
0.3690	0.5458	0.7032	120	1.0031	1.0115
0.5458	0.7032	0.8319	120	1.0035	1.0117
0.7032	0.8319	0.9216	120	1.0040	1.0118
0.1790	0.5458	0.8319	240	1.0037	1.0117
0.3690	0.7032	0.9216	240	1.0036	1.0116
			mean:	1.0040	1.0117

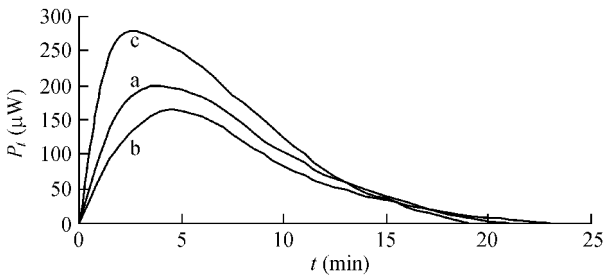

Fig. 1 Power-time curves of different concentration of metal ion to amylase catalyzed reaction at 310 K and pH = 5.24. (a) $c = 0$; (b) $c = 5.0$ mmol·L^{-1} Ni^{2+}; (c) $c = 0.2$ mmol·L^{-1} Ca^{2+}.

Results of inhibition

Inhibitory type

Power-time curves of amylase catalyzed reaction were determined at 0.05 mL of amylase, 2 mL of starch solution contained metal ions (Ni^{2+}), 310 K and pH = 5.24. Using Eqs. (8) and (9), values of K, K_m, v_m and v_{am} were gained. The data are listed in Table 2. This curve is shown in Fig. 1b.

According to Eqs. (2), (3) and (4), comparing the results of Tables 1 and 2, we defined that inhibitory type is competitive inhibition. With the same method for Co^{2+}, inhibitory type also is competitive inhibition.

Inhibitory effect of metal ions (Co^{2+}, Ni^{2+})

From Eq. (2), in existence of different concentration of metal ion, data of amylase catalyzed reaction are different. Values of K_m and v_m are listed in Table 3.

On the basis of data in Table 3, the relationships between K_m and c were established.

for inhibitor of Co^{2+}

$$K_m = 1.0227 \times 10^{-3} + 8.2676 \times 10^{-6}\, c$$

$$R = 0.9955$$

for inhibitor of Ni^{2+}

$$K_m = 2.9648 \times 10^{-3} - 1.3912 \times 10^{-4}\, c$$

$$R = 0.9998$$

With or without inhibitors, according to the power-time curves, v_m and K (K_m) were obtained with thermokinetic theory and reduced extent method. v_m are similar but K and K_m are different. But K_m is bigger than K. These results proved that the inhibitor of Ni^{2+}, Co^{2+} was competitive inhibition.

In the same concentration of metal ions, it is clear that inhibitory rule is $Ni^{2+} > Co^{2+}$, and Ni^{2+} shows a strength inhibitory effect.

Results of activation

Active type

Power-time curves of amylase catalyzed reaction were determined at 0.05 mL of amylase, 2 mL of starch solution containing activator (Ca^{2+}, Li^{+}), 310 K and pH = 5.24. Using Eqs. (8) and (9), values of K, K_m, v_m and v_{am} were gained. The data are listed in Table 4. This curve is shown in Fig. 1c.

According to Eqs. (2), (3) and (4), comparing the results of Tables 1 and 4, active type is analogy with competitive inhibition type. We defined active type is competitive activation. With the same method for Li^{+}, active type also is competitive activation.

Table 2 Values of v_m, v_{am}, K and K_m of amylase catalyzed reaction at 310 K, pH = 5.24 and 5.0 mmol·L^{-1} Ni^{2+}

φ_1	φ_2	φ_3	Δt (s)	Competitive inhibition		Non-competitive inhibition		Counter competitive inhibition	
				$K_m \times 10^3$ ($g \cdot L^{-1}$)	$v_m \times 10^5$ ($g \cdot L^{-1} \cdot s^{-1}$)	$K_m \times 10^3$ ($g \cdot L^{-1}$)	$v_{am} \times 10^6$ ($g \cdot L^{-1} \cdot s^{-1}$)	$K \times 10^3$ ($g \cdot L^{-1}$)	$v_{am} \times 10^6$ ($g \cdot L^{-1} \cdot s^{-1}$)
0.3552	0.4652	0.5636	120	3.5811	1.0176	3.5811	2.8530	1.0097	2.8530
0.4652	0.5636	0.6499	120	3.5512	1.0108	3.5512	2.8339	1.0013	2.8339
0.5636	0.6499	0.7239	120	3.5758	1.0161	3.5758	2.8487	1.0082	2.8487
0.6499	0.7239	0.7860	120	3.5689	1.0145	3.5689	2.8443	1.0063	2.8443
0.3552	0.5636	0.7239	240	3.5687	1.0147	3.5687	2.8448	1.0062	2.8448
0.4652	0.6499	0.7860	240	3.5382	1.0094	3.5382	2.8300	0.9976	2.8300
			mean:	3.5609	1.0138	3.5609	2.8425	1.0053	2.8425

Active effect of metal ions (Ca^{2+}, Li^{+})

From Eq. (2), in existence of metal ion, data of amylase catalyzed reaction is different. Values of K_m and v_m were obtained and shown in Table 5.

On the basis of data in Table 5, the following relationships between K_m and c were established.

for activator of Ca^{2+}

$$K_m = 1.063 \times 10^{-7} c^2 - 1.8311 \times 10^{-6} c + 9.3058 \times 10^{-6}$$

$$R = 0.9999$$

for activator of Li^{+}

$$K_m = 5.6300 \times 10^{-8} c^2 - 1.5329 \times 10^{-6} c + 1.2662 \times 10^{-5}$$

$$R = 0.9999$$

According to the power-time curves, the values of v_m and K (K_m) were obtained. v_m is similar, but K and K_m are different. K_m is smaller than K. These results proved that the activator of Li^{+}, Ca^{2+} is similar to inhibitory type. We called competitive active type. Comparing the results in the same concentrations of metal ions, active rule is $Ca^{2+} > Li^{+}$, and Ca^{2+} shows the strenuous active effect.

In general, it is a new method to define the active or inhibitory type and effect of amylase catalyzed reaction. It has important theoretical value and broad application in future for the study of enzyme catalyzed reaction.

Table 3 Values of K_m and v_m of amylase catalyzed reaction at 310 K, pH = 5.24 and different concentrations of metal ions

Concentration	$K_m \times 10^3$ (g·L^{-1})		$v_m \times 10^5$ (g·L^{-1}·s^{-1})	
c (mmol·L^{-1})	Co^{2+}	Ni^{2+}	Co^{2+}	Ni^{2+}
5.0		3.5609		1.0138
20.0		5.8945		1.0141
30.0	1.2678		1.0128	
50.0	1.4537	9.8747	1.0116	1.0129
80.0	1.6507		1.0105	
100.0	1.8684	16.876	1.0103	1.0110

Table 4 Values of v_m, v_{am}, K and K_m of amylase catalyzed reaction at 310 K, pH = 5.24 and 0.20 mmol·L^{-1} Ca^{2+}

φ_1	φ_2	φ_3	Δt (s)	Competitive inhibition		Non-competitive inhibition		Counter competitive inhibition	
				$K_m \times 10^6$ (g·L^{-1})	$v_m \times 10^5$ (g·L^{-1}·s^{-1})	$K_m \times 10^6$ (g·L^{-1})	$v_{am} \times 10^9$ (g·L^{-1}·s^{-1})	$K \times 10^6$ (g·L^{-1})	$v_{am} \times 10^9$ (g·L^{-1}·s^{-1})
0.34310	0.46630	0.58942	60	9.1588	1.0193	9.1588	1.1369	1.0216	1.1369
0.46630	0.58942	0.71237	60	9.0831	1.0299	9.0831	1.1487	1.0131	1.1487
0.58942	0.71237	0.83496	60	9.0141	1.0299	9.0141	1.1488	1.0054	1.1488
0.71237	0.83496	0.95620	60	8.7546	1.0296	8.7546	1.1484	0.9765	1.1484
0.34310	0.58942	0.83496	120	8.8350	1.0298	8.8350	1.1486	0.9854	1.1486
		mean		8.9691	1.0277	8.9691	1.1463	1.0004	1.1463

Table 5 Values of K_m and v_m of amylase catalyzed reaction at 310 K, pH = 5.24 and different concentrations of metal ions

Concentration	$K_m \times 10^6$ (g·L^{-1})		$v_m \times 10^5$ (g·L^{-1}·s^{-1})	
c (mmol·L^{-1})	Ca^{2+}	Li^{2+}	Ca^{2+}	Li^{2+}
0.20	8.9691		1.0265	
1.00	7.5513	11.203	1.0277	1.0282
4.00	3.6523	7.4009	1.0228	1.0316
5.00	2.8501		1.0316	
6.00		5.4615		1.0325
8.00	1.4504	4.0608	1.0291	1.0336
12.00		2.3594		1.0308

References

1 Belaich, A.; Belaich, J. P. *J. Bacteriol.* **1976**, *125*(1), 19.

2 Liu, J. S.; Zeng, X. C.; Tian, A. M.; Deng, Y. *Thermochim. Acta* **1995**, *253*, 275.

3 Saileri, G.; Vinci, G.; Antonelli, M. L. *Anal. Chim. Acta* **1995**, *300*, 287.

4 Zhang, H. L.; Liu, Y. J.; Sun, H. T.; Sun, X. F.; Shan, Q. Z.; Xie, C. L.; Qu, S. S. *Acta Phys.-Chim. Sin.* **1993**, *9*(6), 836 (in Chinese).

5 Deng, Y. *Chem. J. Chin. Univ.* **1985**, *6*(7), 621 (in Chinese).

6 Shen, T.; Wan, J. Y. *Biochemistry*, High Education Press, Beijing, **1999** (in Chinese).

7 Liang, Y.; Wang, C. X.; Wu, D. Q.; Qu, S. S. *Thermochim. Acta* **1995**, *268*, 17.

8 Liu, J. S.; Zeng, X. C.; Deng, Y.; Tian, A. M. *Acta Chim. Sinica* **1994**, *52*, 767 (in Chinese).

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