The Effect of Some Ions on Glucose Oxidase and Their Kinetics

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Abstract: The enzymatic oxidation reaction of *β*-D-glucose in aqueous solution by glucose oxidase (GOD) obtained from *Aspergillus niger* was studied by the use of spectrophotometric method depend on oxidation of o-toluidine. Some experiments were carried out using some matters and their kinetic parameters were calculated. It was found that this enzymatic reaction was inhibited by $NaNO_3$. The results obtained in the experiments carried out with $HgCl_2$, Hg_2Cl_2, $CuCl_2$ and CuCl with the same method fitted nonessential activation.

Keywords: glucose oxidase, *β*-D-glucose, kinetics.

1. INTRODUCTION

Glucose oxidase is a dimeric and glycosilated flavoprotein. GOD is selective for glucose in clinical applications and biological fluids [1-3]. So, GOD has been intensively used in biosensors the determination of glucose concentration [3-5]. GOD catalyzes the oxidation of *β*-D-glucose by molecular oxygen producing hydrogen peroxide and glucano-δ-lacton which hydrolyzes spontaneously to gluconic acid [6-8]. In the enzymatic oxidation reaction, coenzyme flavin adenin dinucleotide (FAD) is reduced to $FADH_2$ in the presence of glucose. $FADH_2$ catalyzes the reduction of O_2 and $FADH_2$ is reoxidized to FAD [8-10]. The hydrogen peroxide produced reacts immediately with iodide in the presence of ammonium molybdate catalyst to form triiodide. The absorbance increase followed as spectrophotometric is a measure of oxidation rate of triiodide formation. The iodide obtained forms a colored product with o-toluidine [11,12,16].

The simplest mechanism for enzyme reaction containing one-substrate is indicated as follows.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_p} E + P$$

and its Lineweaver-Burk equation is

$$\frac{1}{v} = \frac{K_m}{V_m[S]} + \frac{1}{V_m} \qquad (1)$$

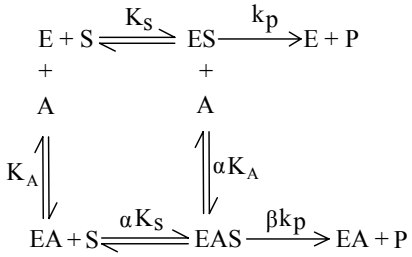
Henry-Michaelis-Menten equation for competitive inhibition is [13,15]

$$\frac{v}{V_m} = \frac{[S]}{K_m(1 + \frac{[I]}{K_i}) + [S]} \qquad (2)$$

The velocity equation for the inhibition is

$$\frac{1}{v} = \frac{K_m}{V_m}\left(1 + \frac{[I]}{K_i}\right)\frac{1}{[S]} + \frac{1}{V_m} \qquad (3)$$

Nonessential activation is a type of activation in which the enzymatic reaction can occur in the absence of the activation.



By taking Henry-Michaelis-Menten equation into consideration, the activation equation is [14]

$$\frac{v}{V_m} = \frac{[S]}{K_m\dfrac{1+\dfrac{[A]}{K_A}}{1+\dfrac{\beta[A]}{\alpha K_A}} + [S]\dfrac{1+\dfrac{[A]}{\alpha K_A}}{1+\dfrac{\beta[A]}{\alpha K_A}}} \qquad (4)$$

2. EXPERIMENTAL

2.1. Materials

GOD (23000 U/g-*Aspergillus niger*), *β*-D-glucose, o-toluidine were provided Sigma. Other chemicals such as ammonium molybdate, sodium nitrate, mercury(II) chloride, mercury(I) chloride, copper(II) chloride, copper(I) chloride, potassium iodide, sodium acetate, acetic acide were purchased from Merck.

2.2. Instrument

Schimadzu UV-160A spectrophotometer was used in determinations of absorbtion spectra for the reaction and in examination of the reaction kinetic.

2.3. Preparation of reaction mixture

The reaction mixture containing 0.15 mL o-toluidine (0.075 $mgmL^{-1}$), 0.10 mL GOD, 1.50 mL %1(w/v) ammonium molybdate, 1.50 mL %0.5(w/v) potassium iodide and *β*-D-glucose, 0.50 M, pH 5.0 acetate buffer and deionized water is prepared and this mixture is kept at 25 °C for the same time was added in the mixture (final volume is 4 mL) to start the reaction. This procedure was repeated for each series of experiments with inhibition or activator.

3. RESULTS AND DISCUSSION

The main enzymatic reaction is

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic Acid} + H_2O_2$$

and H_2O_2 formed in this reaction enzymically oxidizes I_2 by the use of ammonium molybdate.

$$H_2O_2 + 2H^+ + 2I^- \xrightarrow{Mo\,(IV)} I_2 + H_2O$$

I_2 formed in the result of this reaction catalyzes the oxidation of o-toluidine to form a colored product having an maximum absorption at 650 nm.

$$I_2 + \text{o-toluidine(red)} \longrightarrow 2HI + \text{o-toluidine(ox)}$$

Optimum enzyme concentration was obtained as 0.14 mgmL^{-1} for 10^{-4} M β-D-glucose. K_m and V_m values of the reaction without inhibitor and activator, a series of experiments were carried out by trying various substrate concentration. After the reaction was started, the increase of absorbance with respect to time was observed until the reaction was completed. For each study, absorbance (A) graph against time (t) graph was drawn, initial rates of the reactions were found from the slopes on t_0 points of the curves. So, 1/v and 1/[S] graph drawn. The intersection points of the curve on 1/v and 1/[S] axis in the graph are $1/V_m$ and $-1/K_m$ respectively. The slope of curve is K_m/V_m. V_m and K_m were calculated 3.46×10^{-3} ΔAsec^{-1} and 8.37×10^{-6} M, respectively. For studies with inhibitor or activator, this method was repeated.

For the aim of studying the effect of $NaNO_3$ on the enzymatic reaction rate, its solution was added into the mixture before the start of the reaction. Data obtained from the experiments were shown in Table 1. The inhibition constant, K_i, was calculated 3.07×10^{-3} M for $NaNO_3$.

Table 1 Data obtained from the experiments for $NaNO_3$.

[$NaNO_3$] (M)	[S] (M)	$[S]^{-1}$ (M^{-1})	v ($Msec^{-1}$)	v^{-1} ($secM^{-1}$)
2.00×10^{-4}	0.25×10^{-4}	40000	2.47×10^{-3}	405
	0.50×10^{-4}	20000	2.74×10^{-3}	365
	0.75×10^{-4}	13300	3.00×10^{-3}	333
	1.00×10^{-4}	10000	3.10×10^{-3}	327
	1.25×10^{-4}	8000	3.11×10^{-3}	321
3.00×10^{-4}	0.25×10^{-4}	40000	2.50×10^{-3}	400
	0.50×10^{-4}	20000	2.65×10^{-3}	377
	0.75×10^{-4}	13300	2.98×10^{-3}	335
	1.00×10^{-4}	10000	3.12×10^{-3}	320
	1.25×10^{-4}	8000	3.19×10^{-3}	313
4.00×10^{-4}	0.25×10^{-4}	40000	2.40×10^{-3}	416
	0.50×10^{-4}	20000	2.41×10^{-3}	414
	0.75×10^{-4}	13300	2.92×10^{-3}	342
	1.00×10^{-4}	10000	3.43×10^{-3}	291
	1.25×10^{-4}	8000	3.07×10^{-3}	326

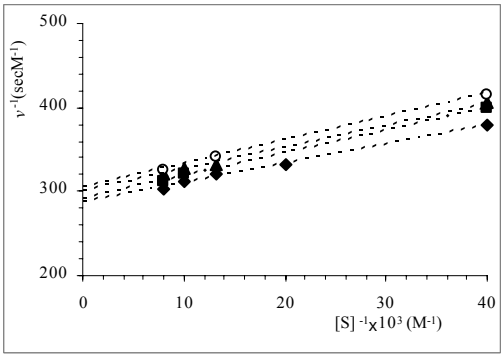


Fig. 1. Effect of $NaNO_3$ on the enzymatic oxidation reaction rate (♦:without $NaNO_3$, ■:3.0×10^{-4} M $NaNO_3$, ▲:2.0×10^{-4} M $NaNO_3$, ○:4.0×10^{-4} M $NaNO_3$)

It was determined that $HgCl_2$, Hg_2Cl_2, $CuCl_2$, CuCl activated the enzymatic oxidation of β-D-glucose by GOD in the experiments done and data obtained from the experiments were shown in Table 2,3,4,5. The results obtained in the

experiments with $HgCl_2$, Hg_2Cl_2, $CuCl_2$, CuCl fitted nonessential activation. The activation contstants, K_A, for $HgCl_2$, Hg_2Cl_2, $CuCl_2$, CuCl were 7.76×10^{-5} M, 3.26×10^{-4} M, 1.12×10^{-4} M, 1.88×10^{-4} M, respectively.

Table 2 Data obtained from the experiments for $HgCl_2$.

$[HgCl_2]$ (M)	[S] (M)	$[S]^{-1}$ (M^{-1})	v ($Msec^{-1}$)	v^{-1} ($secM^{-1}$)
0.25×10^{-4}	0.25×10^{-4}	40000	3.51×10^{-3}	285
	0.50×10^{-4}	20000	3.77×10^{-3}	265
	0.75×10^{-4}	13300	3.93×10^{-3}	254
	1.00×10^{-4}	10000	4.10×10^{-3}	244
	1.25×10^{-4}	8000	4.22×10^{-3}	237
0.50×10^{-4}	0.25×10^{-4}	40000	4.03×10^{-3}	248
	0.50×10^{-4}	20000	4.22×10^{-3}	237
	0.75×10^{-4}	13300	4.31×10^{-3}	232
	1.00×10^{-4}	10000	4.35×10^{-3}	230
	1.25×10^{-4}	8000	4.42×10^{-3}	226
0.75×10^{-4}	0.25×10^{-4}	40000	4.59×10^{-3}	218
	0.50×10^{-4}	20000	4.63×10^{-3}	216
	0.75×10^{-4}	13300	4.61×10^{-3}	217
	1.00×10^{-4}	10000	4.83×10^{-3}	207
	1.25×10^{-4}	8000	4.90×10^{-3}	204

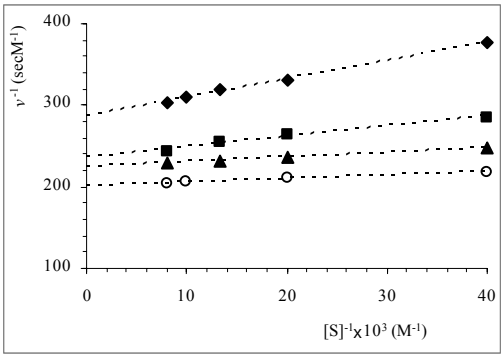


Fig. 2. Effect of $HgCl_2$ on the enzymatic oxidation reaction rate (♦:without $HgCl_2$, ■: 0.25×10^{-4} M $HgCl_2$, ▲:0.50×10^{-4} M $HgCl_2$, ○: 0.75×10^{-4} M $HgCl_2$)

Table 3 Data obtained from the experiments for Hg_2Cl_2.

$[Hg_2Cl_2]$ (M)	[S] (M)	$[S]^{-1}$ (M^{-1})	v ($Msec^{-1}$)	v^{-1} ($secM^{-1}$)
0.25×10^{-4}	0.25×10^{-4}	40000	3.10×10^{-3}	323
	0.50×10^{-4}	20000	3.31×10^{-3}	302
	0.75×10^{-4}	13300	3.40×10^{-3}	294
	1.00×10^{-4}	10000	3.00×10^{-3}	333
	1.25×10^{-4}	8000	3.46×10^{-3}	289
0.50×10^{-4}	0.25×10^{-4}	40000	3.19×10^{-3}	313
	0.50×10^{-4}	20000	3.37×10^{-3}	297
	0.75×10^{-4}	13300	3.44×10^{-3}	291
	1.00×10^{-4}	10000	3.49×10^{-3}	286
	1.25×10^{-4}	8000	3.60×10^{-3}	278
0.75×10^{-4}	0.25×10^{-4}	40000	3.40×10^{-3}	294
	0.50×10^{-4}	20000	3.48×10^{-3}	287
	0.75×10^{-4}	13300	3.49×10^{-3}	286
	1.00×10^{-4}	10000	3.52×10^{-3}	284
	1.25×10^{-4}	8000	3.57×10^{-3}	280

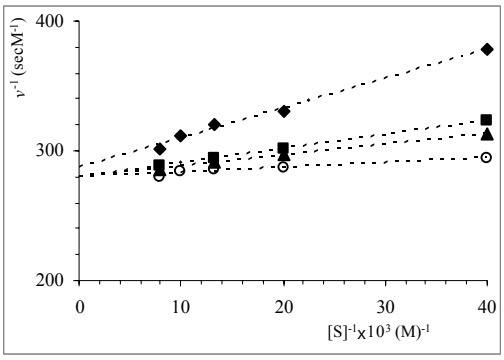


Fig. 3. Effect of Hg_2Cl_2 on the enzymatic oxidation reaction rate (♦:without Hg_2Cl_2, ■:0.25×10^{-4} M Hg_2Cl_2, ▲:0.50×10^{-4} M $HgCl_2$, ○: 0.75×10^{-4} M Hg_2Cl_2)

Table 4 Data obtained from experiments for $CuCl_2$.

$[CuCl_2]$ (M)	[S] (M)	$[S]^{-1}$ (M^{-1})	v ($Msec^{-1}$)	v^{-1} ($secM^{-1}$)
0.25×10^{-4}	0.25×10^{-4}	40000	2.78×10^{-3}	359
	0.50×10^{-4}	20000	3.19×10^{-3}	313
	0.75×10^{-4}	13300	3.30×10^{-3}	303
	1.00×10^{-4}	10000	3.40×10^{-3}	294
	1.25×10^{-4}	8000	3.47×10^{-3}	288
0.50×10^{-4}	0.25×10^{-4}	40000	3.70×10^{-3}	270
	0.50×10^{-4}	20000	3.19×10^{-3}	313
	0.75×10^{-4}	13300	3.78×10^{-3}	264
	1.00×10^{-4}	10000	3.70×10^{-3}	270
	1.25×10^{-4}	8000	3.80×10^{-3}	263
0.75×10^{-4}	0.25×10^{-4}	40000	3.42×10^{-3}	292
	0.50×10^{-4}	20000	3.88×10^{-3}	258
	0.75×10^{-4}	13300	3.91×10^{-3}	256
	1.00×10^{-4}	10000	3.80×10^{-3}	263
	1.25×10^{-4}	8000	4.00×10^{-3}	250

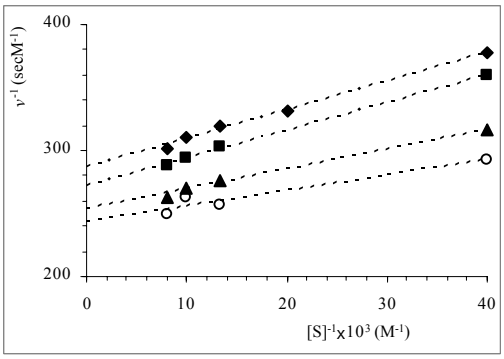


Fig. 4. Effect of $CuCl_2$ on the enzymatic oxidation reaction rate (♦:without $CuCl_2$, ■:0.25×10^{-4} M $CuCl_2$, ▲:0.50×10^{-4} M $CuCl_2$, ○: 0.75×10^{-4} M $CuCl_2$)

4. CONCULIONS

The enzymatic oxidation reaction of *β*-D-glucose in aqueous solution by GOD was investigated by using $NaNO_3$, $HgCl_2$, Hg_2Cl_2, $CuCl_2$ and CuCl. In the experiments, competitive inhibition of $NaNO_3$ was observed. Besides, it was found that the enzymatic reation was activated by $HgCl_2$, Hg_2Cl_2, $CuCl_2$ and CuCl.

In the studies which were done to explain the inhibition mechanism of GOD, it was found that there was an interaction between the FAD group of GOD and the inhibitor when a competetive inhibitor was added to the reaction mixture. This interaction changed the polarization of

enzyme around FAD group and inhibited the enzyme reaction.

Different explanations were done on the determination of the activation mechanism of GOD. GOD containing two moles of FAD group per mole forms ESS inactive complex with substrate. But it forms EAS complex which is more active when activator is added in the reaction mixture. Activator makes a conformation change by forming a complex with FAD which forms active center of the GOD. This change facilitates the transfer of electrons from the substrate to the electron acceptor.

Table 5 Data obtained from the experiments for CuCl .

[CuCl] (M)	[S] (M)	$[S]^{-1}$ (M^{-1})	v ($Msec^{-1}$)	v^{-1} ($secM^{-1}$)
0.25×10^{-4}	0.25×10^{-4}	40000	2.83×10^{-3}	353
	0.50×10^{-4}	20000	3.19×10^{-3}	313
	0.75×10^{-4}	13300	3.40×10^{-3}	294
	1.00×10^{-4}	10000	2.60×10^{-3}	385
	1.25×10^{-4}	8000	3.50×10^{-3}	286
0.50×10^{-4}	0.25×10^{-4}	40000	2.98×10^{-3}	335
	0.50×10^{-4}	20000	3.30×10^{-3}	303
	0.75×10^{-4}	13300	3.49×10^{-3}	286
	1.00×10^{-4}	10000	3.54×10^{-3}	282
	1.25×10^{-4}	8000	3.60×10^{-3}	278
0.75×10^{-4}	0.25×10^{-4}	40000	3.40×10^{-3}	294
	0.50×10^{-4}	20000	3.60×10^{-3}	278
	0.75×10^{-4}	13300	3.70×10^{-3}	270
	1.00×10^{-4}	10000	3.77×10^{-3}	265
	1.25×10^{-4}	8000	3.80×10^{-3}	263

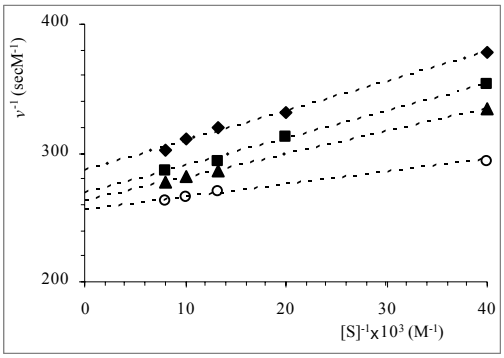


Fig. 5. Effect of CuCl on the enzymatic oxidation reaction rate (♦:without CuCl, ■:0.25×10^{-4} M CuCl, ▲:0.50×10^{-4} M CuCl, ○: 0.75×10^{-4} M CuCl)

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