EFFECT OF SOME POLLUTED METALS ON THE ACTIVITY AND KINETICS OF IMMOBILIZED GLUCOSE ISOMERASE (SWEETZYME TYPE-T) FROM *Streptomyces murinus.*
BY

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ABSTRACT

The effect of sodium (Na^+), potassium (K^+) and manganese (Mn^{++}) ions on the reaction activity of immobilized glucose isomerase (Sweetzyme type-T) indicated that these ions act as activators in the reaction mixture. The reaction activity was stimulated with increasing the above ions concentration up to 0.04M in the presence of (0.01M) Mg^{++} ions. The percentage increment of activity were 95.31%, 86.48% and 115.21% relative to control at concentration 0.04M of Na^+, K^+ and Mn^{++} ions, respectively. However, the results indicated that Cu^{++}, Hg^{++}, Ca^{++} and Fe^{+++} ions act as inhibitors in the reaction media. The sweetzyme type-T lost 98.22%, 98.31, 99.91% and 96.24 of its activity after the addition of 0.7 M of Ca^{++}, Fe^{+++}, Cu^{++} and Hg^{++} ions, respectively.

Reaction kinetics of immobilized glucose isomerase enzyme (sweetzyme type-T) was found to be greatly affected by Cu^{++}, Hg^{++}, and Mn^{++} ions. The maximum reaction velocity (V_{max}) of the enzyme was decreased from 63.89 mM/L/min (in the optimum condition of the enzyme) to 0.99 and 0.47 mM/L/min for Cu^{++} ions and to 7.33 and 4.67 mM/L/min for Hg^{++} ions at concentration of 0.01 and 0.04 M, respectively. On the other hand, the maximum reaction velocity of this enzyme was increased from 63.89 mM/L/min to 119.06 and 137.5 mM/L/min in presence of 0.01 and 0.04 M of Mn^{++} ions, respectively and to 102.78 and 127.9 mM/L/min in the absence of 0.01M Mg^{++} ions under the above concentrations of Mn^{++}, respectively. Michaelis constant (K_m = 0.37mM) was constant in reactions with different concentrations in absence and presence of metal ions. The results showed that noncompetitive type of inhibition occurred in all cases.

INTRODUCTION

The annual consumption of sucrose in Egypt reached to about 1.59 million tons and the major part of this sugar is mainly imported (Ministry of Agriculture, 1994).

Recently, the production of high fructose syrups from hydrolyzed starch by enzymatic isomerization process can certainly cover some shortage of sucrose sugar in the Egyptian market. High fructose corn syrups (HFCS) have rapidly developed as alternatives to sucrose for use in sweetening food products (Coker and Venkata, 1985).

Olivier and du Toit (1986) reported that Mg^{++}, Mn^{++} and Co^{++} were effective on the activity of immobilized enzyme, where as Co^{++} ions most probably fulfill a stabilizing rather than an activating function.

Novo (1992) found that the activity of immobilized glucose isomerase enzyme (Sweetzyme type T) was stimulated in the presence of Mg^{++} ion in the reaction mixture at rate of 8×10^{-4} M. This effect depends upon the substrate concentration, at low glucose concentration the effect of Mg^{++} ion was very great, whereas at high glucose concentration, the enzyme showed a considerable activity even in the absence of Mg^{++} ion.

Schafhauser and Storey (1992) showed that the isomerizing enzyme for glucose required magnesium (Mg^{++}) for the reaction rate of coimmobilized glucose isomerase on biobone.

Converti and Del Borghi (1998) evaluated the kinetic parameters of immobilized glucose isomerase (sweetzyme type-T) from *Streptomyces murinus*. They found that Michaelis constant (K_m) and the maximum reaction velocity (V_{max}) were 0.70 mol/L and 4750 µmol/min/L, respectively. The reaction mixture was carried out under optimal condition at 60°C and pH 7.5 for 30 min.

Hess and Kelly (1999) found that the Michaelis constant (K_m) and the maximum reaction velocity (V_{max}) of soluble and immobilized xylose isomerase, were 15.9 and 27.0 mM and 52.2 and 56.0 U/mg, respectively when using xylose as substrate. While, these parameters $(K_m$ and $V_{max})$ were 88.5 and 115.0 mM and 22.4 and 22.0 U/mg, respectively with glucose as substrate. The reaction was carried out under the optimal conditions at pH 7.0 and 95°C for both of soluble and immobilized forms.

Visuri, *et al.,* (1999) studied the stabilities of native and cross-linked crystalline glucose isomerase forms of *Streptomyces rubiginosus*. They found that the cross-linked crystalline glucose isomerase forms of enzyme was more stable in the presence of substrate while, in a buffer solution the native enzyme was more stable. In the presence of high substrate concentration, the inactivation was related to browning reaction between the enzyme and the reactive sugar, resulting in soluble sugar-protein complexes.

The aim of the present work is to study the effect of sodium (Na^+), potassium (K^+) and manganese (Mn^{++}) ions and other polluted metals i.e. Cu^{++}, Hg^{++}, Ca^{++} and Fe^{+++} on the activity of immobilized glucose isomerase enzyme (Sweetzyme type-T). These metals might contaminate as trace in the media of reaction from the tanks and polluted water during the action of this enzyme. Also,

evaluation the effect of metals on the kinetics of the enzyme under investigation is very useful for determining the mode of action, since these parameters are very important from the industrial and economical point of view.

MATERIALS AND METHODS

1. Immobilized glucose isomerase enzyme (sweetzyme type-T), E.C.5.5.99.1.
D. glucose-ketal-isomerase was supplied by NOVO Industria A/S, Denmark. It was obtained from a strain of *Streptomyces murinus*. Glucose and fructose sugars (Sigma Chem., Co.) were used as standards. All other chemical of metals ions used in this work were obtained commercially and highest purity available.

2.1. Kinetic of immobilized glucose isomerase enzyme (sweetzyme type-T) in the absence of metals ions:
Different concentrations of glucose solutions were used, i.e. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mM. The optimum conditions for enzyme activity was carried out at 60°C with incubation periods of 60 min at pH 7.5 by 0.1 M Tris-buffer (Converti and Del Borghi, 1998). The produced fructose sugar was determined by L-Cysteine Carbazole-H_2SO_4 method as described by Dische and Borefreund (1951).

2.2. Measurement of the activity of immobilized glucose isomerase enzyme (sweetzyme type-T) in the presence of metals ions:
Different concentrations of each Na^+, K^+, Mn^{++}, Cu^{++}, Hg^{++}, Ca^{++}, and Fe^{+++} ions were used, i.e. 0.01, 0.04 and 0.07 M. This experiments were carried out under at the same conditions (substrate concentration 0.9mM, pH 7.5, temperature 60°C and incubated for 60 min). The obtained fructose content was determined by the method as mentioned before. A blank experiment was carried out under the same conditions.

3.1. Kinetics of sweetzyme (type-T) enzyme in the presence of Cu^{++} and Hg^{++} ions:-
Two concentrations of metals ions i.e. 0.01 and 0.04M were used in the kinetics experiment with different concentrations of glucose as substrate, i.e., 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mM. The reaction activity of sweetzyme (type-T) was determined by the methods as described by Novo (1993).

The resulted fructose was estimated according to the method of Dische and Borefreund (1951). Michaelis constant (K_m), enzyme inhibitor dissociation constant (K_i) were obtained according to the method of calculation which mentioned by Plummer (1978).

RESULTS AND DISCUSSION

1- Effect of Mn^{++}, Na^+ and K^+ ions on the reaction activity of immobilized glucose isomerase (Sweetzyme type-T) in the presence of 0.01M Mg^{++} ions.

Different concentrations of the above ions i.e 0.01, 0.04 and 0.07 M were used in reaction mixture of isomerizing glucose to high-fructose by using sweetzyme type-T. The obtained results in Table (1) show that Mn^{++}, Na^+ and K^+ ions act as activators. At 0.04 M concentration of ions gave the highest percentage increment of activity (115.21, 95.31 and 86.48%) while, at concentration of 0.07 M, the percentage increment in activity reached (89.20, 75.80 and 70.05%) relative to control.

This increment was higher than that obtained by Benaiges *et al.* (1986) using 0.03M Mg^{++} ions with the same concentration of Mn^{++} ions.

Table (1): Effect of Mn^{++}, Na^+ and K^+ ions on the reaction activity of immobilized glucose isomerase enzyme in the presence of (0.01 M) Mg^{++} ion.

Metal ions concentration [M]	Fructose content mM/L			Reaction activity mM/L/min			Percentage increment of activity (%)		
	Mn^{++}	Na^+	K^+	Mn^{++}	Na^+	K^+	Mn^{++}	Na^+	K^+
0.00 (Control)	63.89	63.89	63.89	1.065	1.065	1.065	-	-	-
0.01	102.78	98.27	87.21	1.713	1.638	1.454	60.84	53.80	36.53
0.04	137.50	124.81	119.15	2.292	2.080	1.986	115.21	95.31	86.48
0.07	121.87	112.24	108.68	2.015	1.871	1.811	89.20	75.80	70.05

This phenomenon might be attributed to the autoinhibition effect occurred by excess of metal ions, Olivier and du Toit (1986). On the other hand, the percentage increment of activity reached to 95.31% and 86.48% relative to control at concentration 0.04 M of Na^+ and K^+, respectively.

2. Effect Cu^{++}, Hg^{++}, Ca^{++} and Fe^{+++} ions on the reaction activity of Sweetzyme type-T.

Three different concentrations of the above metal ions i.e. 0.01, 0.04 and 0.07 M were used to study the effect of these metal ions on the reaction activity of the enzyme. The results are shown in Table (2). A great decrease was noticed in reaction activity at 0.07 M concentration of Cu^{++} and Hg^{++} metal ions i.e from 1.065 mM/L/min (without metal ions, control) to 0.001 mM/L/min, 0.04 mM/L/min for Cu^{++} and Hg^{++} ions, respectively. However, the enzyme lost 99.8% of its activity after the addition of (0.01 M) Cu^{++} ions and 88.5% of its activity at the same concentration of Hg^{++} ions. On the other hand, Ca^{++} and Fe^{+++} ions were found to more effected on the activity of immobilized glucose isomerase than Hg^{++} ions.

Table (2): Effect of metal ions on the reaction activity of immobilized glucose isomerase enzyme in the presence of (0.01 M) Mg^{++} ion.

Metal ions conc. [M]	Fructose content mM/L				Reaction activity mM/L/min				Percentage loss of activity (%)			
	Ca^{++}	Fe^{+++}	Cu^{++}	Hg^{++}	Ca^{++}	Fe^{+++}	Cu^{++}	Hg^{++}	Ca^{++}	Fe^{+++}	Cu^{++}	Hg^{++}
0.00 (Control)	63.89	63.89	63.89	63.89	1.065	1.065	1.065	1.065	-	-	-	-
0.01	5.72	4.61	0.14	7.22	0.095	0.077	0.002	0.122	91.08	92.77	99.81	88.54
0.04	2.17	2.89	0.30	2.39	0.036	0.480	0.005	0.040	96.62	95.49	99.53	96.24
0.07	1.13	1.06	0.04	2.44	0.019	0.018	0.001	0.040	98.22	98.31	99.91	96.24

The inhibition effect produce by Cu^{++}, Hg^{++}, Ca^{++} and Fe^{+++} ions on sweetzyme type-T might be attributed to the binding effect with some specific function groups on the enzymatic protein chain, Hughes (1981). These ions can bind as chelating agent with sulfhydryl (-SH), hydroxyl (-OH), and carboxylic (-COOH) groups on the protein enzyme chain. Such binding might cause a change in its conformation leading to less performance in enzyme action. The same interpretation was introduced by El-Asar and Abbas (1988). However, Ca^{++} and Fe^{+++} ions might be chelated towards protein enzyme molecule more than Cu^{++} and Hg^{++} ions.

3. **Effect Cu^{++}, Hg^{++} and Mn^{++} ions at different concentrations on reaction kinetics of sweetzyme type -T:**
Different concentrations of the above metal ions i.e. 0.01 and 0.04 M were used to study the reaction kinetics of enzyme (K_m, K_i and V_{max}) by using different concentrations of glucose syrup i.e., 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mM. All the experiments were carried out at optimum conditions of this enzyme i.e. pH 7.5, at 60°C and incubation time of 60 min. The obtained results are shown in Tables (3, 4, 5 and 6). From these results the maximum reaction velocity (V_{max}) of sweetzyme type-T was greatly decreased at different concentrations of Cu^{++} and Hg^{++} ions, while the effect of Mn^{++} ions on the reaction velocity was greatly increased comparing with control. The maximum reaction velocity (V_{max}) of the enzyme were 63.89 mM/L/min (without Cu^{++} and Hg^{++} ions), while it reached to 0.99 and 0.47 mM/L/min for Cu^{++} ions and 7.33 and 4.67 mM/L/min for Hg^{++} ion at concentrations 0.01 and 0.04 M for the above metal ions, respectively.

On the other hand, the V_{max} of sweetzyme at 0.01 and 0.04 M of Mn^{++} ions in the presence of 0.01 M Mg^{++} ions were 119.06 mM/L/min and 137.5 mM/L/min. But in absence of Mg^{++} ions, V_{max} were 102.78 mM/L/min and 127.9 mM/L/min with 0.01 M and 0.04 M Mn^{++} ions, respectively. The maximum reaction velocity (V_{max}) of the enzyme was higher with Mn^{++} ions and Mg^{++} ions at 0.01 M in reaction mixture, this may be due to competition between the two different ions in the reaction media which may be favoured for Mn^{++} ions (Bell, 1977).

Lineweaver-Burk (1954) plots 1/(S) against 1/V of enzyme in absence and presence of inhibitors and activators are shown in Figures (1, 2 and 3 a, b).

Table (3): Effect of Cu^{++} ions at different concentrations on the reaction kinetics of glucose isomerase (sweetzyme type-T) in the presence of 0.01 M Mg^{++} ions.

Substrate	Fructose content mM/L			Reaction velocity (v)				$1/v \times 10^{-2}$		
Conc. (S) mM	Conc. of added Cu^{++} ions			Concentrations of Cu^{++} ions			1/(S)	Conc. of Cu^{++} ions		
	Normal	0.01M	0.04M	Normal	0.01M	0.04M		Normal	0.01M	0.04M
0.1	13.33	0.46	0.27	13.62	12.49	9.43	10.00	7.34	8.01	10.60
0.2	19.44	0.84	0.42	22.46	20.59	15.55	5.00	4.45	4.86	6.43
0.3	22.22	0.90	0.43	28.66	26.27	19.84	3.33	3.49	3.81	5.04
0.4	27.78	0.99	0.43	33.25	30.48	23.02	2.50	3.01	3.28	4.34
0.5	40.28	0.86	0.47	36.78	33.72	25.46	2.00	2.72	2.97	3.93
0.6	44.44	0.54	0.43	39.59	36.29	27.41	1.67	2.53	2.76	3.65
0.7	49.99	0.47	0.37	41.87	38.38	28.99	1.43	2.39	2.61	3.45
0.8	61.11	0.45	0.33	43.76	40.11	30.30	1.25	2.29	2.43	3.30
0.9	63.89	0.44	0.30	45.35	41.57	31.40	1.11	2.21	2.41	3.18
1.0	45.83	0.32	0.29	46.72	42.83	32.35	1.00	2.41	2.33	3.09

Table (4): Effect of Hg^{++} ions at different concentrations on the reaction kinetics of sweetzyme type-T in the presence of 0.01 M Mg^{++} ions.

Substrate	Fructose content mM/L			Reaction velocity (v)				$1/v \times 10^{-2}$		
Conc. (S) mM	Conc. of added Hg^{++} ions			Concentrations of Hg^{++} ions			1/(S)	Conc. of Hg^{++} ions		
	Normal	0.01M	0.04M	Normal	0.01M	0.04M		Normal	0.01M	0.04M
0.1	13.33	2.11	2.78	13.62	12.38	8.67	10.00	7.34	8.08	11.53
0.2	19.44	3.28	3.56	22.46	20.42	14.29	5.00	4.45	4.90	7.00
0.3	22.22	4.11	3.61	28.66	26.05	18.24	3.33	3.49	3.84	5.48
0.4	27.78	5.06	4.39	33.25	30.23	21.16	2.50	3.01	3.31	4.73
0.5	40.28	5.56	4.67	36.78	33.44	23.41	2.00	2.72	2.99	4.27
0.6	44.44	5.50	4.52	39.59	35.99	25.19	1.67	2.53	2.78	3.97
0.7	49.99	6.67	3.78	41.87	38.06	26.64	1.43	2.39	2.63	3.75
0.8	61.11	7.11	3.06	43.76	39.78	27.85	1.25	2.29	2.51	3.59
0.9	63.89	7.33	2.39	45.35	41.23	28.86	1.11	2.21	2.43	3.47
1.0	45.83	7.22	2.06	46.72	42.47	29.73	1.00	2.41	2.35	3.36

Table (5) Effect of Mn⁺⁺ ions at different concentrations on the kinetic of glucose isomerase enzyme (sweetzyme type-T)

Mn⁺⁺ ion concentrations 0.01M

Substrate Conc. mM (S)	1/(S)	P* Fructose content mM/L	P* Reaction Velocity (V)	P* 1/V ×10⁻²	P* Reaction activity mM/L min	P* Increment of activity %	A* Fructose content mM/L	A* Reaction Velocity (V)	A* 1/V ×10⁻²	A* Reaction activity mM/L/min	A* Increment of activity %
0.10	10.0	38.33	24.20	4.13	0.64	-40.0	32.22	22.64	4.42	0.54	-49.6
0.20	5.00	60.89	40.22	2.49	1.02	-4.7	39.72	37.11	2.69	0.58	-45.6
0.30	3.33	65.44	51.62	1.94	1.10	+2.4	57.46	47.16	2.12	0.96	-10.1
0.40	2.50	79.17	60.13	1.66	1.32	+23.0	62.50	54.54	1.83	1.04	-2.2
0.50	2.00	81.20	66.79	1.50	1.35	+27.0	67.25	60.19	1.66	1.12	+5.3
0.60	1.67	83.33	72.01	1.39	1.39	+30.3	69.44	64.65	1.55	1.16	+8.6
0.70	1.43	92.07	76.32	1.31	1.54	+44.1	78.68	68.27	1.47	1.31	+23.1
0.80	1.25	119.06	79.91	1.25	1.99	+86.3	102.78	71.27	1.40	1.71	+60.9
0.90	1.11	77.86	82.94	1.21	1.30	+21.9	86.61	73.78	1.36	1.44	+35.6
1.00	1.00	56.94	85.53	1.17	0.95	-10.9	59.72	75.92	1.32	1.00	-6.5

P* Presence of (0.01 M) Mg⁺⁺

Table (5): Continued

Mn⁺⁺ ion concentrations 0.04M

Substrate Conc. mM (S)	1/(S)	P* Fructose content mM/L	P* Reaction Velocity (V)	P* 1/V ×10⁻²	P* Reaction activity mM/L/min	P* Increment of activity %	A* Fructose content mM/L	A* Reaction Velocity (V)	A* 1/V ×10⁻²	A* Reaction activity mM/L/min	A* Increment of activity %
0.10	10.00	98.61	23.58	4.24	1.64	+54.4	88.21	21.75	4.60	1.47	+38.0
0.20	5.00	115.28	40.26	2.48	1.92	+80.4	104.72	37.18	2.69	1.75	+63.9
0.30	3.33	120.56	52.68	1.90	2.01	+88.6	105.50	48.69	2.05	1.76	+65.1
0.40	2.50	122.22	62.29	1.61	2.04	+91.3	106.94	57.61	1.74	1.78	+67.3
0.50	2.00	127.78	69.94	1.43	2.13	+100.0	114.83	64.73	1.54	1.91	+79.7
0.60	1.67	130.56	76.18	1.31	2.18	+104.3	116.67	70.53	1.42	1.94	+82.6
0.70	1.43	135.80	81.36	1.23	2.26	+112.5	123.31	75.36	1.33	2.06	+92.9
0.80	1.25	137.50	85.74	1.17	2.29	+115.2	127.87	79.44	1.26	2.13	+100.0
0.90	1.11	126.70	89.48	1.12	2.11	+98.3	102.78	82.93	1.21	1.71	+60.9
1.00	1.00	104.17	92.72	1.08	1.74	+63.0	91.67	85.95	1.16	1.53	+43.5

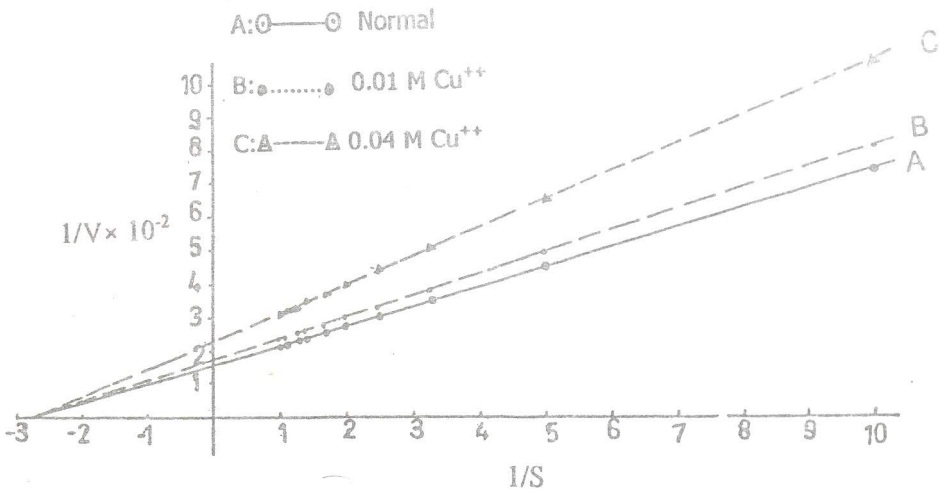P* Presence of (0.01 M) Mg⁺⁺   A* Absence of (0.01 M) Mg⁺⁺

Fig (1): Lineweaver-Burk plot of glucose isomerase enzyme (Sweetzyme type-T) in the presence of Cu^{++} ions.
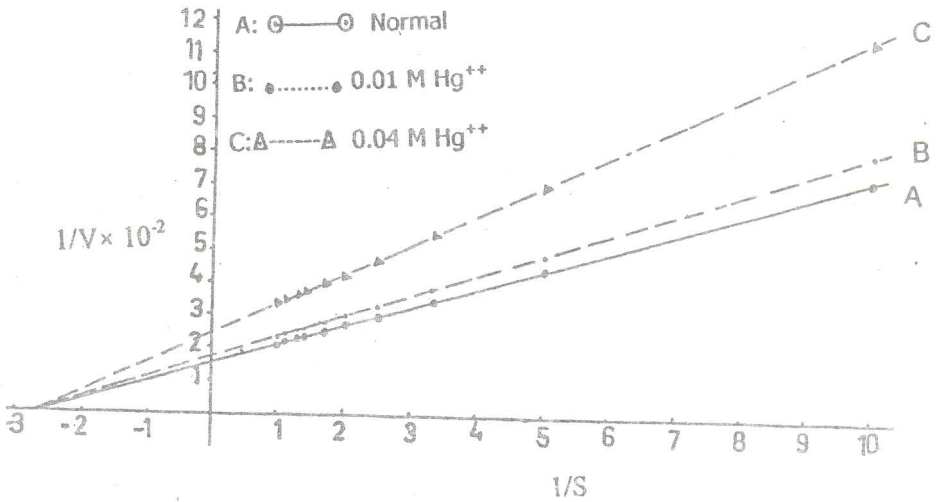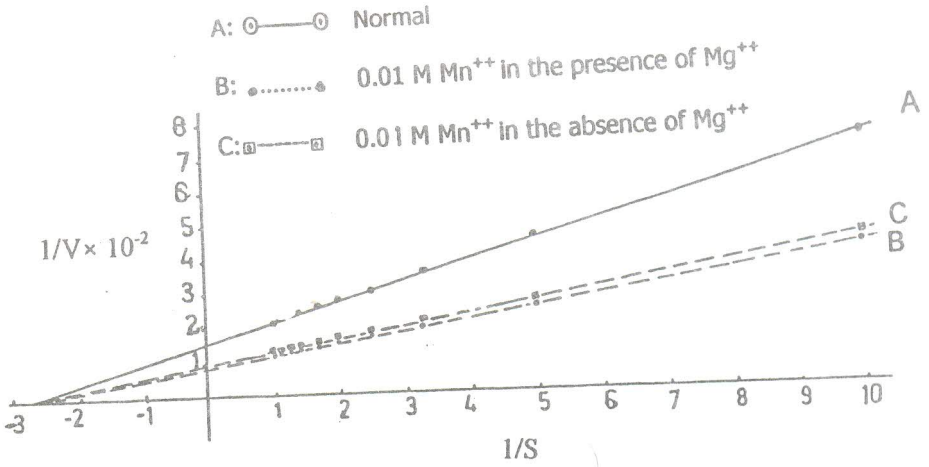

Fig (2): Lineweaver-Burk plot of glucose isomerase enzyme (Sweetzyme type-T) in the presence of Hg^{++} ions.
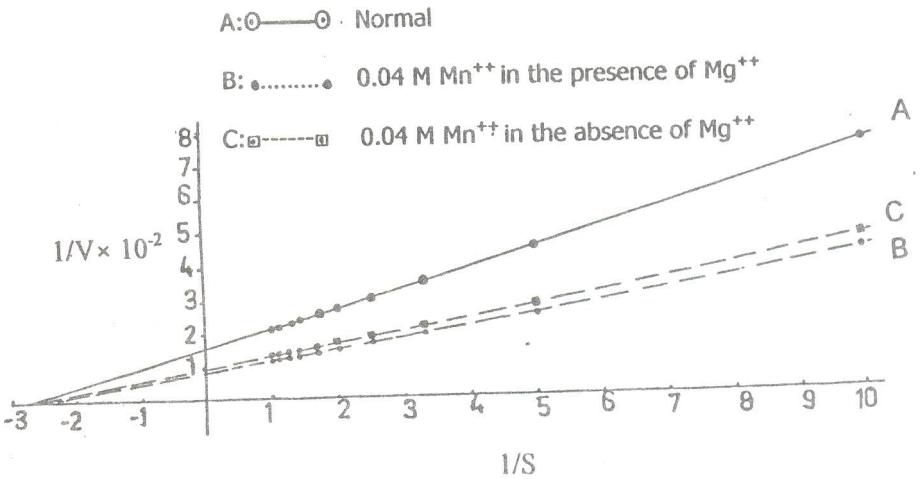
a

b

Fig (3 a, b): Effect of Mn++ ions on the Lineweaver-Burk plot of glucose isomerase enzyme (Sweetzyme type-T) in the presence and absence of Mg++ ions.

From these Figures, it might be observed that V_{max} of sweetzyme type-T in the absence and presence of inhibitors and activators was greatly affected while, K_m was constant and equaled to 0.37 mM. It can be concluded that the inhibition is non-competitive type in all cases. These results are consident with those obtained by Converti and Del Borghi (1998) and those mentioned by Plummer (1978).

Table (6): Effect of metal ions on K_i and V_{max} of immobilized glucose isomerase enzyme (sweetzyme type-T) in the presence of 0.01 M Mg^{++} ions.

Concentration of metal ions [M]	K_i (mM)		V_{max} (mM/L/min)	
	Cu^{++}	Hg^{++}	Cu^{++}	Hg^{++}
0.01	0.90	0.07	0.99	7.33
0.04	0.11	0.10	0.47	4.67

The most common type of noncompetitive inhibition is given by reagents that can combine reversibly with some functional group of the enzyme (Outside the active site) that is essential for maintaining the catalytically active three-dimensional conformation of the enzyme molecule. Some enzymes possessing an essential-SH group are noncompetitively inhibited by heavymetal ions suggesting that such-SH groups must be intact for the enzyme to retain its normal conformation (Segel, 1975).

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تأثير بعض أيونات المعادن الملوثة على نشاط وحركية إنزيم الجلوكوز
أيزوميريز المحمل (سويت زيم-ت) المنتج من الأستيربتوميس مورونيس

فرحات فوده على فوده ، أحمد على أحمد عبد الرحمن
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يهدف هذا البحث إلى دراسة تأثير أيونات الصوديوم والبوتاسيوم والمنجنيـــز والكالسيوم وبعض أيونات المعادن الثقيلة مثل الزئبقيك والنحاسيك والحديديــك علــى درجة نشاط إنزيم الجلوكوز أيزوميريز المحمل (سويت زيم- ت) وكذلك دراسة تـأثير بعض هذه الأيونات مثل النحاس والزئبقيك على الكينيتكية لهذا الإنزيم حيــث أن هــذه الأيونات يمكنها أن تأتى إلى وسط التفاعل مــن المـاء المسـتخدم أو مــن خزانـات المتفاعلات المستخدمة لإنتاج شراب عالى الفراكتوز.

وقد أظهرت النتائج أن أيونات الصوديـوم والبوتاسيوم والمنجنيـز تعمــل كمنشطات فى وسط التفاعل وذلك فى وجود أيون الماغنسيوم تحت تركيز ٠.٠١ مولر حيث أدى وجود هذه الأيونات إلى زيادة درجة النشاط الإنزيمــى بمقـدار ٣١ر٩٥%, ٤٨ر٨٦%, ٢١ر١١٥% على التوالى تحت تركيز ٠.٠٤ مولر من الأيونـات سـابقة الذكر. بينما أوضحت النتائج أن أيونات الكالسيوم والحديديك والنحاسيك والزئبقيك تقوم بعامل التثبيط فى وسط التفاعل وتزداد درجة التثبيط بزيادة تركيز هذه الأيونات حيـث أن درجة التثبيط وصلت إلى ٦٢ر٩٦%, ٤٩ر٩٥%, ٥٣ر٩٩%, ٢٤ر٩٦% للأيونـات سابقة الذكر على التوالى تحت تركيز ٠.٠٤ مولر ويتضح من هذه النتائج أن لأيــون النحاسيك درجة تثبيطة مرتفعة حتى فى التركيزات المنخفضــة حيــث فقـد الأنزيم

91ر99% من درجة نشاطه تحت تركيز 01ر0 مولر بينما أيون الحديديك أدى إلــــى فقد 31ر98% من درجة نشاط الأنزيم تحت تركيز 07ر0 مولر.

وقد أظهرت الدراسة الحركية لإنزيم الجلوكوز أيزوميريز المحمل أن السرعة القصوى (V_{max}) لهذا الإنزيم قد تأثرت بدرجة كبيرة فى وجـــــود أيونـــات النحاسيك والزئبقيك وذلك فى وجود أيون الماغنسيوم 01ر0 مولـــر فقـد إنخفضـت السـرعة القصوى من 89ر63 ملليمول/لتر (وذلك فى عدم وجود الأيونات المثبطة) إلى 99ر0 ملليمول/لتر, 47ر0 ملليمول/لتر فى وجود تركيزات 01ر0 مولر , 04ر0 مولر مـن أيون النحاسيك على التوالى. بينما فى وجود أيون الزئبقيك وتحت نفـس التركيـزات سابقة الذكر إنخفضت السرعة القصــوى مـن 89ر63 ملليمـول/لـتر إلـى 33ر7 ملليمول/لتر, 67ر4 ملليمول/لتر على التوالى . ومن ناحية أخرى أظهرت النتـائج أن السرعة القصوى لهذا الإنزيم إزدادت من 89ر63 ملليمـول/لـتر (الحالـة القياسـية للأنزيم) إلـى 06ر119 , 50ر137 ملليمـول/لـتر , إلـى 78ر102 , 90ر127 ملليمول/لتر تحت تركيزات 01ر0 , 04ر0 مولرمن أيونات المنجنيز وذلك فى وجود أوغياب 01ر0 مولر من أيونات الماغنسيوم على التوالى. كما أظهرت النتائج أن ثابت ميكاليس (K_m) ثابت ولم يتأثر فى حالة وجود أو غياب هــذه الأيونـات بينمـا تتـأثر السرعة القصوى للإنزيم لذلك يتضح أن نوع التثبيط فى الحالات السابقة مـــن النـوع الغير متنافس ويرجع ذلك إلى أن هذه الأيونات يمكنها أن ترتبط بالمجـاميع الفعالـة للأنزيم خارج مراكز النشاط وتحدث تغير فى الشكل التكوينى لجزئ الأنزيــم والــذى يؤثر على درجة النشاط الأنزيمى.