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# 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 $8\times10^{-4}$ 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. | Fructose content mM/L | | | | Reaction activity mM/L/min | | | | Percentage loss of activity (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| [M] | $Ca^{++}$ | $Fe^{+++}$ | $Cu^{++}$ | $Hg^{++}$ | $Ca^{++}$ | $Fe^{+++}$ | $Cu^{++}$ | $Hg^{++}$ | $Ca^{++}$ | $Fe^{+++}$ | $Cu^{++}$ | $Hg^{++}$ |
| 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 Conc. (S) mM | Fructose content mM/L | | | Reaction velocity (v) | | | 1/(S) | $1/v \times 10^{-2}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Conc. of added $Cu^{++}$ ions | | | Concentrations of $Cu^{++}$ ions | | | | 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 Conc. (S) mM | Fructose content mM/L | | | Reaction velocity (v) | | | 1/(S) | $1/v \times 10^{-2}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Conc. of added $Hg^{++}$ ions | | | Concentrations of $Hg^{++}$ ions | | | | 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)

| Substrate Conc. mM (S) | 1/(S) | $Mn^{++}$ ion concentrations 0.01M P* Fructose content mM/L | Reaction Velocity (V) | 1/V ×10⁻³ | Reaction activity mM/L/min | Increment of activity % | A* Fructose content mM/L | Reaction Velocity (V) | 1/V ×10⁻³ | Reaction activity mM/L/min | 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 | -6.6 |

Table (5): Continued

| Substrate Conc. mM (S) | 1/(S) | Mn++ ion concentrations 0.04M P* Fructose content mM/L | Reaction Velocity (V) | 1/V ×10⁻³ | Reaction activity mM/L/min | Increment of activity % | A* Fructose content mM/L | Reaction Velocity (V) | 1/V ×10⁻³ | Reaction activity mM/L/min | 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) $Mg^{++}$

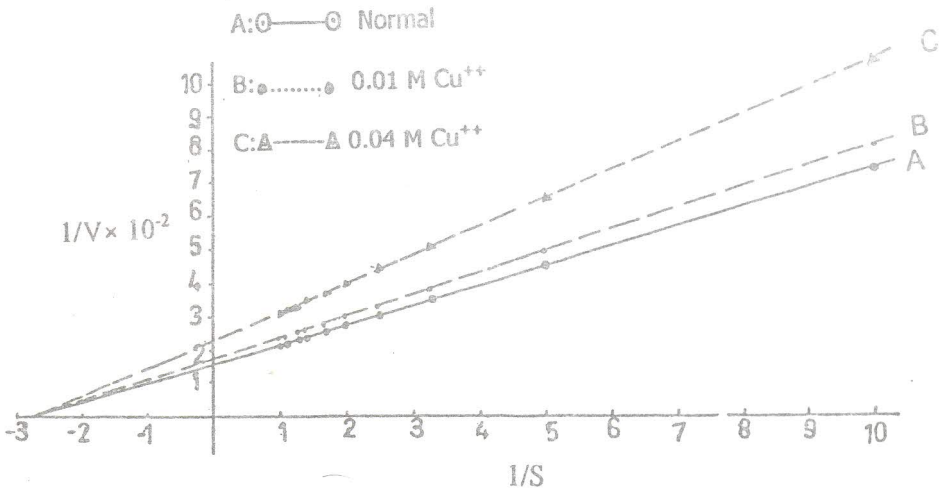



Fig (1): Lineweaver-Burk plot of glucose isomerase enzyme (Sweetzyme type-T) in the presence of $Cu^{++}$ ions.

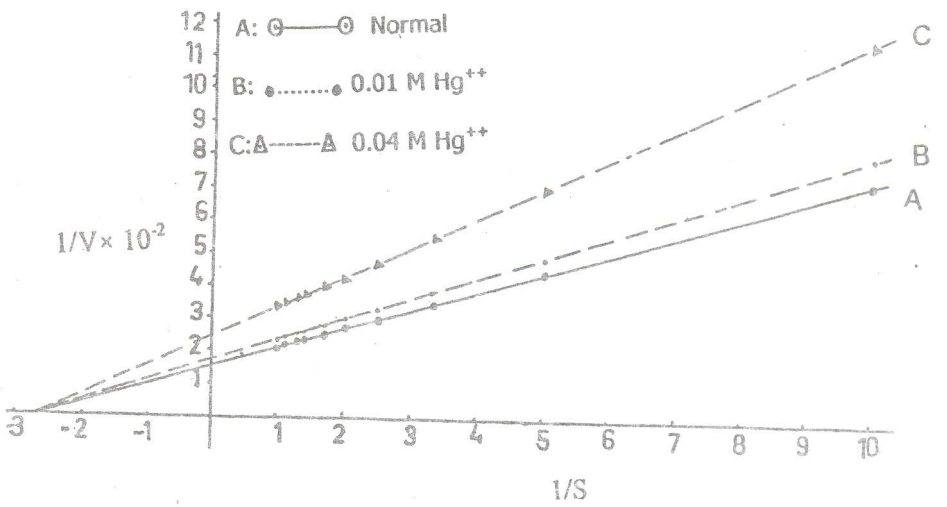
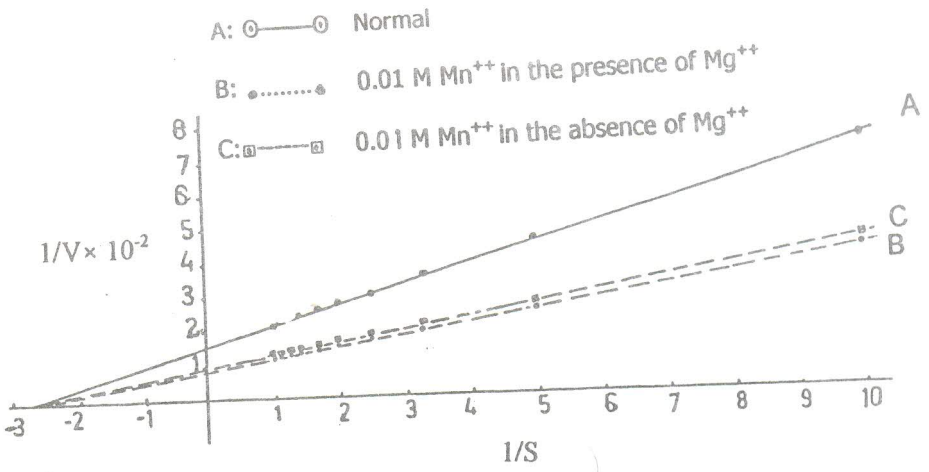



Fig (2): Lineweaver-Burk plot of glucose isomerase enzyme (Sweetzyme type-T) in the presence of $Hg^{++}$ ions.

a




b

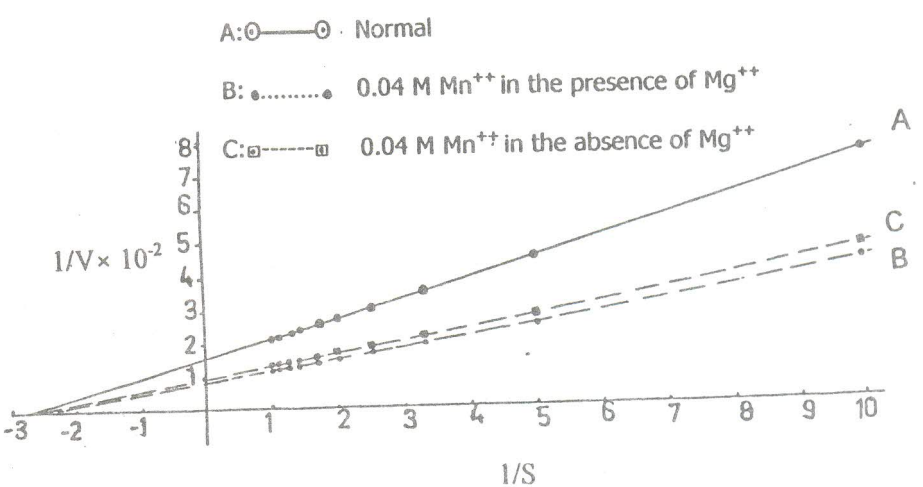



Fig (3 a, b): Effect of Mn++ ions on the Lineweaver-Burk plot of glucose isomerase enzyme (Sweetzyme type-T) in the presence and absence of $Mg^{++}$ ions.

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 | $K_i$ (mM) | | $V_{max}$ (mM/L/min) | |
|---|---|---|---|---|
| [M] | $Cu^{++}$ | $Hg^{++}$ | $Cu^{++}$ | $Hg^{++}$ |
| 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 الأستيربتوميسس مورونيس

**فرحات فوده على فوده ، أحمد على أحمد عبد الرحمن**

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يهدف هذا البحث إلى دراسة تأثير أيونات الصوديوم والبوتاسيوم والمنجنيز والكالسيوم وبعض أيونات المعادن الثقيلة مثل الزئبقيك والنحاسيك والحديديك على درجة نشاط إنزيم الجلوكوز أيزوميريز المحمل (سويت زيم- ت) وكذلك دراسة تأثير بعض هذه الأيونات مثل النحاس والزئبقيك على الكينيتكية لهذا الإنزيم حيث أن هذه الأيونات يمكنها أن تأتى إلى وسط التفاعل من الماء المستخدم أو من خزانات المتفاعلات المستخدمة لإنتاج شراب عالى الفراكتوز.

وقد أظهرت النتائج أن أيونات الصوديوم والبوتاسيوم والمنجنيز تعمل كمنشطات فى وسط التفاعل وذلك فى وجود أيون الماغنسيوم تحت تركيز ٠ر٠١ مولر حيث أدى وجود هذه الأيونات إلى زيادة درجة النشاط الإنزيمى بمقدار ٣١ر٩٥%, ٤٨ر٨٦%, ٢١ر١١٥% على التوالى تحت تركيز ٠ر٠٤ مولر من الأيونات سابقة الذكر. بينما أوضحت النتائج أن أيونات الكالسيوم والحديديك والنحاسيك والزئبقيك تقوم بعامل التثبيط فى وسط التفاعل وتزداد درجة التثبيط بزيادة تركيز هذه الأيونات حيث أن درجة التثبيط وصلت إلى ٦٢ر٩٦%, ٤٩ر٩٥%, ٥٣ر٩٩%٢٤ر٩٦% للأيونات سابقة الذكر على التوالى تحت تركيز ٠ر٠٤ مولر ويتضح من هذه النتائج أن لأيون النحاسيك درجة تثبيطة مرتفعة حتى فى التركيزات المنخفضة حيث فقد الأنزيم

٩١ر٩٩% من درجة نشاطه تحت تركيز ٠١ر٠ مولر بينما أيون الحديديك أدى إلى فقد ٣١ر٩٨% من درجة نشاط الأنزيم تحت تركيز ٠٧ر٠ مولر.

وقد أظهرت الدراسة الحركية لإنزيم الجلوكوز أيزوميريز المحمل أن السرعة القصوى ($V_{max}$) لهذا الإنزيم قد تأثرت بدرجة كبيرة فى وجود أيونات النحاسيك والزئبقيك وذلك فى وجود أيون الماغنسيوم ٠١ر٠ مولر فقد إنخفضت السرعة القصوى من ٨٩ر٦٣ ملليمول/لتر (وذلك فى عدم وجود الأيونات المثبطة) إلى ٩٩ر٠ ملليمول/لتر, ٤٧ر٠ ملليمول/لتر فى وجود تركيزات ٠١ر٠ مولر , ٠٤ر٠ مولر من أيون النحاسيك على التوالى. بينما فى وجود أيون الزئبقيك وتحت نفس التركيزات سابقة الذكر إنخفضت السرعة القصوى من ٨٩ر٦٣ ملليمول/لتر إلى ٣٣ر٧ ملليمول/لتر, ٦٧ر٤ ملليمول/لتر على التوالى . ومن ناحية أخرى أظهرت النتائج أن السرعة القصوى لهذا الإنزيم إزدادت من ٨٩ر٦٣ ملليمول/لتر (الحالة القياسية للأنزيم) إلى ٠٦ر١١٩ , ٥٠ر١٣٧ ملليمول/لتر , إلى ٧٨ر١٠٢ , ٩٠ر١٢٧ ملليمول/لتر تحت تركيزات ٠١ر٠ , ٠٤ر٠ مولرمن أيونات المنجنيز وذلك فى وجود أوغياب ٠١ر٠ مولر من أيونات الماغنسيوم على التوالى. كما أظهرت النتائج أن ثابت ميكاليس ($K_m$) ثابت ولم يتأثر فى حالة وجود أو غياب هذه الأيونات بينما تتأثر السرعة القصوى للإنزيم لذلك يتضح أن نوع التثبيط فى الحالات السابقة من النوع الغير متنافس ويرجع ذلك إلى أن هذه الأيونات يمكنها أن ترتبط بالمجاميع الفعالة للأنزيم خارج مراكز النشاط وتحدث تغير فى الشكل التكوينى لجزئ الأنزيم والذى يؤثر على درجة النشاط الأنزيمى.