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Studies on the combined action of amylases and glucose isomerase on starch and its hydrolyzate Part I. Production, extraction, purification and kinetic behavior of glucose isomerase

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With 15 figures and 11 tables

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1. Introduction

The annual consumption of sucrose in A.R.E. exceeds 15 million tons and a major part of this sugar is imported. The shortage of sucrose can be partially covered by enzymatic conversion (glucose isomerase) of glucose syrup which is manufactured of low price corn starch. Glucose isomerase has many other applications (1).

First report for isomerization of free glucose by microorganism was shown by *Marshall* and *Kooi* (2) and *Marshall* (3). Many investigators studied various microorganisms as a source of glucose isomerase *(Marshall* and *Kooi* (2), *Tsumura* and *Sato* (4), *Natake* and *Yoshimura* (5, 6, 7)) and all reports give evidence that *Streptomyces* sp. is often used to produce glucose isomerase. Many methods were used for purification of extracted crude glucose isomerase by *Sumura* and *Sato* (8, 9, 10), *Nataka* (11), *Yamanaka* (12), *Danno* (13) and *Standberg and Smiley* (14). They also studied some factors affecting reaction velocity of glucose isomerase.

Materials and methods

Materials

1. Source of microorganism. *Streptomyces phaeochromogenes* which produces glucose isomerase enzyme was kindly supplied the Agriculture Research Service Culture Collection, Peoria, Illinois, U.S.A.

2. Glucose oxidase enzyme was obtained from a strain of *Penicillium crysogenum*-811 and was kindly supplied by Enz. Res. Unit, Agric. Res. Center of Egypt, with an activity equal to 500 Unit Perg gram.
3. D(+) glucose and D(-) fructose chromatographically pure were obtained from B.D.H. (England).

Methods

A. Microbiological methods

1. Medium: The medium recommended by *Tsumura* and *Sato* (10) was used for enzyme production.
2. Fermentation: Fermentation was carried out aerobically at 28 °C for 24 hrs, using glass fermentor 5 liter (bioflo chemstat, New Burnewick). The medium (3 L.) in the fermentor was sterilized at 120 °C for 20 min. Then inoculated with the seeding material (30 ml). At the end of fermentation period, the cells were harvested by centrifugation at 5000 r.p.m. for 15 min.
3. Preparation of enzyme extract: The washed cells were suspended in distilled water and subjected to ultrasonic wave at 10 KC for 10 min using MSE 100 Watt Ultrasonic Disintegrator N. 26131, London.

B. Chemical methods

1. Fructose was determined in presence of glucose according to the method of *Dische* et al. (15) and modified by *Marshall* and *Kooi* (2).
2. Glucose isomerase activity was measured according to the method of *Standberg* and *Smiley* (14).
3. Glucose was determined in presence of other reducing sugars by the method of *Brady* and *Zagorski* (16).
4. pH was reading by using Beckman Zeromatic pH meter.
5. Precipitation of glucose isomerase by salting out with ammonium sulphate (*Dixon* and *Webb* [17]).
   Twelve concentrations of ammonium sulphate, i.e., 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60%, were tested for precipitating glucose isomerase. In all experiments enzyme extract (10 ml, 340 units/ml) was salted out with $(NH_4)_2 SO_4$. The mixture was kept for 6 hrs at 4 °C before separation by centrifugation. The enzyme precipitate was dissolved in distilled water (10 ml) and the enzyme recovery was determined.
6. Precipitation by using organic solvents (*Dixon* and *Webb* [17]).
   Ethanol, isopropanol and acetone were used separately in different concentrations 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 vol. of solvent per vol. of enzyme extract. The mixture was kept for 6 hrs at 4 °C. The precipitate was separated by centrifugation, then dissolved in distilled water (10 ml) and the recovered enzyme was determined.

Factors affecting the kinetic behavior of recovered glucose isomerase

1. Effect of substrate concentration

Different quantities of glucose were dissolved in tris-buffer (0.05 M, pH 8.0) to give final concentrations of 0.1, 0.3, 0.5, 0.7, 0.9 and 1.1 M. The magnesium chloride ($MgCl_2 \cdot 7\ H_2O$) was 0.07 M in all cases. The reaction was carried out under constant temperature 70 °C for one hour, then perchloric acid (1 ml, 0.5 M) was added to stop the reaction. The mixture was then diluted and fructose was determined. A blank experiment was carried out under the same conditions without glucose.

2. Effect of pH

Five solutions of D-glucose in tris-buffer were adjusted to pH values of 5, 6, 7, 8 and 9. High concentration of D-glucose (0.8 M) was used besides magnesium chloride ($MgCl_2 \cdot 7\ H_2O$) at a concentration of 0.07 M. The reaction was carried out as mentioned above.

3. Effect of temperature

Seven different temperatures, i.e., 40, 50, 60, 70, 80, 90 and 100 °C were tested. All experiments were carried out at constant pH (8.0), D-glucose concentration (0.8 M) and $MgCl_2 \cdot H_2O$ (0.07 M).

4. Effect of metal ions

Different concentrations of Mg^{++}, i.e., 0.01, 0.03, 0.05, 0.07, 0.1, 0.13, 0.16, 0.19 and 2.2 M were used separately and all other factors were standardized as pH, temperature and high glucose concentration (0.8 M).

5. Antagonistic effect of some metal ions

Some metal ions, i.e., K^+, Na^+, Ca^{++} and Fe^{+++} were tested for its antagonistic effect with Mg^{++} ion. The concentrations used for each metal ions were 0.01, 0.04, 0.08 and 0.12 M in presence of Mg^{++} ion (0.07 M) and all conditions were optimized as before.

Results and discussion

Glucose isomerase is an enzyme which catalyzes the transformation of D-glucose to D-fructose (Aldo-Keto-isomerization).

In this work *Streptomyces phaeochromogenes* which is an efficient microorganism in the production of glucose isomerase was used. Fermentation was carried out aerobically on a medium containing D-xylose. At the end of incubation period, the cells were harvested by centrifugation and the crude glucose isomerase as endocellular enzyme was extracted by ultrasonic disintegration. The crude enzyme was partially purified by fractional precipitation with ammonium sulphate or organic solvents.

Twelve concentrations of ammonium sulphate for precipitating glucose isomerase as described above were tested. Results in table 1 illustrat-

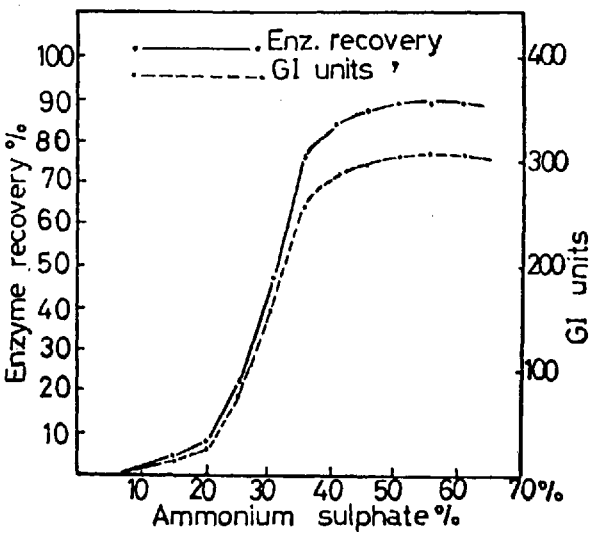

Fig. 1. Effect of ammonium sulphate concentration on precipitation of glucose isomerase.

ed by figure 1 show that ammonium sulphate at a concentration of 50% (0.65 saturation) gave maximum amount of enzyme recovery (88.8%). It was also observed that a negligible amount of the enzyme (7.6%) was recovered in the precipitate at low concentrations of ammonium sulphate (20%).

According to these findings, partial purification of glucose isomerase enzyme was achieved by fractional precipitation through two steps. The first step was done by using (0.26 saturation) ammonium sulphate for precipitating a protein fraction with low enzymatic activity. In the second step the ammonium sulphate concentration was raised to 50% (0.65 saturation) to precipitate the active enzyme fraction.

These results are in line with those obtained by *Tsumura* et al. (10) and *Danno* (13) who precipitated glucose isomerase enzyme by salting out with ammonium sulphate at 0.3–0.5 saturation without any appreciable loss in the activity.

Organic solvents (Ethanol, isopropanol and acetone) were also tested to precipitate glucose isomerase. Because most enzymes are inactivated by organic solvents at room temperature, the precipitation was carried out at 4 °C (*Dixon* & *Webb* [17]).

The results recorded in table (2) and illustrated by figure 2 indicate that acetone gave maximum amount of enzyme recovery (66.2%) when used at a concentration of 3 volumes/volume of enzyme extract. Ethanol and isopropanol gave 45% and 35% of the enzyme recovery when used at concentrations of 2.5 and 2.0 (v/v) respectively.

It could be concluded that the most suitable organic solvent for the precipitation of glucose isomerase is acetone. Ethanol and isopropanol at high concentrations have a slightly effect on enzyme. When ethanol concentration was increased from 2.5 to 3.0 (v/v), the activity was decreased from 45% to 35.9% respectively. The same trend was observed when the concentration of isopropanol was increased. The activity decreased from

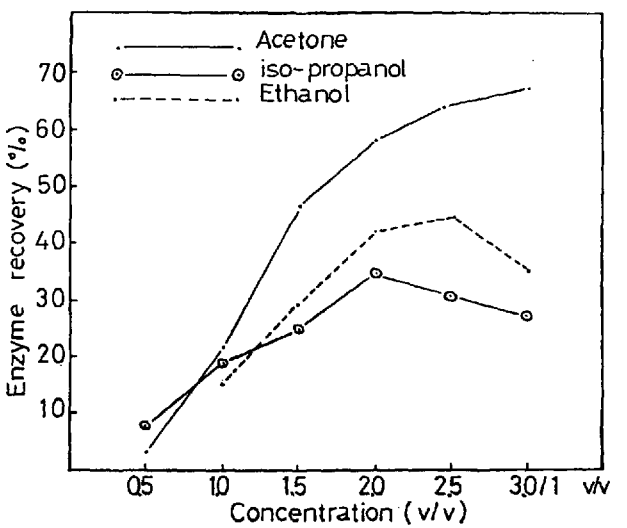

Fig. 2. Effect of organic solvents on precipitation of glucose isomerase.

Table 1. Effect of ammonium sulphate concentration on precipitation of glucose isomerase.

Ammonium sulphate		Re-dissolved precipitate activity (units/ml)**)	Enzyme recovery %
Concentration %	Saturation*)		
6	0.06	0	0
10	0.13	5	1.5
15	0.20	14	4.1
20	0.26	26	7.6
25	0.33	76	22.3
30	0.39	161	47.3
35	0.46	257	75.6
40	0.52	288	84.6
45	0.59	295	86.8
50	0.65	302	88.8
55	0.72	302	88.8
60	0.79	302	88.8

*) Calculated by taken 77% ammonium sulphate equal to 1.0 saturation.
**) Initial activity 340 units/ml (as 100% enzyme activity).

35% to 27.9%, when the concentration of isopropanol was increased from 2 to 3 (v/v) respectively. This may be due to its precipitating effect on inactive protein which contaminated the active enzyme, or to the partial denaturation of enzyme protein.

Taking in consideration the results just reported before, partial purification of glucose isomerase could be achieved by fractional precipitation using acetone in two steps. The first step was done by adding 0.5 v/v acetone and discarding the precipitate, then the acetone concentration was increased to 3.0 v/v in the supernatant to precipitate the active enzyme protein.

Purification technique for glucose isomerase enzyme

On the basis of the results mentioned in tables 1 and 2 regarding the precipitation of glucose isomerase, the enzyme was purified by the following technique.

Table 2. Effect of organic solvents on precipitation of glucose isomerase.

Concentration (v/v)	Acetone		Ethanol		Isopropanol	
	GI/ml*)	Enz. recovery %	GI/ml	Enz. recovery %	GI/ml	Enz. recovery %
0	340	100	340	100	340	100
0.5/1	12	3.5	0	0	27	7.9
1.0/1	73	21.5	52	15.3	64	18.8
1.5/1	158	46.5	71	29.0	85	25.0
2.0/1	198	58.2	144	42.3	119	35.0
2.5/1	218	64.1	152	45.0	105	30.9
3.0/1	225	66.2	122	35.9	95	27.9

*) GI/ml: Glucose isomerase units per ml.

Table 3. Purification technique for glucose isomerase enzyme.

Step	Protein concentration mg	%	Enzyme activity units	%	Specific activity**)
Crude extract*)	950	100	20,500	100	21.6
Concentrated extract	906	95.3	19,920	97.2	22.0
Dialysis	906	95.3	19,110	93.2	21.0
$(NH_4)_2 SO_4$					
0.0 –0.26	250	27.0	800	3.9	3.2
0.26–0.59	610	63.9	18,000	87.8	29.0
Amerbilite CG-50	400	42.1	17,100	83.4	42.0
Acetone	200	30.5	15,300	49.7	52.0

*) Initial volume of crude extract 500 ml.
**) Specific activity = units/mg protein.

The experiment was carried out by using enzyme extract (specific activity equal to 21.6). The extract was concentrated under vacuum at 40 °C, then dialyzed against distilled water for 24 hrs at 4 °C. Ammonium sulphate was added at a concentration of 50% (0.65 saturation) and allowed to stand over night at 4 °C, then the precipitate was collected and redissolved in distilled water and passed through column of Amberlite IRA-400 previously charged with acetate.

The enzyme solution was then passed through an Amberlite CG-50 column which had been equilibrated with tris-buffer pH 7.0, 0.05 M (*Natake* [11]). The column was then washed with the same buffer to remove the inactive protein. The enzyme adsorbed on the column was eluted with 0.4 M sodium acetate buffer pH 6.0. Acetone was added to the elute at a concentration of 3.0 v/v and left for 15 minutes at 4 °C. The precipitate was separated by centrifugation, then redissolved in distilled water.

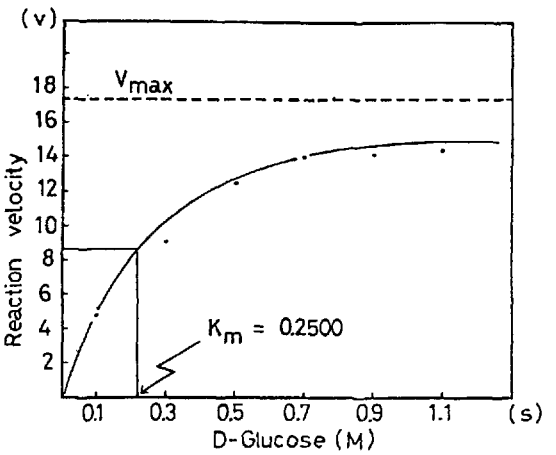

Fig. 3. Substrate conc. versus reaction velocity.

Table 4. Effect of substrate concentration on reaction velocity of glucose isomerase.

D-glucose (s) mole	Reaction velocity (v)	1/S	1/v ($\times 10^{-2}$)	S/v ($\times 10^{-2}$)	v/s	-Log (s) (ps)
0.1	4.94	10.00	20.2	2.0	49.40	1.0000
0.3	9.11	3.33	10.9	3.2	30.36	0.5229
0.5	12.51	2.00	7.9	3.9	25.02	0.3010
0.7	14.05	1.43	7.1	4.9	20.07	0.1549
0.9	14.20	1.11	7.0	6.3	15.77	0.0458
1.1	14.36	0.90	6.9	7.6	13.05	0.0414

Table 3 summarized the steps used in enzyme purification. The enzyme was obtained in an apparently pure form for further studies.

B. The kinetic behavior of glucose isomerase

Kinetics show how the rate of enzyme catalyzed reactions is related to various environmental factors. So, these factors (substrate concentration, pH and temperature, effect of metal ions) were studied.

Substrate concentration is one of the most important factors which affects the velocity of the enzyme reaction. Table 4 and figure 4 show that an increase of substrate concentration results at first a very rapid rise in velocity of reaction rate (first order reaction). As the substrate concentration was increased, the rate of reaction fell down (mixed of first and zero order kinetic), while at larger substrate concentration no further change in velocity was observed (zero order kinetics, *Segel* [18]). This can be attributed to that the enzyme-catalyzed reaction at different substrate concentrations is diphasic. At low concentration the active site of the enzyme was not saturated (phase 1) and as number of substrate molecules increase, the active sites are covered to greater degree until no more active sites are available and the enzyme is working at full capacity. Then the rate is independent of substrate concentration (phase II, *Dixon* and *Webb* [17]).

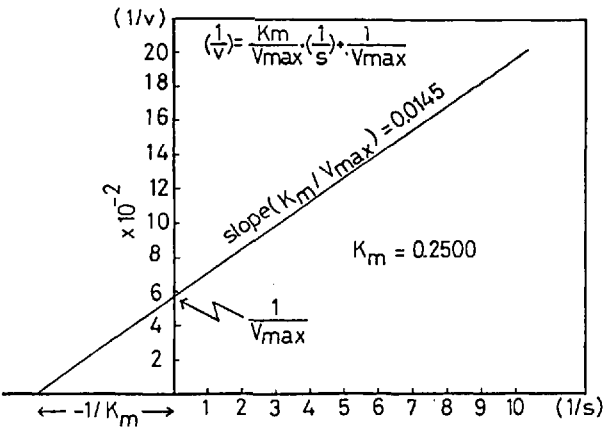

Fig. 4. 1/s versus 1/v.

Table 5. Different technique for calculating Km and Vmax for glucose isomerase enzyme.

Plots	Slope	Intercepts		Vmax	Km
		Ordinate	Abscissa		
(1/v) VS (1/S)	Km/V = 0.0140	L/v = 0.056	−1/Km = 4.0	17.85	0.250
(v) VS (v/S)	−Km = 0.250	V = 18.20	V/Km = 72.8	18.20	0.250
(S/v) VS (S)	1/V = 0.0583	Km/V = 0.0145	−Km = 0.250	17.15	0.250

The Michaelis constant for glucose isomerase enzyme was calculated by four methods (Lineweaver & Burk technique, Edia and Hofstee technique, Hanes technique and logarithmic of Michaelis equation – *Dowd* and *Riggs* [19]). The results are summarized in table 5 and illustrated in figures 5, 6, 7, 8. It was found that the average K_m value obtained from these techniques was equal to 0.250 M.

Since enzymes are protein in nature, pH changes will highly affect the ionic character of the protein and will markedly affect the catalytic nature

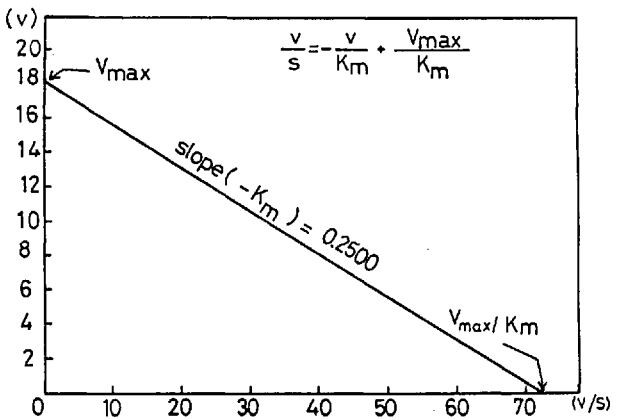

Fig. 5. (v/s) versus (v).

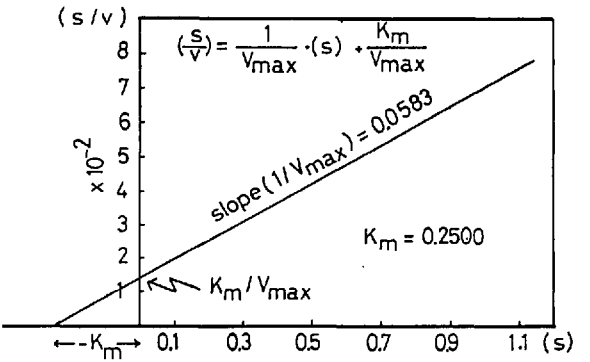

Fig. 6. (s) versus (s/v).

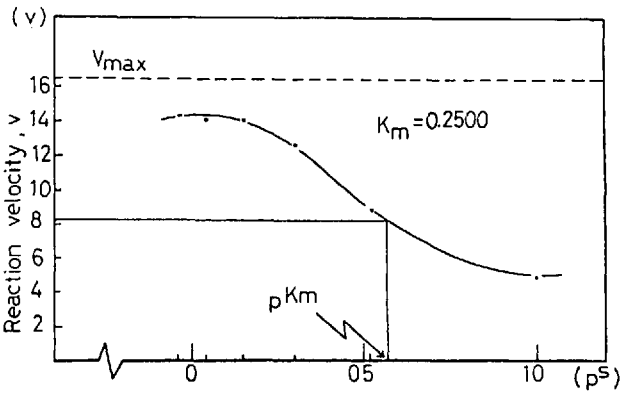

Fig. 7. p^s(-Log s) versus (v).

of enzyme. Besides this effect, low or high pH value can cause considerable denaturation and inactivate the enzyme protein. The activity of glucose isomerase was tested at different pH values. The effect of pH on the affinity was eliminated by using a sufficiently glucose concentration (0.8 M) to saturate the active sites of enzyme at all pH's. Table 6 and figure 9 show that the optimum pH is 7.5 and the velocity falls on either side of that pH. The results are in agreement with those mentioned by *Tsumura* and *Sato* (9); *Natake* and *Yoshimura* (6).

Seven different temperatures were investigated at constant pH, glucose concentration and magnesium ion concentration. Table 7 illustrated

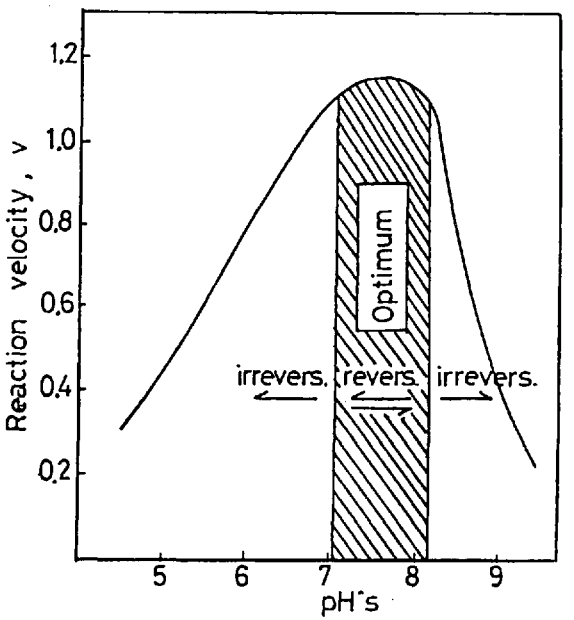

Fig. 8. Effect of pH on the reaction velocity.

Table 6. Effect of pH on the reaction velocity of glucose isomerase.

pH	Reaction velocity (μM fructose)
5.0	0.42
6.0	0.82
7.0	1.07
8.0	1.15
9.0	0.46

by figure 10 shows that the enzymes activity was accelerated as the temperature increased until 80 °C and then fall, giving an apparent optimum temperature. From figure 11 the optimum temperature for glucose isomerase was between 70 °C–80 °C. These results are in line with those obtained by *Standberg* and *Smiley* (14).

Figure 11 shows that, as the temperature raised the curvature increased until the velocity fall to minimum value. The optimum temperature is determined by the balance between the increase of initial velocity and destruction of the enzyme at high temperature.

To study the equilibrium constant (K) as effect of temperature, the enzymatic isomerization was carried out at temperatures of 40°, 50°, 60° and 70° with D-glucose or D-fructose (0.03%) in tris-buffer (0.05 M, pH 8.0) containing 0.07 Mg^{++}. The reaction mixture was incubated at each of the

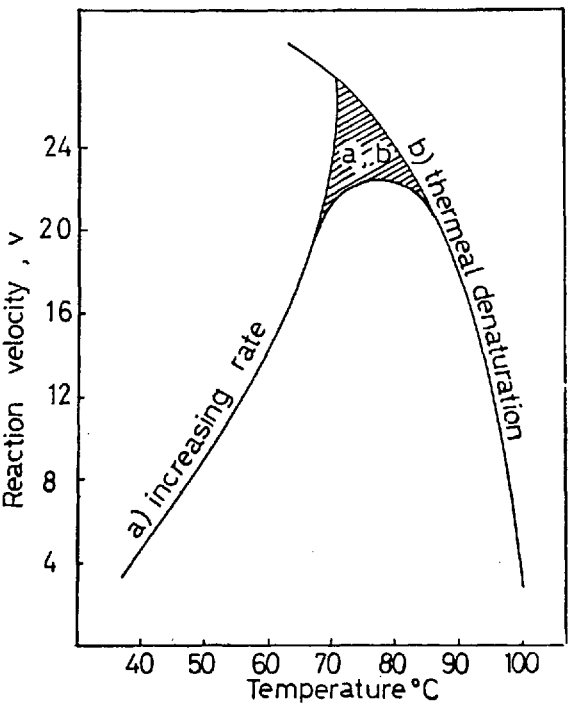

Fig. 9. Effect of temperature on reaction velocity.

Table 7. Effect of temperature on the reaction velocity of glucose isomerase.

Temperature °C	Reaction velocity (μM fructose)
40	4.0
50	8.6
60	15.2
70	20.2
80	22.1
90	17.1
100	3.9

aforementioned temperatures. At definite intervals, a protein of the reaction mixture (0.5 ml) was withdrawn and poured immediately into perchloric acid (0.5 M) to stop the reaction. The concentrations of fructose formed or remained was determined, the reaction was continued until the equilibrium was reached.

Figure 11 shows typical time courses of the glucose isomerase reaction which were carried out at temperatures ranging from 40 °C to 70 °C, starting from either D-glucose or D-fructose. The lower curves represent the time course of formation of D-fructose from D-glucose, and the upper curves show the time courses of the decrease of D-fructose from the initial concentration of D-fructose. It was observed that the equilibrium was reached after 3 hrs incubation period. The equilibrium concentration of D-fructose was obtained directly from its concentration at this time, or it can be obtained simply by the following equation:

$$(\text{D-glucose})_{eq.} = (\text{D-glucose})_{Initial} - (\text{D-fructose})_{eq.}$$

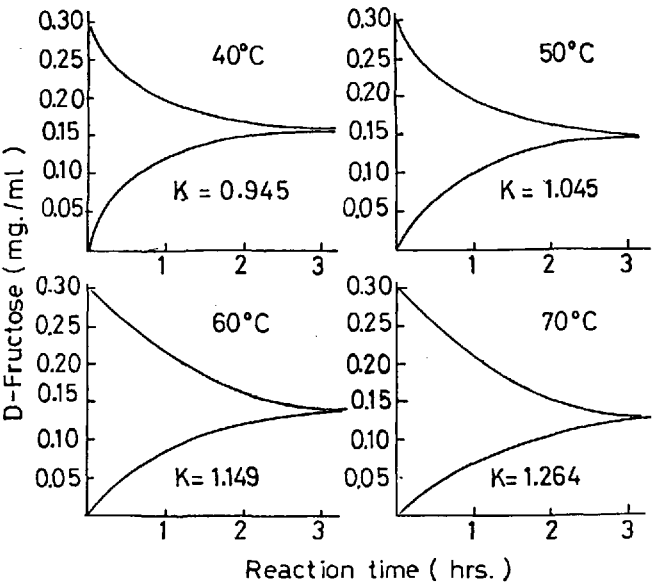

Fig. 10. Typical time course of isomerization reaction.

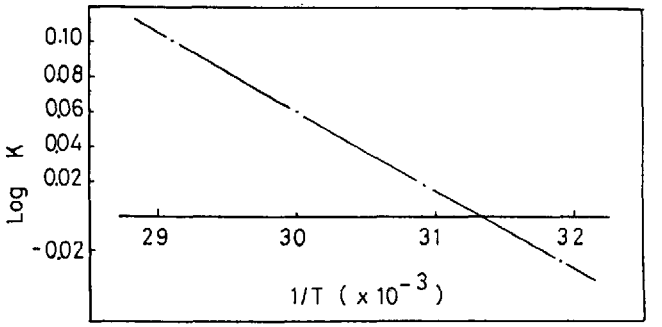

Fig. 11. Log K versus 1/7.

The values of the èquilibrium constant obtained by this technique at temperature range from 40 °C to 70 °C are listed in table (8). According to the Van't Hoff reaction isobar:

$$\frac{d \ln K}{d T} = \frac{H}{RT^2}$$

where H is the heat of the reaction at constant pressure, T is absolute temperature and R is the gas constant. Δ H can be obtained from the slope of the plot of log K against 1/T as shown in figure 12. The curve is linear

Table 8. Kinetic parameters and equilibrium constant of glucose isomerase.

Temperature °C	T*)	1/T (× 10^{-3})	D-glucose mg/ml	%	D-fructose mg/ml	%	K	Log K
40	313	3.19	0.155	51.4	0.145	48.6	0.945	−0.0246
50	323	3.09	0.147	48.9	0.153	51.1	1.045	0.0191
60	333	3.00	0.140	46.6	0.160	53.4	1.149	0.0603
70	343	2.91	0.132	44.1	0.168	55.9	1.264	0.1017

*) T = °C + 273.

Table 9. Effect of Mg^{++} ion concentration on the reaction velocity of glucose isomerase.

Mg^{++} ion (M)	Reaction velocity
0.01	7.0
0.03	13.2
0.05	17.6
0.07	19.1
0.10	16.0
0.13	17.9
0.16	14.5
0.19	13.9
0.22	12.3

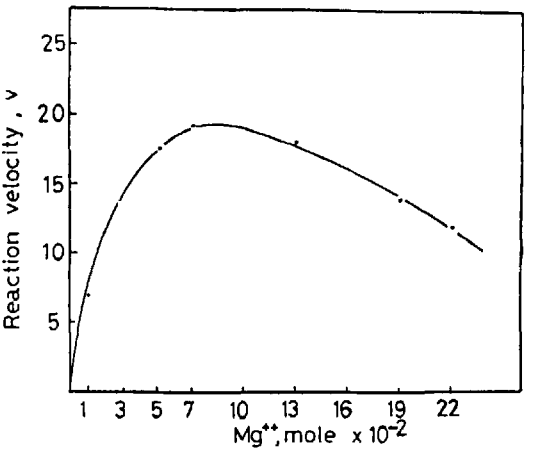

Fig. 12. Effect of magnesium ion concentration on the reaction velocity of glucose isomerase.

over the temperature range 40 °C to 70 °C. The heat of the reaction Δ H when D-fructose is formed from D-glucose, has been calculated from the slope of the Van't Hoff's Plot and was found to be equal to 2900 cal/mole.

It is well known that metallic ions play an important role in the velocity of enzyme reactions. Because of the important role of Mg^{++} ions in activating glucose isomerase enzyme (*Tsumura* and *Sato* [10] and *Standberg* and *Smiley* [13]), different concentrations of Mg^{++} were used at high glucose concentration. Data in table 9 and figure 13 indicate that the reaction velocity increased as the Mg^{++} concentration increased up to 0.07 M. At higher concentration of Mg^{++}, the enzyme activity was decreased due to its autoinhibition at higher concentration.

A trial was done to calculate the K_m of Mg^{++} ions. When $1/Mg^{++}$ was plotted against 1/V a double reciprocal form of Lineweaver and Burk was obtained figure 14. The graph out the vertical axis at a point which gives 1/V max and has a slope of Km/Vmax. The Km was found to be 0.02 M.

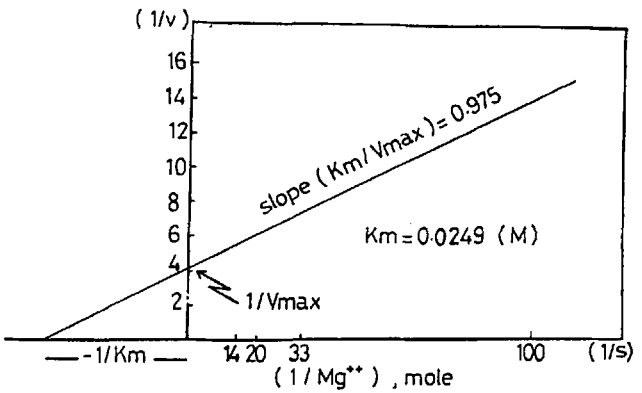

Fig. 13. 1/s versus 1/v (Lineweaver-Burk technique).

Table 10. Effect of different concentration of magnesium ion on the reaction velocity.

D-glucose (s) mole	7×10^{-2} Mg^{++}		1×10^{-2} Mg^{++}		3×10^{-2} Mg^{++}		5×10^{-2} Mg^{++}	
	V	S/v	V	S/v	V	S/v	V	S/v
0.1	5.02	0.020	4.34	0.023	3.84	0.026	3.33	0.030
0.3	9.67	0.031	8.33	0.036	7.50	0.040	6.38	0.047
0.5	4.90	0.042	10.41	0.048	9.26	0.054	7.93	0.063
0.7	12.96	0.054	11.47	0.061	10.14	0.069	8.86	0.079
0.9	14.28	0.063	12.16	0.074	10.84	0.083	9.37	0.096
1.1	14.28	0.077	12.64	0.087	11.34	0.097	9.82	0.112

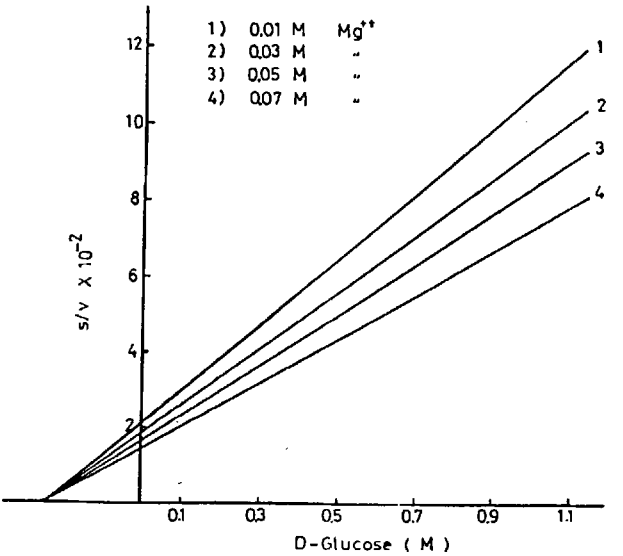

Fig. 14. (S/V) versus (S) at different concentrations of magnesium.

A special interest was given to study the mechanism of activation by magnesium ions. The metal may act as a binder between enzyme and substrate, combining with both and so holding the substrate at the active center of the enzyme. In order to study the affinity of the metal to the

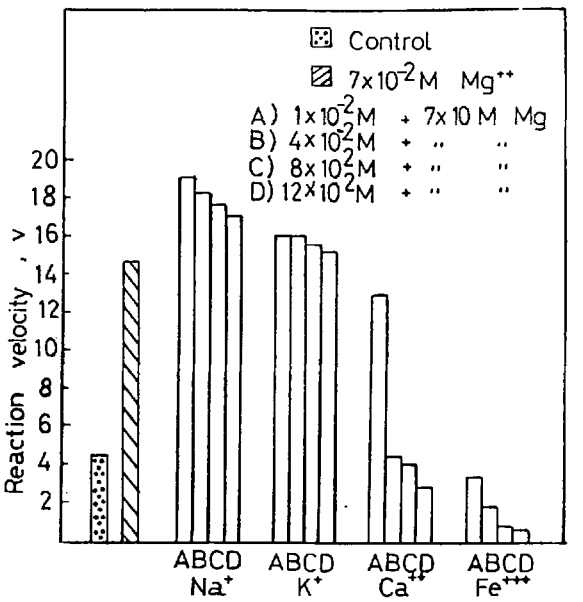

Fig. 15. Antagonistic effect of metal ions on reaction velocity of glucose isomerase.

substrate and enzyme, the velocity of the isomerization was determined by using six different concentrations of D-glucose, i.e., 0.1, 0.3, 0.5, 0.7, 0.9, and 1.1 M in presence of different magnesium ion concentrations, i.e., 0.01, 0.03, 0.05, and 0.07 M for each glucose concentration. Other conditions such as pH and temperature were optimized. The reaction velocity of D-glucose isomerization at the varying levels of magnesium were plotted in double reciprocal form. As shown in table 10 and figure 15 the lines obtained at each concentration level of magnesium gave the same intercepts on abscissa. These results indicate that Km value are not changed (0.024 M) when magnesium concentration was varied while change in Vmax was observed. It could be concluded from these results that the metal ions combined with the enzyme independently of the substrate and the enzymatic reaction can be illustrated as in the following equations:

$$E + M = EM$$
$$EM + S = EMS - EM + P \text{ (Product)}$$

where: E, Enzyme; M, Metal; EM, Enzyme-Metal complex; S, Substrate and EMS, Enzyme-metal-substrate complex.

The metal is attached to the enzyme to form active EM complex, which combines with the substrate to form EMS complex, which decomposes to give the products.

As it is well known in some cases, ions may compete with other ions and have an antagonistic effect. Some metal ions, i.e., K^+, Na^+, Ca^{++} and Fe^{+++}, were tested for their antagonistic effect with Mg^{++} ion. It is clear from table 11 and figure 16 that Na^+ and K^+ act as activators. They increase the activation effect of Mg^{++} particularly at lower concentration. The

Table 11. Antagonistic effect of some metal ions on the reaction velocity of glucose isomerase.

Metal ion concentration ($M \times 10^{-2}$)					Relative activity
Mg^{++}	Na^+	K^+	Ca^{++}	Fe^{+++}	%
7	–	–	–	–	100
7	1	–	–	–	133.8
7	4	–	–	–	128.17
7	8	–	–	–	126.05
7	12	–	–	–	119.79
7	–	1	–	–	142.67
7	–	4	–	–	112.67
7	–	8	–	–	110.56
7	–	12	–	–	108.45
7	–	–	1	–	77.46
7	–	–	4	–	30.98
7	–	–	8	–	28.77
7	–	–	12	–	19.72
7	–	–	–	1	23.94
7	–	–	–	4	13.40
7	–	–	–	8	6.34
7	–	–	–	12	5.21

increase was 33% and 42% at a concentration of 0.01 M using Na^+ or K^+ respectively. On the other hand Ca^{++} and Fe^{+++} act as inhibitors and they have a deleterious effect on the activation of Mg^{++}, the decrease was 80% and 95% at 0.12 M of Ca^{++} and Fe^{+++} respectively.

Summary

Glucose isomerase was produced from *Streptomyces phaeochromogenes* by aerobic fermentation at 28 °C for 24 hrs. The crude enzyme was obtained by disintegrating the harvested cells. It was found that ammonium sulphate at a saturation of 0.3–0.5 gave the maximum enzyme recovery (88.8%) from the crude extract, while acetone gave 66.2% at a concentration of 3/1 (V/V). On this basis the crude enzyme extract was purified following several steps as concentration, dialysis, precipitation with $(NH_4)_2\ SO_4$, then passing through column of Amberlite CG-50, and the eluate was treated with acetone to precipitate the enzyme.

The kinetics behavior was studied and it was found that: optimum D-glucose concentration was 0.8 M, Km was 0.25 M, optimum pH was 7.0 and temperature was 70 °C. Magnesium at concentration of 0.07 M gave the maximum activity and its Km was 0.024 M. Antagonistic effects of Na^+, Ca^{++} and Fe^{+++} in presence of 0.07 M of Mg^{++} were studied.

K_m and V_{max} at different levels of Mg^{++} concentration were determined and no change in K_m value was observed, while V_{max} was affected. These findings indicate that the Mg^{++} combined with enzyme independently of the substrate.

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