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Research Article

Thermostability Studies of *Streptomyces roseiscleroticus* Glucose Isomerase

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ABSTRACT

This study was aimed at investigating the kinetic and thermodynamic properties of Streptomyces roseiscleroticus glucose isomerase and also studying the effect of a few divalent metal ion (Mg^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Ca^{2+} and Zn^{2+}) concentration on the glucose isomerase activity. Glucose isomerase was produced from Streptomyces roseiscleroticus using a submerged fermentation system. The crude glucose isomerase was purified 6.55 fold with specific activity of 696 U/mg protein and percentage yield of 2.17 after ammonium sulphate precipitation (80% saturation), dialysis, ion-exchange chromatography (DEAE-Cellulose) and gel filtration (Sephadex-G100). The purified glucose isomerase had optimum pH and temperature of 7.5 and 60°C, respectively. A simple first order reaction which assumes a biphasic thermo inactivation curve was used to study the kinetic and thermodynamic properties of the enzyme. Activation energy of denaturation (Ea), 19.845 KJ/mol was obtained, with a Z-value of 0.0073. Thermodynamic parameters showed that, the enthalpy of activation of denaturation (ΔH) was 17.243, 17.160, 17.077, 16.993 and 16.911 KJ/mol at 303°K, 323°K, 333°K, 343°K and 353°K, respectively. The Gibbs free energy values were greater than zero (ΔG>0) at all the temperatures under study, while the entropy of inactivation were less than zero (ΔS<0) at all the temperatures under study. Studies on the effect of divalent metal ions on glucose isomerase activity showed that, Mg^{2+}, Mn^{2+}, Co^{2+} and Cu^{2+} enhanced glucose isomerase activity at increasing concentration, while Ca^{2+} and Zn^{2+} decreased glucose isomerase activity with Ca^{2+} showing the greatest inhibition at all concentrations. These findings indicate that Streptomyces roseiscleroticus glucose isomerase is a stable enzyme and could be useful for industrial purposes and applications, especially for sugar syrup production and bioethanol fermentation.

Keywords: Glucose isomerase; Specific activity; Kinetics; Thermodynamics; Metal ion

INTRODUCTION

Glucose isomerase is an intracellular oxidoreductase that catalyzes the reversible isomerization of D-glucose to D-fructose [1] The isomerization ability of glucose isomerase is characterized by a hydride shift mechanism which involves ring opening of the substrate, isomerization via a metal ion induced hydride shift from carbon-2 to carbon-1 of the substrate, followed by the ring closure of the product formed [2]. Glucose isomerase has the ability to isomerize a wide variety of substrates such as pentoses, hexoses, sugar alcohols, and sugar phosphates, although, with varying substrate specificities [3]. The enzyme however, utilizes D-glucose and D-xylose as its most common substrates [3]. Glucose Isomerase has the largest market in the food industry because of its application in the production of syrups particularly, high fructose corn syrup (HFCS), an equilibrium mixture of glucose and fructose [4]. Fructose being a sweetener is currently employed world over as an alternative to sucrose or invert sugar in the food and beverage industry [5]. The importance of glucose isomerase also extends to the production of ethanol from raw materials (hemicellulosic biomass) via isomerization of xylose to xylulose. Many microorganisms have been reported to be good producers of glucose isomerase [6], most of which require xylose or glucose as an enzyme inducer. Glucose isomerase can be sourced from a number of prokaryotes, especially bacteria, however, most industrially used glucose isomerases are obtained from the Streptomyces spp [7] largely because of their very high potentials and credentials to produce secondary metabolites [8]. Metal ions binding to proteins are one of the factors responsible for protein stabilization [9]. A number of divalent metal ions have be reported to serve as electron donors or lewis acids, as they participate directly in the catalytic mechanism of enzymes, thus,

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enhancing the metalloenzyme activities of enzymes [10]. As a result of the importance of glucose isomerase, it has become highly desirable to enhance its activity in other to increase or broaden its useful applications and utilization. This research, therefore, was aimed at studying the thermostability properties of Streptomyces roseiscleroticus glucose isomerase and also investigating the effect of divalent metal ions on glucose isomerase activity.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals used in this research were of analytical grade and were obtained directly from the manufacturers. Major chemicals such as carbazole, cysteine hydrochloric acid, dodecyl trimethyl ammonium bromide (DTAB), sephadex G 100, Diethyl ammonium ethyl cellulose (DEAE-cellulose), Bovin serum albumin (BSA) and Folin-ciocalteau reagent were obtained from Sigma Aldrich, Germany.

Sample collection and isolation of the micro organism

Soil sample (5 g) was collected from garden soil obtained from Department of Soil Science, University of Nigeria, Nsukka, using a sterile container. Isolation of the microorganism was carried out as described by [11].

Screening for glucose isomerase producing organism

Glucose isomerase producing organism was screened by adopting the method as described by [12], in which 2, 3, 5-triphenyltetrazolium solution was used as the chromogenic substrate. Also, the isolated microbial strain was further subjected to molecular characterization as described in the previous work reported by [13], using 16S rRNA sequencing technique.

Glucose isomerase production

Submerged fermentation (SmF) technique was employed for glucose isomerase production. Erlenmeyer flask (250 ml) containing 50 ml of sterile basal medium charged with 1% peptone, 0.25% yeast extract, 1% D-xylose, 0.5% NaCl, 0.5% beef extract, 0.05% MgSO4.7H2O and adjusted to pH 6.8 was inoculated using a four day old pure culture of the Streptomyces isolate. Five discs of the Streptomyces isolate were plugged into the fermentation broth using a cork borer of diameter 10 mm. The fermentation broth was incubated at room temperature on a shaker for seven (7) days. The microbial biomass was separated by centrifugation at 10000 rpm for 15 min and washed severally with distilled water. The cells were further suspended in 0.05 M sodium phosphate buffer pH 7.0 containing 0.1% dodecyltrimethylammonium bromide (DTAB) and then incubated at room temperature for 24 hr after which it was centrifuged and filtered. The filtrate was used as a crude enzyme.

Glucose isomerase assay

Glucose isomerase assay was carried out as described by [14]. An aliquot (0.1 ml) of enzyme solution was mixed with the reaction mixture. The reaction mixture contained 0.5 ml of 0.1 M potassium phosphate buffer pH 8.0, 0.2 ml of 0.05 M MgSO4.7H2O and 0.2 ml of 1 M glucose solution. After incubation in water bath at 60°C for 30 min, 1 ml of 0.5 M perchloric acid was added to stop the reaction. Fructose formed was determined by the method of [15] in which 0.2 ml of 1.5% cysteine hydrochloride, 6 ml of 70% H2SO4 and 0.2 ml of 0.12% alcoholic carbazole were added to the reaction mixture. The purple colour developed was measured spectrophotometrically at 560 nm. The absorbance was converted to glucose isomerase (GI) activity using a fructose standard curve. One unit of glucose isomerase (GI) activity (U) is defined as the amount of enzyme that produced one µmol of D-fructose per min under the assay conditions.

Protein determination

Protein content of the crude enzyme was determined by the method of [16], using Bovine Serum Albumin (BSA) as standard.

Purification of the Streptomyces roseiscleroticus glucose isomerase

Ammonium sulphate precipitation: Ammonium sulphate precipitation was carried out as described by. [17], the crude enzyme solution was precipitated at pH 4.0 and pH 8.0 using eighty percent (80%) ammonium sulphate saturation. The ammonium sulphate – crude enzyme solutions were kept at 4°C for 24 hr, it was thereafter centrifuged at 10000 rpm for 30 mins, the precipitates were collected and re-dissolved in 0.1 M phosphate buffer pH 7.5. Glucose isomerase activity was assayed and protein contents were determined as described above.

Dialysis

The precipitate was desalted by dialysis. A 10 cm pretreated dialysis bag was used and activated by rinsing in distilled water. The dialysis bag was suspended in a beaker containing 0.01 M sodium phosphate buffer pH 7.0. Dialysis was carried out for 12 hr with continuous stirring and buffer changed every 6 hr with a view to removing low molecular weight substances and other ions that may interfere with enzyme activity. Glucose isomerase activity was assayed on the dialyzate as described above, while protein content of the dialyzate was also determined.

Ion exchange chromatography

Ion exchange chromatography was carried out by adapting the method as described by [17]. The dialyzate enzyme solution (10 ml) was applied to a column (23.5 x 2 cm) of DEAE-cellulose which was previously equilibrated with 0.05 M phosphate buffer pH 7.5. The column was washed with the same buffer followed by a stepwise elution with 0.05 M phosphate buffer pH 7.5 containing a linear gradient of NaCl (0.1 to 1.0 M). Fractions were collected in a test tube at a flow rate of 5 ml per 17 mins; these fractions were assayed for glucose isomerase activity as described above. Fractions containing enzyme activity were pooled together and

stored for further use.

Gel filtration

A volume (10 ml) of the enzyme solution was introduced into a sephadex G 100 column (80 cm by 2.0 cm) pre equilibrated with 0.05 M phosphate buffer pH 7.5. Fractions were collected at a flow rate of 5 ml per 20 mins. The protein concentration of each fraction was monitored spectrophotometrically at 280 nm. Glucose isomerase activity of each fraction was assayed as described above with the active fractions pooled together and stored at -4°C.

Characterization of the Streptomyces roseiscleroticus glucose isomerase

Effect of pH on glucose isomerase activity: Effect of pH on glucose isomerase activity was determined as described by

[18], using 20 mM sodium acetate buffer pH 3.5-5.0, 20 mM phosphate buffer pH 6.0-7.0 and 20 mM Tris-HCl buffer pH 8.0-10.0. An aliquot (0.1 ml) of the enzyme was incubated with 0.2 ml of 1 M glucose, 0.2 ml MgSO4.7H2O (0.05 M) in 20 mM of each buffer for 60 min. Glucose isomerase activity at these various pH intervals was then monitored as described above.

Effect of temperature on glucose isomerase activity

Effect of temperature on glucose isomerase activity was determined as described by [18] at temperatures of 30°C to 90°C at 10°C interval. An aliquot (0.1 ml) of enzyme was incubated with 0.2 ml of 1 M glucose at the pre-determined pH at the various temperature ranges. Glucose isomerase activity at these temperature ranges was monitored just as glucose isomerase activity was monitored as described above.

Thermostability studies of Streptomyces roseiscleroticus glucose isomerase

Thermal inactivation study of glucose isomerase: The heat stability of the purified glucose isomerase was tested as described by [19,20]. This was achieved by incubating the enzyme in 0.1 M phosphate buffer, pH 7.5 at temperature range of 30°C to 80°C respectively for a period of 120 min in a water bath. Aliquots (0.1 ml) of the sample were collected at 0, 15, 30, 45, 60, 75, 90, 105 and 120 min and assayed for glucose isomerase activity respectively. The residual glucose isomerase activities were determined under optimum conditions of pH and temperature. In all cases, the initial activity was assumed 100% and used to calculate the activities as percentages of the initial activity during the incubation period.

Percentage residual activity= Glucose isomerase activity at each time interval /Glucose isomerase activity at time zero×100

The first order inactivation constant, k values were obtained from the slope of first order enzyme inactivation equation according to the method described by of [21] as follows:

$$\text{Log(\% residual activity} = -(k/2.303)t$$

Where: t is the time of inactivation.

Half-life (t1/2) of inactivation was calculated from,

$$t^{1/2} = \ln(2)/k$$

The D-value (defined as the time needed for 90% reduction of the initial activity) was calculated from the relationship:

$$\text{D-value} = \ln 10/k$$

The Z-value is the temperature increase needed for 90% decrease in the D-value. The Z-value was obtained by plotting log D value at different treatment time against corresponding temperature. The slope of the line is equal to the negative reciprocal of Z-value.

Arrhenius law used to describe the temperature dependence of the rate constants (k-values) was:

$$lnk = Ae^{-Ea/RT}$$

Where Ea is the activation energy, A is the Arrhenius constant, R is the universal gas constant (8.314 J/mol.K) and T is the absolute temperature.

The values of the activation energy (Ea) and Arrhenius rate constant (k) were used to determine different thermodynamic parameters such as Gibbs free energy change (ΔG), the entropy change (ΔS) and the enthalpy change (ΔH) using the following expressions:

$$\Delta G_{inact} = -RTln(kh/K_b T)$$

$$\Delta S_{inact} = (\Delta H_{inact} - \Delta G_{inact})/T$$

$$\Delta H_{inact} = Ea_{inact} - RT$$

Where K_B Boltzmann's constant (1.3806 x 10-23 J/K), h is Plank's constant (6.6260 x 10^{-34} J.s).

Effect of divalent metal ions on Streptomyces roseiscleroticus glucose isomerase activity

Divalent metal ion concentrations of 10 mM, 30 mM and 50 mM of metal salts ($MgCl_2$, $MnCl_2$, $CoCl_2$, $CaCl_2$, $CuCl_2$, and $ZnCl_2$) were prepared as described by [19]. The reaction consisted of 0.1 ml of enzyme solution, the reaction mixture (0.5 ml of 0.1 M potassium phosphate buffer pH 8.0 and 0.2 ml of 1 M glucose solution) together with 200 µl of each metal ion solution respectively, all incubated at the optimum conditions of pH and temperature. The control was carried out without metal ions.

RESULTS AND DISCUSSION

Microbial strain was isolated from garden soil obtained from the Department of Soil Science, University of Nigeria, Nsukka, using starch casein agar as a differential media. The isolated microbial strain was screened for glucose isomerase producing ability using 2, 3, 5-triphenyltetrazolium solution as chromogenic substance.

The microbial strain was identified as Streptomyces species based on their morphological and microscopic characteristics (Plate 1). The isolated microorganism was a gram positive cocci, and tested positive to catalase, citrate utilization, nitrate reduction and sugar/starch fermentation tests (Table 1). The isolated Streptomyces strain was further subjected to molecular characterization using 16S rRNA sequencing and were subsequently confirmed as Streptomyces roseiscleroticus [13].

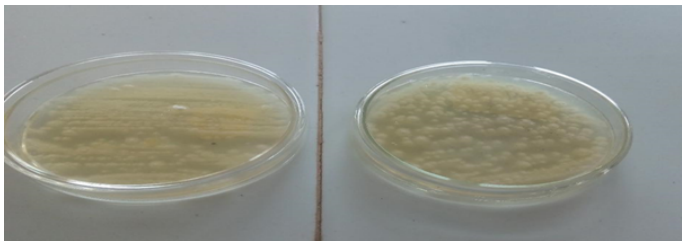

Plate 1: Two day old isolates of Streptomyces species obtained from soil by serial dilution, the Streptomyces colonies observed on the nutrient agar were sporulated, white to milky in colour and grows singly, in clusters and sometimes, linearly.

Table 1: Biochemical tests/screening of the isolated microorganism

Tests	Results
Citrate utilization	Positive
Catalase	Positive
Nitrate reduction	Positive
Starch hydrolysis	Positive
Sugar fermentation(glucose,fructose, xylose, Galactose)	Positive

Glucose isomerase was produced from Streptomyces roseiscleroticus using a submerged fermentation system. The crude glucose isomerase had a specific activity of 106.511 U/mg (Table 2). Purification of the crude glucose isomerase using ammonium sulphate showed that eighty percent (80%) ammonium sulphate saturation was found most suitable to precipitate proteins with maximum glucose isomerase activity at both acidic and basic pH regions (Figure 1) with a specific activity of 137.599 U/mg, purification fold and percentage yield of 1.9 and 18.70% respectively (Table 2).

Table 2: Purification table for glucose isomerase

	Volume (ml)	Total Protein (mg)	Total Activity µmol/min	Specific activity (U/mg)	Purification fold	% Yield
Crude Enzyme	1000	480.86	50984.83	106.511	1	100
Ammonium Sulphate precipitation	400	69.312	9537.27	137.59	1.29	18.7
Dialysis	80	12.81	2608.61	203.68	1.919	5.11
Ion exchange (DEAE cellulose)	50	3.92	2271.23	579.39	5.45	4.45
Gelfiltration (Sephadex G 100) U=µmol/min	30	1.59	1106.87	696.14	6.56	2.17

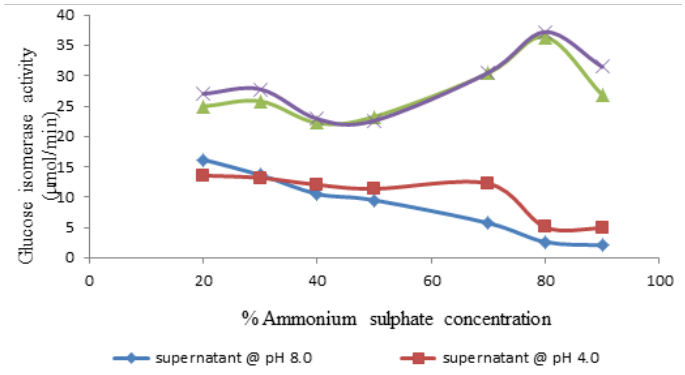

Figure 1: Percentage (%) Ammonium sulphate precipitation of glucose isomerase. Eighty percent (80%) ammonium sulphate saturation precipitated proteins with maximum glucose isomerase activity.

The elevated concentration of ammonium salt needed for the precipitation of proteins with glucose isomerase activity could be suggestive of the nature and type of the hydrophilic amino acid residues present of the protein [22]. Ammonium sulphate precipitation is a useful method for enzyme concentration as it provides some levels of purification of proteins away from non-proteins. Hydrophobic proteins however, precipitates at a lower salt concentration than hydrophilic proteins because protein solubility is a function of their ionic strength and pH of the solution they are contained. [3,18] in their research both reported an ammonium sulphate saturation of seventy percent (70%) suitable for the precipitation of proteins with glucose isomerase activity.

The dialyzate was purified to 1.91 fold with specific enzyme activity of 203.67 U/mg and 5.11% yield (Table 2). Increased glucose isomerase activity of the dialyzate could be attributed to some levels of purification of the dialyzate and also the removal of the ammonium salts that could saturate the enzyme active site. Enzyme purification by dialysis often increases the volume of the enzyme solution, because of the initial osmotic effect of the ammonium sulphate utilized [23].

The specific activities after ion exchange chromatography and gel

filtration were 579.39 and 696.14 U/mg respectively, with 5.45 and 6.55 purification folds, respectively, and percentage yields of 4.45 and 2.17% respectively (Table 2). Single enzyme activity peak of the ion exchange chromatography and gel filtration suggests that isoenzymic forms arising from anionic and physical heterogeneity respectively are not present (Figures 2 and 3) [17].

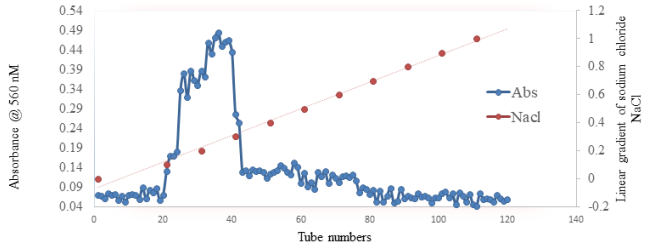

Figure 2: Elution profile of glucose isomerase on DEAE Cellulose, A volume of 5 ml of 120 fractions were collected, stepwise elution with 0.05 M phosphate buffer pH 7.5 containing a linear gradient of NaCl (0.1 to 1.0 M) was used. Glucose isomerase activity was assayed in all the fractions collected.

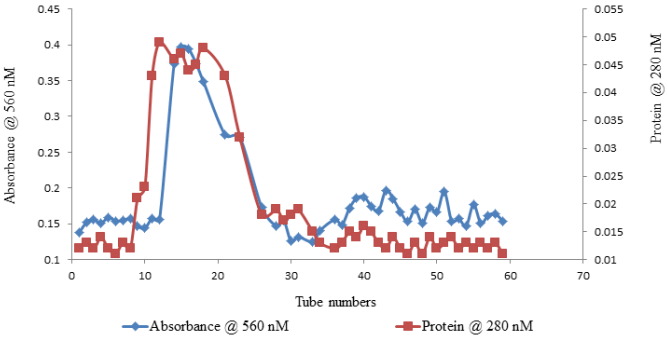

Figure 3: Elution profile of glucose isomerase during gel filtration on sephadex G 100. A volume of 5 ml of 60 fractions were collected at an elution rate of 5 ml per 20 min. Glucose isomerase activity and protein determination was assayed in each of the fractions collected.

Also, elution profiles with single enzyme activity peak have been reported to depict some levels of purity of proteins [15]. The increase in specific activity after each purification step could possibly suggests that for a purification procedure to be successful, the specific activity of the present purification step should exceed the specific activity of the previous purification procedure [17,24].

Studies on the effect of pH (Figure 4) on glucose isomerase activity showed that an optimum pH of 7.5 was obtained. Optimum pH of 7.5 for glucose isomerase is suggestive of a maximum ionization of the imidazole group of the proton abstracting base which forms hydrogen bonds with the hydroxyl group of the substrate [5,25] Chanitnun and Pinphanichakarn (2012) reported an optimum pH 7.0 for glucose isomerase from streptomyces sp. Ch7 grown on agricultural residues, [26,27] Sathya and Ushadevi (2014) reported an optimum pH 7.5 for glucose isomerase from Streptomyces species. Similarly, Prashant et al (2010) reported optimum pH 7.0 for glucose isomerase from a Streptomyces species. Also, (18) obtained a promising glucose isomerase activity (84 to 100% of the highest activity) over the pH range of 6.5 to 8.0, with an optimum pH of 7.0. Glucose isomerase have been reported to be stable across a broad pH range (5.5-8.5) (2), this characteristic gives glucose isomerase good credentials for industrial importance and utilization.

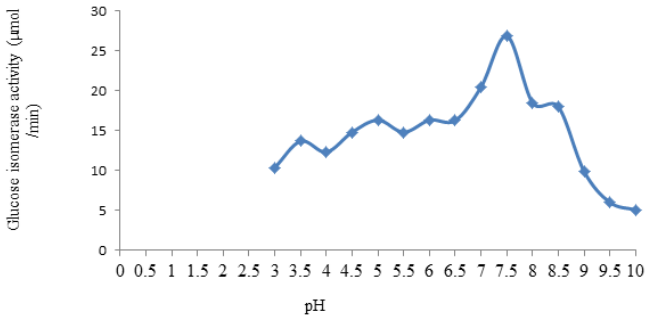

Figure 4: Effect of pH on glucose isomerase activity. Glucose isomerase activity was assayed using 20 mM sodium acetate buffer pH 3.5-5.0, 20 mM phosphate buffer pH 6.0-7.0 and 20 mM Tris-HCl buffer pH 8.0-10.0. Optimum pH was obtained at pH 7.5

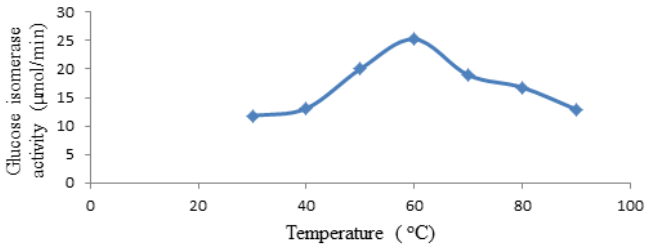

Figure 5: Effect of temperature on glucose isomerase activity. Glucose isomerase activity was assayed at temperatures of 30°C to 90°C at 10°C interval at the pre-determined pH. Optimum temperature was obtained at 60°C

Studies on the effect of temperature on glucose isomerase activity showed that an optimum temperature of 60°C was obtained (Figure 5). Optimum temperature of glucose isomerase have been reported within the ranges from 60°C to 80°C (18,27). Increase in glucose isomerase activity as temperature increases could be suggestive of an increase in the kinetic energy of the reacting species or substrates [4,28] in their work reported an optimum temperature of 50°C for glucose isomerase obtained from *bacillus thuringiensis*, similarly, Ogbo and Odibo (2007) reported an optimum temperature of 60°C for glucose isomerase from Saccharococcus caldoxylosilyticus. Microbial glucose isomerases especially from bacteria sources such as Bacillus species, Actinoplanes missouriensis, Streptomyces species and the thermus thermosulfurogenes have been reported to have high optimum temperatures within the ranges from 60°C to 80°C (Figure 6) [2].

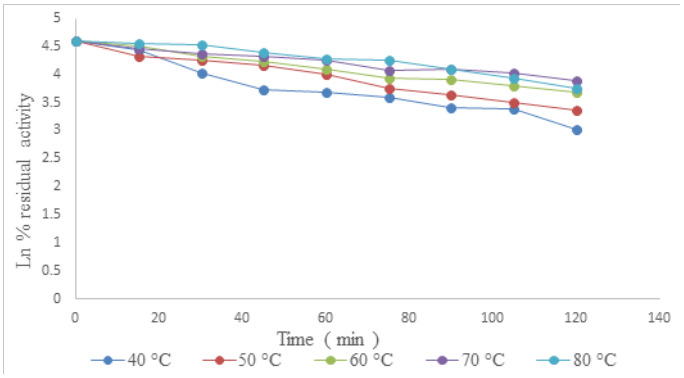

Figure 6: Thermal inactivation of Streptomyces roseiscleroticus glucose isomerase at different temperatures.

Studies on the various kinetics and thermodynamics parameters of Streptomyces roseiscleroticus glucose isomerase showed that the rate of enzyme inactivation (Kd) decreased with increase in temperature (Table 3). This is a likely characteristic of a thermostable enzyme [29]. An increased rate of enzyme inactivation (Kd) at high temperature indicates that the enzyme or protein is less thermostable at increasing temperatures [29,30]. Similarly, longer half-life (t ½) was observed at high temperatures. This is suggestive of a thermal stable enzyme or protein at elevated temperatures [19]. Streptomyces roseiscleroticus glucose isomerase showed a half-life (t½) of 126 min at 70°C. Also, at the enzyme optimum temperature (60°C), Streptomyces roseiscleroticus glucose isomerase had a half-life of 90 min. This is suggestive of a thermostable enzyme at higher or elevated temperatures [21]. Also, the duration of heat treatment needed for 90% reduction of the initial enzyme activity (that is, D-value) increased with increase in temperature (Table 3). This indicates an increase in denaturation time with a corresponding increase in temperature. It also suggests that the enzyme is thermally stable at elevated temperature [29]. Furthermore, thermal denaturation of enzyme requires an input of a minimum amount of energy known as activation energy of denaturation (Ea) which must be supplied to the enzyme in order to initiate the denaturation process. When the initial input of energy is less than the activation energy (Ea), the unstable intermediate can fold back or revert into the native state upon cooling [8]. Activation energy of denaturation, (Ea) of 19.845 KJ/mol was obtained from the Arrhenius plot of the rate of thermal denaturation (Figure 7).

Table 3: Kinetics and thermodynamic parameters of thermal inactivation of Streptomyces roseiscleroticus glucose isomerase.

T (°K)	Kd (min-1)	t ½ (min)	D-Value (min)	ΔH(KJ/mol)	ΔG(KJ/mol)	ΔS(KJ/mol)
40 (313)	0.012	57.762	191.916	17.243	19355.667	-61.897
50 (323)	0.01	69.315	230.301	17.16	20612.208	-63.872
60 (333)	0.0077	90.019	299.09	17.077	21908.262	-65.846
70 (343)	0.0055	126.026	418.727	16.993	23243.832	-67.82
80 (353)	0.007	99.021	329.011	16.911	24618.915	-69.794

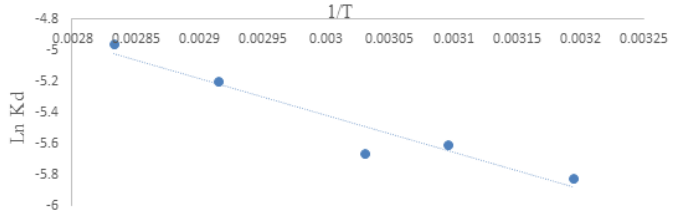

Figure 7: Arrhenius plot for thermal inactivation of Streptomyces roseiscleroticus glucose isomerase

A large value of Ea indicates that more energy is required to inactivate the enzyme [21]. Activation energy of denaturation (Ea) is directly related to the enthalpy of activation of denaturation (ΔH). Enthalpy is the total amount of energy required to bring the enzyme from the ground state to the activated intermediate. ΔH values were found to be greater than zero (ΔH>0) and decreasing as the temperature increased (Table 3). A positive enthalpy is attributed to an endothermic reaction, as the enzyme can withstand the amount of heat treatment required by the reaction [31,32].

Gibbs free energy of activation of denaturation (ΔG) is a more reliable indicator for enzyme stability [29,33]. The Gibbs free energy of activation of denaturation was positive (ΔG>0) and increased with increase in temperature (Table 3). A positive Gibbs free energy was obtained at all temperatures (Table 3). Positive Gibbs free energy indicates that the enzyme is thermally stable at high temperature and the reaction it catalyzes is non-spontaneous, hence, a thermostable enzyme is required to catalyze the reaction [21,29]. A smaller or negative value of change in Gibbs free energy indicates spontaneity of a reaction and a low thermostable enzyme, as such, the reaction it catalyzes does not require a lot of heat [33,34]. The thermal denaturation of enzyme is also dependent on the entropy (ΔS) of the reaction. Entropy measures the randomness or disorderliness of a reaction or molecule [31,35]. Negative entropy (ΔS0) was obtained in this study at all temperatures. Negative entropy often suggests a reversible reaction process and availability of an aggregation process in which a few inter and intramolecular bonds are formed [36]. Large or positive entropy often indicates an irreversible reaction process as a large number of intramolecular bonds are formed [29].

A thermal destruction time (Z-value) of 0.0073 was obtained (Figure 8). A low magnitude of the thermal destruction time (Z-value) has been reported to indicate the sensitivity of the protein or enzyme to an increase in temperature. Similarly, a high magnitude of the thermal destruction time (Z-value) indicates a more sensitivity of the protein or enzyme to the duration of heat treatment [37].

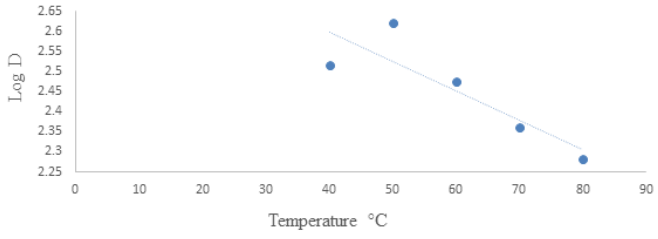

Figure 8: Temperature dependence of the decimal reduction time of Streptomyces roseiscleroticus glucose isomerase for calculating Z- value.

Studies on the effect of various divalent metal ions (Mg^{2+}, Mn^{2+}, Co^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}) on the activity of Streptomyces roseiscleroticus glucose isomerase showed that Mg^{2+}, Mn^{2+}, Co^{2+} and Cu^{2+} enhanced glucose isomerase activity at increasing concentrations, while Ca^{2+} and Zn^{2+} decreased glucose isomerase activity with Ca^{2+} showing the greatest inhibition at all concentrations.

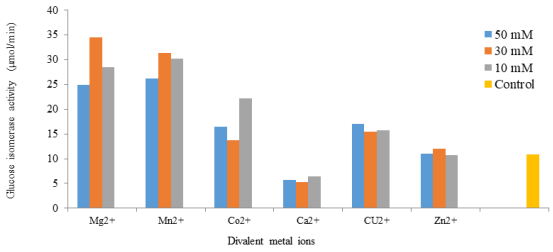

Figure 9: Effect of divalent metal ion on glucose isomerase activity

Ogbo and Odibo (2007) reported that divalent metal ions (Mn^{2+}, Mg^{2+} and Co^{2+}) enhanced the activity of glucose isomerase obtained from Saccharococcus caldoxylosilyticus while Cu^{2+} inhibited the enzyme activity. Similarly, (4) Hamed et al (2016) reported that glucose isomerase from *bacillus thuringiensis* requires divalent metal ions (Mn^{2+} and Mg^{2+}) as cofactor to enhancing glucose isomerase activity. Also, Yassien et al. (2013) reported the presence of the combination of Mg^{2+} and Co^{2+} ions in enhancing and improving glucose isomerase activity. The enhanced glucose isomerase activity caused by these divalent metal ions could possibly be attributed to the ability of these divalent metal ions to serve as an electron donor as they have been reported to participate directly in the catalytic mechanism of the enzyme (18,29),. Also, decrease in glucose isomerase activity caused by some divalent metal ions (Ca^{2+} and Zn^{2+}) could be attributed to the interaction of these divalent metal ions with the amino acid residues involved in the enzyme catalysis, as this could possibly result in the competition between the exogenous cations and the protein associated cations, hence, resulting in the reduction of metalloenzyme activity [10].

CONCLUSION

From the experimental results, the kinetic and thermodynamic parameters indicates that glucose isomerase, has good credentials for industrial utilities. Also, divalent metal ions such as Mg^{2+}, Mn^{2+}, Co^{2+} and Cu^{2+} could enhance glucose isomerase activity for an improved use.

DECLARATIONS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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