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Stabilizers Enhanced the Activity of Mutarotase Chracterized from Bovine Kidney Cortex

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Abstract

The crude extract of mutarotase having 5.491 U/mL activity (1.264 U/mg specific activity) was subjected to sephadex G-150 column and obtained 16.518 U/mg specific activity. Optimum pH and temperature were 7.5 and 40 ^{0}C having maximum activity of 4.273 U/mL and 4.918 U/mL respectively. It was noted that, 200 mg substrate concentration showed highest activity 5.607 U/mL and that of 5.519 U/mL at 500 μL enzyme concentration. The study on effect of stabilizers showed that glycerol (15 %) as best one among sodium benzoate and sodium citrate with the activity of 3.71 U/mL even after 30 days.

Key Words: Mutarotase; Characterization; Stabilizers

Introduction

Aldose-1-epimerase/mutarotase (EC 5.1.3.3) is a key enzyme of carbohydrate metabolism catalyzing the interconversion of the α into β-anomer of hexose sugars such as glucose and galactose [1]. This enzyme also acts on L-arabinose, D-xylose, D-galactose, D-maltose and D-lactose [2]. It has been isolated and purified from various animal tissues such as bovine kidney and liver [2] kidney, liver and small intestine of rats [3] human kidney [1] and hog kidney cortex [4]. The analytical and clinical use of mutarotase shows its high specificity and stability. Mutarotase is stable at pH 6.5-7.5 and 30-37^{o}C temperature, as the stability towards heat is upto 50^{o}C [5]. This is used for the rapid determination of glucose in conjunction with glucose oxidase and peroxidase, which is the chief application of the said enzyme [6].

In view of the importance of mutarotase enzyme in quality control laboratories of pharmaceutical, food industries and clinical diagnostic, there is a great need to produce/ purify this enzyme at local level to meet our requirements as well as to save the economy of our country.

Materials and Methods

Homogenization

Fresh bovine kidney cortex (100 g) was homogenized with 5 mM EDTA buffer pH 7.4 (500 mL) in a blender for 1 minute at 2^o C. It was centrifuged at 7,000 rpm (Kokusan-Japan, Model H-200 NR, Rotor BN) for 10 min. at 0^{o}C and supernatant was separated having the enzyme [6].

Purification of mutarotase

The enzyme was purified by ammonium sulfate precipitation [2] and sephadex G-150 for gel filtration chromatography [6-7] at room temperature.

Analytical Method

Mutarotase activity was determined by the method [8] using α-D-glucose as substrate. All fractions of the enzyme were subjected to Biuret reaction [9] for measurement of protein contents.

Kinetics of Mutarotase

5 mM EDTA buffers of different pH (5, 5.5, 6, 6.5, 7, 7.5 and 8) were used for enzyme assay to determine the optimum pH. Enzyme assay with optimized pH was carried out at different temperatures (0, 4, 10, 30, 35, 40, 45 and 50 ^oC) to determine the optimum one. Various substrate (α-D-glucose) concentrations (55.5, 111, 166.5, 222, 277.5, 333, 388.5, 444, 499.5 and 555 mM in 5 mM EDTA buffer) were used to study the effect of substrate concentration on enzyme activity. To determine the effect of enzyme concentration, various concentrations (0.139, 0.278, 0.555, 0.832, 1.11, 1.387, 1.665, 1.942, 2.22, 2.497 and 2.775 U/mg) were used. All the analysis was carried out at polarimeter (Erma-Japan).

Effect of Stabilizers

To determine the effect of stabilizers on enzyme activity, glycerol, sodium benzoate, and sodium citrate were used in 0, 15, 30 and 45 % concentrations. So, stability as well as enhancement in activity was observed with increase in time period (0-30 days).

Results and Discussion

Mutarotase catalyzes the mutarotation of D- glucose and other related sugars. Enzyme was extracted from bovine kidney cortex by blending it for 1 minute at 2^o C and centrifugation at 7,000 rpm for 10 min. Crude extract having the activity of 5.491 U/mL with 4.345 mg/mL protein contents was subjected to ammonium sulfate precipitation technique. Ammonium sulfate is the most commonly used reagent for salting out the desired enzymes due to its hygroscopic nature and shielding effect [10]. Desalted enzyme (4.174 U/mg specific activity) was subjected to sephadex G-150 column and obtained the enzyme with 13.07 fold purification (Table 1). These findings are in accordance [9-6] but on the other hand the specific activity was 250.46 U/mg [2]. This high value is due to the application of various purification techniques like DEAE-cellulose, hydroxyl apatite, gel filtration, bio gel P-100 chromatographic and double ammonium sulfate precipitation. Moreover, the experiments were conducted at room temperature, so, enzyme activity may lost during purification.

Table 1. Summary of Mutarotase purification.

Samples	Activity (U/mL)	Protein contents (mg/mL)	Specific activity (U/mg)	Fold Purification
Crude	5.491	4.345	1.264	1
$(NH_4)_2SO_4$ (Desalted)	4.091	0.980	4.174	3.30
After gel filtration chromatography	2.775	0.168	16.518	13.07

The purified enzyme was subjected to different kinetic parameters i.e. pH, temperature, substrate concentration, enzyme concentration. Optimum pH was found to be 7.5 that showed maximum activity 4.273 U/mL of mutarotase (Fig.1). This finding also supports that optimum pH range of mutarotase is 6.5-7.5 when analyzed from bovine kidney cortex [2]. It is further reported that optimum pH of mutarotase was 7.4 with activity of 3.14 U/mL [6]. The optimum temperature of enzyme has been recorded as 40 ^oC with activity of 4.918 U/mL (Fig.2). Moreover, optimum temperature of mutarotase was 43 ^oC [2] and also 43 ^oC with 2.82 U/mL activity in another study [6].

Maximum activity of enzyme was 5.607 U/mL when analyzed at 111 mM (200 mg) substrate concentration. Detailed results in (Fig.3), indicate that with increasing the substrate concentration, activity of the enzyme was increased but a stage when there was no change in activity of enzyme with more increase in substrate concentration. But by adding the substrate concentration the enzyme was inhibited [2]. It was also showed in this regard that the activity of enzyme at 500 mg substrate concentration was 2.47 U/mL proving to be optimum [6]. The maximum activity (5.519 U/mL) of mutarotase enzyme in the present study was optimum at 1.387 U/mg (500 μL) enzyme concentration as shown in (Fig.4). It is in accordance that 500 μL indicating

optimum enzyme concentration obtained 3.034 U/mL activity of bovine kidney mutarotase [6].

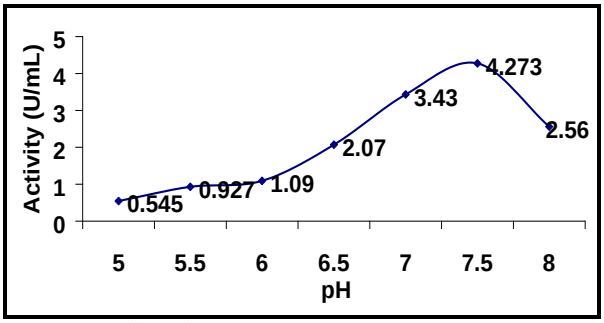

Figure 1. Effect of pH on mutarotase activity

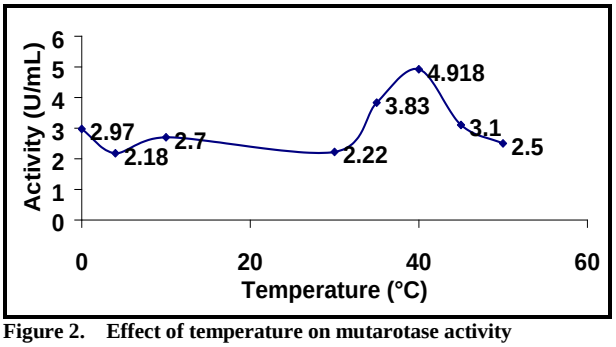

Figure 2. Effect of temperature on mutarotase activity

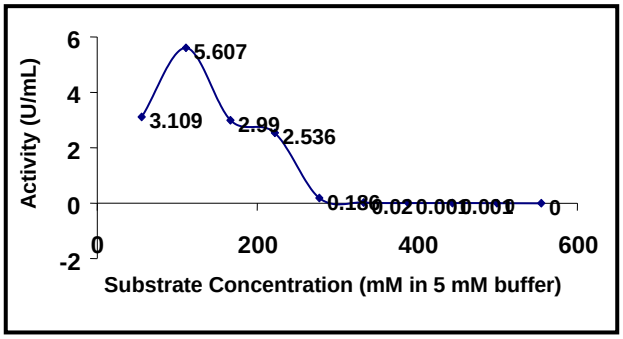

Figure 3. Effect of substrate concentration on mutarotase activity

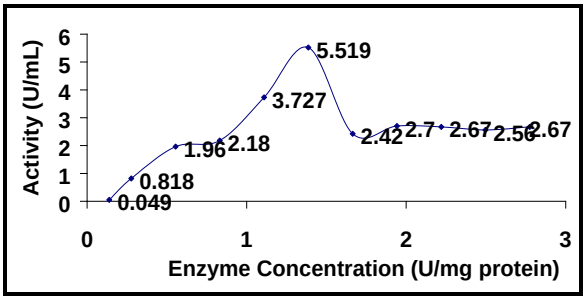

Figure 4. Effect of enzyme concentration on mutarotase activity

The study on effect of stabilizers showed that glycerol is the best one stabilizer among sodium citrate and sodium benzoate. After 30 days, 15% glycerol containing enzyme showed maximum activity 3.71 U/mL (Table 2) while sodium benzoate and sodium citrate obtained 0.13 U/mL (Table 3) and 0.22 U/mL (Table 4) respectively. Both sodium benzoate and sodium citrate showed their maximum results at 30% concentrations, as the activity of enzyme was decreased day by day.

Table 3. Effect of varying percentage of sodium benzoate on mutarotase activity.

Days	Mutarotase Activity (U/mL)			
	0 % Sodium benzoate	15 % Sodium benzoate	30 % Sodium benzoate	45 % Sodium benzoate
0	5.519	2.54	1.96	1.03
1	5.203	1.81	1.41	0.72
2	5.221	1.63	1.37	0.68
3	5.010	1.46	1.70	0.76
4	4.786	1.33	1.65	0.61
5	4.104	0.85	1.4	0.43
10	3.968	0.79	1.22	0.31
20	3.12	0.54	1.05	0.24
30	1.09	0.13	1.01	0.09

Table 4. Effect of varying percentage of sodium citrate on mutarotase activity.

Days	Mutarotase Activity (U/mL)			
	0 % Sodium citrate	15 % Sodium citrate	30 % Sodium citrate	45 % Sodium citrate
0	5.519	2.72	2.05	1.13
1	5.203	1.33	1.10	0.81
2	5.221	1.30	1.02	0.73
3	5.010	1.36	1.13	0.79
4	4.786	0.90	1.15	0.62
5	4.104	0.98	1.2	0.67
10	3.968	0.74	1.09	0.46
20	3.12	0.62	0.85	0.28
30	1.09	0.22	1.00	0.00

Hence, after purification and kinetic studies, it is concluded that bovine kidney is a rich source of mutarotase enzyme. There is a great need to exploit natural sources for the isolation and commercial production of beneficial enzymes to meet the requirements of our industry, diagnosis results as well as treatment. Moreover this work will also help towards the stabilization of mutarotase when used especially in glucose diagnostic kit.

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