

Purification and Characterization of Uricase Enzyme Produced by

Gliomastix gueg



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Abstract

Uricase enzyme was produced and purified from *Gliomastix gueg* (NRC 1A) fermentation using ammonium sulphate selective precipitation (70% saturation) followed by ion exchange chromatography on DEAE- cellulose column. Results showed that pure enzyme produced during ammonium sulphate 70% saturation proved to be high uricolytic activity (146.84 U/ml) and specific activity (157.13 U/mg protein). Uricase produced from *Gliomastix gueg* (NRC 1A) was bound to the ion exchange column chromatography at pH 8.5 and eluted with linear NaCl (0-1M). Most of the uricolytic activity was found in the second protein peak in the 0.5 - 0.6M NaCl gradient. The enzyme was purified almost 33.2- fold with a specific activity 428.70U/mg protein. The molecular weight of the purified uricase was estimated by SDS-poly-acrylamide gel electrophoresis technique (60kDa). The maximum purified uricase activity was detected at 35°C and pH 9.0, the enzyme was stable at 35°C at different times tested. Maximum increase of uricase activity was obtained in the presence of Ca²⁺ ions reaching 387.05U/ml followed by Mn²⁺ (162.67U/ml). Inhibitors such as P.CMBA and cystine reduced the uricase activity by 15.26 and 12.50 U/ml, respectively. The effect of substrate concentration as well as enzyme concentration was also investigated. Results demonstrated that, 10µg of uric acid substrate and 100 µl (231U/ml) of the purified enzyme per reaction was enough to be used in the reaction mixture when incubated for 20 minutes. Results showed that the rate of uric acid consumption in the reaction mixture by purified uricase enzyme increase with increasing the incubation time.

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1. Introduction

Urate oxidase or uricase (urate: oxygen oxidoreductase, EC 1.7.3.3), the enzyme that catalyzes specifically the oxidation of uric acid to allantoin and plays an important part in nitrogen metabolism [17]. Uricase is used in medicine and clinical biochemistry as a diagnostic reagent for measurement of uric acid in blood and other biological fluids [2]. Higher primates (apes and

humans) lack functional uricase and excrete uric acid as the end product of purine degradation [8,18]. In some individuals, uric acid precipitate, leading to gout symptoms. Gout treatments generally include allopurinol, a potent competitive inhibitor of xanthine dehydrogenase, the enzyme that catalyses the conversion of hypoxanthine to xanthine and xanthine to uric acid [12]. In the case of gout associated with renal complications, direct injection of urate oxidase

allows a much more rapid resorption of urate nephrolithiasis. Such injections are done to prevent or treat hyperuricemia disorders that may occur during chemotherapy. Although several microbial sources of uricase have been proposed for this clinical indication, only one has actually been used commercially under the trade mark of uricozyme and is isolated and purified from *Aspergillus flavus*.

Gout is a painful disorder, characterized by uricemia, recurrent attacks of acute arthritis, deposition of sodium urate in and around joints, and in many cases, formation of uric acid calculi [10].

This enzyme is widely present in most vertebrates, but uricase is absent in humans and other higher primates [15]. It was first found in bovine kidney. Various natural sources such as bacteria [11], fungi [7] and eukaryotic cells [13] have also been found to be uricase producers.

Uricase was originally isolated from mammalian organisms. Recently interest was concentrated on microbial preparations from various fungi, yeast and bacteria. The microbial enzyme is inducible and therefore, the presence of uric acid or some other inducer in the medium is necessary for enzyme production [2].

In various microorganisms uricase synthesis is regulated by components of the growth medium and the ability to degrade uric acid and to use it for growth is an inducible property of these microorganisms [16]. Moreover, it was suggested that uricase formation might be controlled by a repression in which a metabolite derived from both the nitrogen and carbon sources may participate [4].

Several investigators [2,14] studied the purification and characterization of uricase enzyme by microorganisms.

This paper describes the purification and properties of microbial uricase from *Gliomastix gueg* (NRC 1A).

2. Materials and methods

Organism:

The fungal strain used in this study was brought from culture collection unit of the Department of Chemistry of Natural and Microbial products at the National Research Center. This fungal strain was

tested for uricase production using uric acid medium: uric acid 1.0g; K₂HPO₄ 1.0g; MgSO₄ 0.5g; NaCl 0.5g; FeSO₄ 0.01g and Sucrose 20.0g [1]. This medium was dissolved in 1 liter distilled water and pH was adjusted at 8.0. Uricase was extracted into borate buffer (pH 8.5)

Uricase assay:

Uricase activity was measured according to the procedure described by Adamek method [2]. To 2 ml of a solution containing uric acid (10 µg per ml of borate buffer 0.2M, pH 8.5), 0.8 ml of water and 0.1 ml of crude enzyme at 25°C were added. After 10 min., 0.2 ml of 0.1 M potassium cyanide solution was added to the mixture to stop the enzyme reaction. In the reference sample, the solution of potassium cyanide was added to the mixture before addition of the crude enzyme. The absorbance of both samples was measured at 293 nm. The difference between the absorbance of the sample and reference is equivalent to the decrease in uric acid during the enzyme reaction. One unit of uricase enzyme was equal to the amount of enzyme which convert 1 µmol of uric acid to allantoin per min at 30°C.

Purification of uricase:

The following steps were performed for purification of uricase produced by *Gliomastix gueg* (NRC1A) under all optimal conditions.

Uricase production and preparation of cell free filtrate:

Gliomastix gueg (NRC1A) was allowed to grow on uric acid fermentation medium under all previous studied optimal conditions (at 30°C on rotatory incubator shaker at 150 rpm for 8 days). At the end of the incubation period, the obtained extract was filtrated by centrifugation at 5000-6000 rpm for 15 min at 4°C to obtain the cell free filtrate. Uricase activity and protein content were determined and then the obtained cell free filtrate was preserved in the refrigerator as a crude uricase enzyme.

Crude enzyme fractional precipitation by ammonium sulphate:

Different concentrations of ammonium sulphate (40, 60, 70, and 80% w/v) were used. Solid ammonium sulphate was added slowly to the culture

filtrate with gentle stirring on ice bath until the required saturation of ammonium sulphate was reached after which the mixture was allowed to stand at 4°C overnight. The mixture was then centrifuged at 8000 rpm. for 30 minutes at 4°C. Further ammonium sulphate was added to the culture filtrate and the process repeated until the final saturation reached 80%. Both enzyme activity and protein content were determined for each separate fraction.

Uricase concentration using Dialysis:

This purification step was carried out to remove the traces of ammonium sulphate. The resultant precipitate was dissolved in 5 ml 0.02 M Tris-HCl buffer pH 8.5 and dialyzed overnight against 2 liters of the same buffer in a cellophane bag [14]. The concentrated and dialyzed cell free supernatant became ready to be applied on further purification step, i.e., column chromatography technique.

Purification of uricase by ion exchange chromatography on DEAE-cellulose:

A glass column (1.5×60 cm) was packed with DEAE-cellulose. The concentrated and dialyzed cell free supernatant was applied to a column, which previously was equilibrated with 0.01M borate buffer (pH 8.5). The column was washed with 3 times volumes of 0.01 M borate buffer, pH 8.5 at a flow rate of 40ml/hour and the bound proteins were eluted with a linear NaCl gradient (0-1 M) in the same buffer by using fraction collector (LKB Radi-Rac) and analyzed by UV spectrophotometer at 280 nm. Fractions containing uricase enzyme were pooled and concentrated using ammonium sulphate precipitation method as described above.

Determination of protein content:

Total protein concentrations of cell free supernatant and purified samples were assayed by the method of Bradford [5] using a calibration curve established with bovine serum albumin (BSA) as a standard and compassion brilliant blue dye (CBB) G-250. Appropriate volume (20µl) was taken from the protein solution in 10 ml test tube. The volume in the test tube was completed to 0.1 ml with distilled water (80 µl) then 5ml of CBB dye were added to the tube

and the contents were well mixed. The absorbance of the mixture was measured after 5 minutes at 595 nm against reagent blank. The protein content calculated from the standard curve.

SDS-PAGE protein Electrophoresis:

The purity of the isolated protein was determined by the SDS-PAGE on 10% gel according to the method of Laemmli [9]. This method was used to determine the molecular weight of the purified uricase enzyme.

Determination of molecular weight:

The molecular weight of enzyme was determined by standard protein markers (low molecular weight 14 – 60 kDa) with different molecular weights such as α lactalbumin (14kDa), Trypsin (23kDa), Carbonic anhydrase (29kDa), Glyceraldehy-3-phosphate dehydrogenase (36kDa), Ovalbumin (45kDa), Glutamic dehydrogenase (55kDa) and bovine serum albumin (60kDa) were used for establishing a standard curve.

Properties of the purified uricase:

pH value:

The purified enzyme reaction mixture were incubated at different pH values 7.0, 7.5, 8.0, 8.5, 9.0 and 10.0 using borate buffer (0.2M).

Temperature:

The purified enzyme reaction mixture were incubated at different temperature 30, 35, 40, 45, 50, 55 and 60°C.

Incubation time:

The purified enzyme is incubated at different times (10, 15, 20, 25, 30 and 60 min) to study the best time for reaction.

Thermal stability:

The purified enzyme is incubated with different temperatures (30, 35, 40 and 50°C) at different times (10, 15, 20, 25 and 30 min). At the end of incubation period at those temperature degrees, the replicate tubes were cooled and assayed for uricase activity as previously mentioned.

Metal ions:

All these metal ions Ca²⁺, Co²⁺, Mn²⁺, Mg²⁺, Fe²⁺, PO₄³⁻, were applied into the reaction mixture at a concentration of 10⁻³M.

Inhibitors:

Different inhibitors such as cysteine, cystine, riboflavin, folic acid, ascorbic acid, reduced glutathione, nicotinic acid, arginine, glycine, tryptophane, iodine, sodium cyanide and parachloromercuribenzoic acid (P-CMBA) have been added to the reaction mixture at a concentration of 10⁻³ M, the pH was adjusted to 8.5.

Substrate concentration:

The substrate (uric acid) was applied into the reaction mixture at different concentrations (10, 20, 30, 40, 50 and 60 µg/ml of borate buffer, pH 8.5).

Enzyme concentration:

The purified enzyme was added to the reaction mixture at different concentrations to study the best concentration of enzyme. The reaction mixture contained 25, 50, 75, 100, 125 and 150 µl of the purified enzyme.

Enzyme application (In vitro study):

The produced enzyme was tested to oxidize uric acid (In vitro) as follows: A volume of 2ml of a solution containing 6mg uric acid substrate per 100ml of borate buffer (0.2 M, pH 9), 0.8ml of water and 0.1ml (231U/ml) of the purified enzyme at 35°C were

Ammonium sulphate concentration (%)	Uricase activity (U/ml)	Protein content (mg/ml)	Specific activity (U/mg protein)
Crude	16.23	1.26	12.89
40	0	3.04	0
60	68.29	1.48	46.14
70	146.84	0.93	157.13
80	0	0	0

added at different incubation periods (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 120 min). Then 0.2 ml of 0.1M potassium cyanide solution was added to the mixture to stop the enzyme reaction. In the reference sample, 0.8ml of water was added to the 0.1ml (231 U/ml) purified enzyme only. The

absorbance of both samples was measured at 293 nm. The difference between absorbance of the sample and reference is equivalent of the consumption of uric acid during the enzyme reaction. Uric acid was determined by Domagk and Schlicke method [6] using uric acid standard 6mg/dl (356.9µmol/l) which was purchased from Biocon, Germany.

3. Results and discussion

Purification of uricase:

These experiments were carried out to obtain the pure uricase from the crude compound which was collected from the fermentation experiments of *Gliomastix gueg* (NRC1A).

Precipitation by ammonium sulphate:

Different concentrations of ammonium sulphate (40, 60, 70 and 80% W/V) were added to the crude uricase. The Results presented in Table (1) indicate that ammonium sulphate 70% saturation proved to be high uricolytic activity (146.84 U/ml) and specific activity (157.13 U/mg protein) comparing with crude uricase and other concentrations followed by ammonium sulphate 60% saturation. On the other hand, concentration of 40% ammonium sulphate showing extremely high protein content (3.04mg/ml) rather than other concentrations. However such concentration revealed zero uricase and specific activity. There is no precipitation obtained with 80% ammonium sulphate. These results are in agreement with those of Saeed [Saeed et al., 2004] who found that *P.aeruginosa* uricase enzyme was purified using ammonium sulphate (70% saturation). Also Adamek [2] showed that crude enzyme extract from *C. utilis* was precipitate with 60% ammonium sulphate. These results were in disagreement with those of Azab [3] who reported that the proteins of the culture supernatants (of *Proteus* and *Streptomyces*) were precipitated with 80% ammonium sulphate.

Table (1): Purification of uricase from *Gliomastix gue* (NRC1A) by ammonium sulphate.

Ion exchange chromatography on DEAE-cellulose:

The obtained uricase after partially purified with 70% ammonium sulphate was dialyzed and lyophilized and dissolved in 1ml of 0.01M borate buffer. Then applied to the top of DEAE-cellulose column chromatography (1.5X60cm) which previously equilibrated with 0.01M buffer at pH 8.5 at room temperature. At first, the unbounded protein was removed by washing with 3 times volumes of 0.01M borate buffer. Uricase was bound to the ion exchange column at pH 8.5 eluted with linear NaCl gradient (0-1M). The results illustrated in Fig.(1) show that three protein peaks were detected at fractions (8-13), (20-30) and (33-39), the protein content of these fractions were 0.311, 2.037 and 0.238 mg, respectively. Also most of the uricolytic activity was detected in the second protein peak at fraction (20-30) in the 0.5 - 0.6M NaCl gradient. The

first protein peak fraction (8-13) showed lesser enzyme activity than the second and third protein peak. The purification results which summarized in Table (2) record that the enzyme was purified almost 33.2- fold with a specific activity and recovery of 428.70 and 17.60%, respectively. These results are in disagreement with those of Saeed [13] who showed that *P. aeruginosa* uricase enzyme was purified using ion exchange chromatography on Q-sepharose fast flow anion exchange and sephadex G-50 column. They found that upon using Q- sepharose fast flow column gave the most of uricolytic activity was found in the third protein peak in the 0.5M NaCl gradient and the enzyme was purified almost 29.71-fold with a specific activity and recovery of 592 and 31% respectively.

Purification steps	Volumn (ml)	Enzyme activity (U/ml)	Total units	Protein content (mg/ml)	Total protein	Specific activity (U/mg protein)	Purification -fold	Recovery %
Cell free supernatant (CFS)	500	16.23	8118.9	1.260	630.00	12.89	1	100
Pellet after 70% ammonium sulphate and Dialysis	50	146.84	7341.8	0.934	46.72	157.13	12.19	90
After DEAE-cellulose column	10	143.20	1432.0	0.334	3.34	428.70	33.20	17.60

Table (2): Purification of uricase enzyme using DEAE-cellulose column.

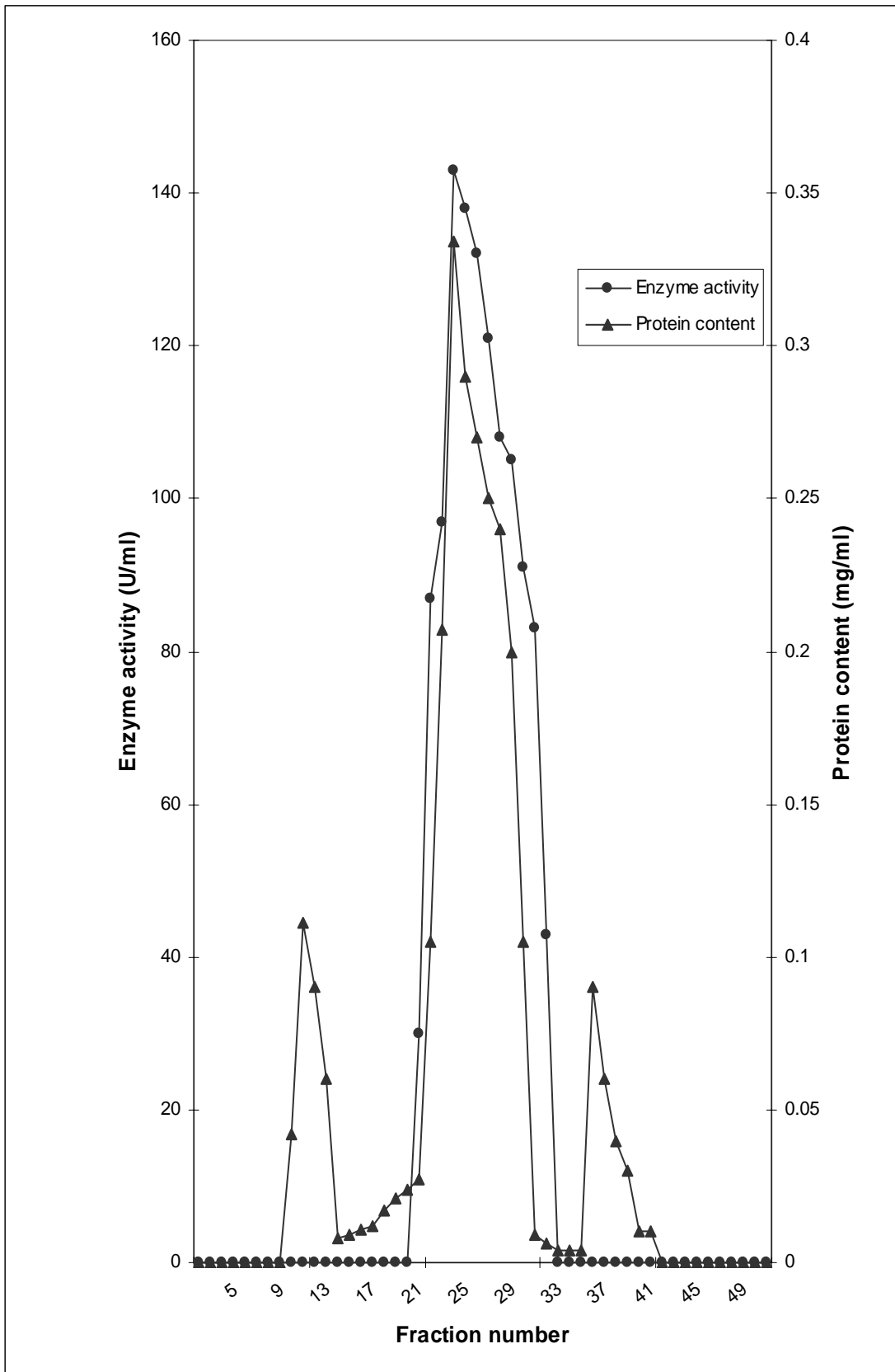


Fig. (1): Fractionation of uricase enzyme on DEAE-Cellulos

On the other hand, upon using sephadex G-50 column demonstrated that, only the second protein peak overlapped with the uricase activity and the enzyme was purified to 31.9-fold and with a specific activity of 636.360. However, Adamek [2] found that *C.utilis* uricase enzyme was purified using ion exchange chromatography on DEAE – cellulose and the active fraction of uricase was released from the column at a low ionic strength (0.1- 0.2M sodium chloride).

Determination of molecular weight for purified uricase:

The molecular weight of the purified uricase was determined by SDS- poly acrylamide gel electrophoresis (SDS-PAGE). This was carried out as

mentioned in material and methods in section (3.9) using standard protein with different molecular weight markers [bovine serum albumin (60kDa), glutamic dehydrogenase (55kDa), ovalbumin (45kDa), glyceraldehydes-3-phosphate dehydrogenase (36kDa), carbonic anhydrase (29kDa), trypsin (23kDa) and alpha lactalbumin (14kDa)] with the purified uricase sample. The results in Fig.(2) showed that the molecular weight of the enzyme was found to be 60kDa. These results are slightly in agreement with those of Saeed [14] who found that the molecular weight of the purified uricase was determined by SDS-PAGE to be 68.0 kDa for one subunit.

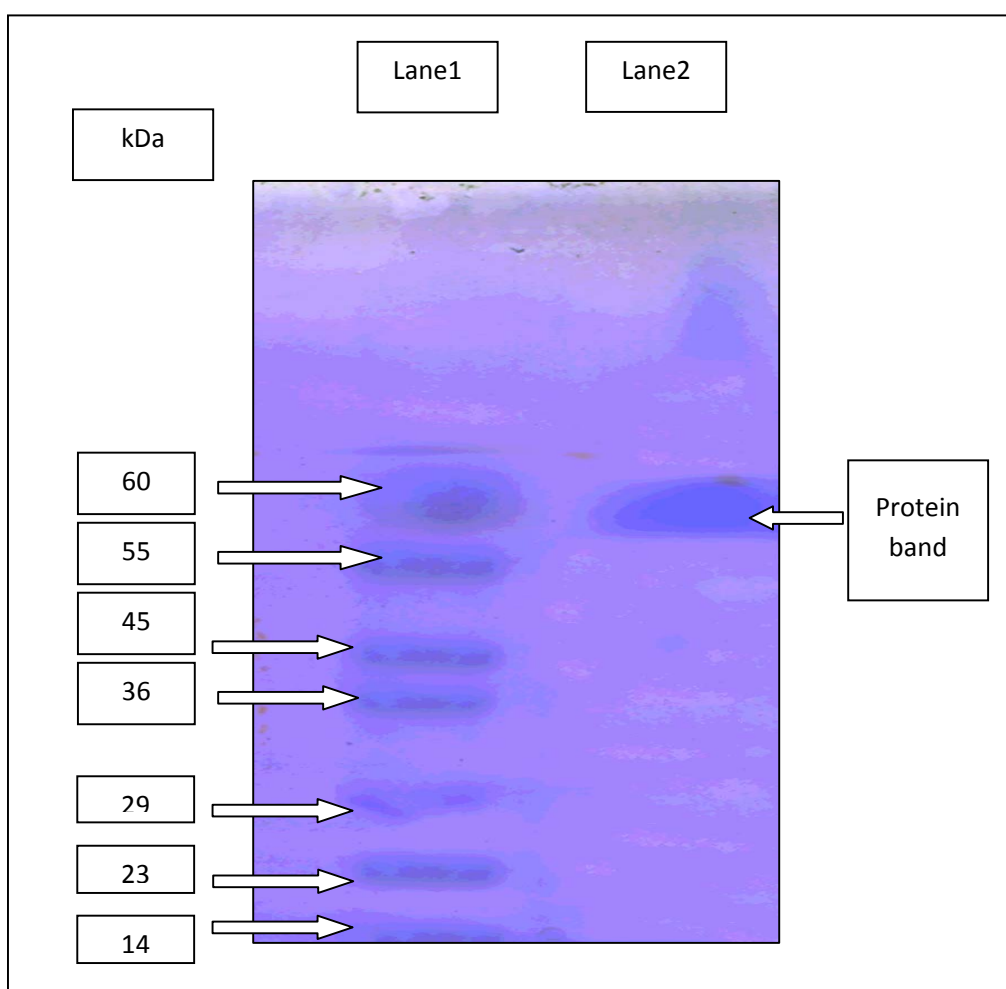


Fig. (2): The molecular weight of the purified uricase enzyme by SDS- poly acrylamide gel electrophoresis (SDS-PAGE) using standard protein with different molecular weight markers.

Lane1: bovine serum albumin (60kDa), glutamic dehydrogenase (55kDa), ovalbumin (45kDa), glyceraldehydes-3-phosphate dehydrogenase (36kDa), carbonic anhydrase (29kDa), trypsin(23kDa) and alpha lactalbumin (14kDa), Lane 2: purified uricase enzyme.

Properties of purified uricase:

a- Effect of different pH-values on the activity of purified uricase:

This experiment was performed to investigate the effect of different pH-values on the purified uricase activity. 1ml of 0.2 M borate buffer at pH values ranging from 7.0 – 10.0 was added at room temperature with 0.1ml of purified enzyme. The enzyme activity was determined as mentioned before. The results in Fig (3) show that the maximum purified uricase activity was detected at pH 9.0 reaching 366.00 U/ml. These results are in agreement with those of [2,14] who found that purified enzyme exhibited maximum uricolytic activity at pH 9.0 and 8.5 respectively.

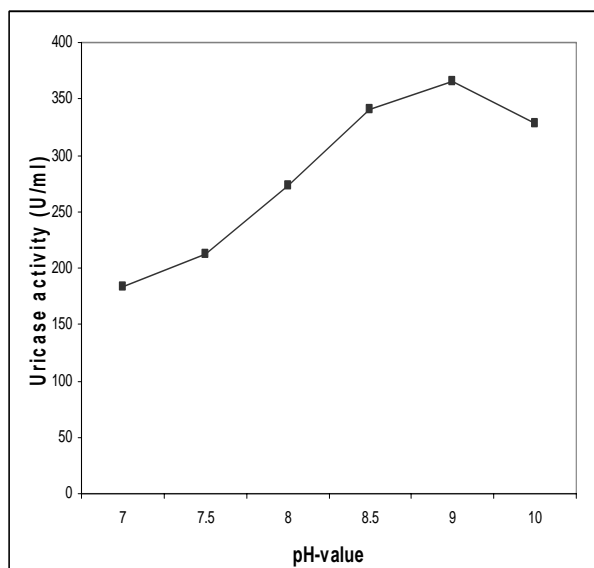


Fig. (3): Effect of different pH-values on the activity of purified uricase produced by *Glomastix gueg* (NRC1A).

b- Effect of reaction incubation temperature on the activity of purified uricase:

This experiment was performed to determine the effect of different incubation temperatures on the purified uricase activity. This was carried out, by incubating reaction mixture at different incubation temperatures viz. 30, 35, 40, 45, 50, 55 and 60°C respectively. Uricase activity was determined as previously mentioned before. It is clear from the results presented in Fig. (4) that purified *Glomastix*

gueg exhibited maximum uricolytic activity (483.50 U/ml) at 35°C. Such findings were in disagreement with those of [2,14] who found that the optimum temperature of the purified uricase activity was found to be 35°C.

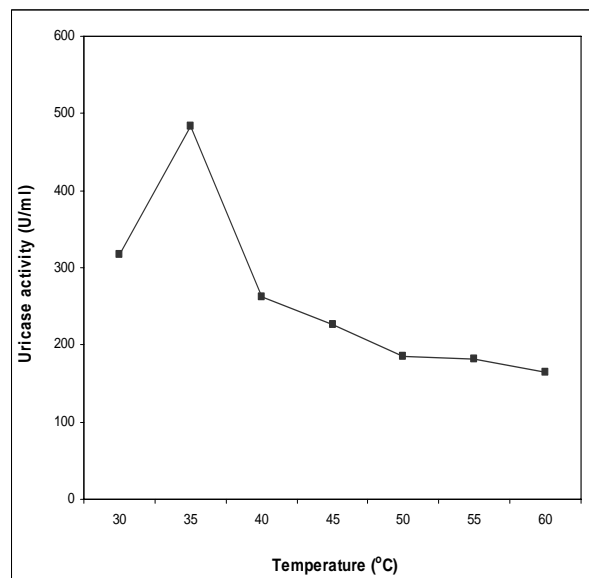


Fig. (4): Effect of reaction incubation temperature on the activity of purified uricase

c- Effect of reaction incubation time on the activity of purified uricase:

This experiment was carried out to determine the optimum incubation time at which the purified enzyme reach its maximum activity. The reaction mixtures were incubated for 10, 15, 20, 25, 30 and 60 min. Assessment of uricase activity was performed as described before. The results in Fig. (5) demonstrate that the maximum purified uricase activity (313.71 U/ml) was detected after 20 min. of incubation time and then decreased with increasing time.

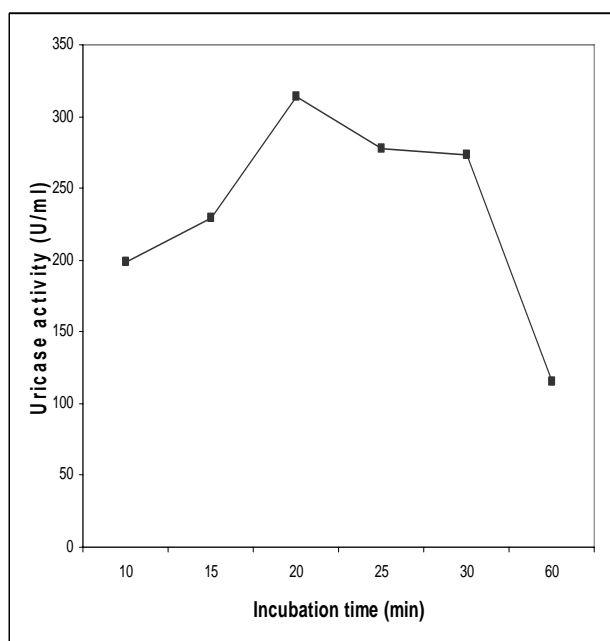


Fig. (5): Effect of reaction incubation time on the activity of purified uricase

d- Thermal stability of purified uricase:

This experiment was designed to determine the temperature range within the partially purified uricase enzyme maintains its activity and also to illustrate the enzyme thermal stability under study. This experiment was carried out by incubating the purified enzyme at different temperatures (30, 35, 40 and 50°C) and different times (10, 15, 20, 25, and 30 min.), at the end of incubation period at those temperature degrees, the enzyme activity was determined as described before. The results presented in Table (3) and Fig.(6) illustrate that purified uricase enzyme retained 93.62% of its activity after exposure to 35°C for 10 minutes. Also retained 62.92% of its activity after exposure for 30 min. at the same temperature, Thus the purified *Gliomastix gueg* uricase enzyme was stable at 35°C at different times. The residual uricase activity showed decrease from 42.75% after 10 min. to 13.36% after 60 min. at 50°C. These results are in disagreement with those of Saeed [14] who found that *P. aeruginosa* uricase enzyme retained 12.4% of its original activity after exposure to 40°C for 50 minutes. Moreover, the purified enzyme lost all of its original activity after 10 minutes of exposure to 70°C.

Also, Zhu [19] reported that purified *C.utilis* uricase enzyme was sensitive to heat.

Incubation time (min)	Residual uricase activity (%)			
	Temperature °C			
	30	35	40	50
10	67.07	93.62	66.06	42.75
15	63.02	71.73	41.74	25.02
20	20.46	71.43	17.42	18.43
25	15.39	69.40	16.41	14.37
30	14.38	62.92	15.30	13.36

Table (3) Thermal stability of the purified uricase from *Gliomastix gueg* (NRC1A).

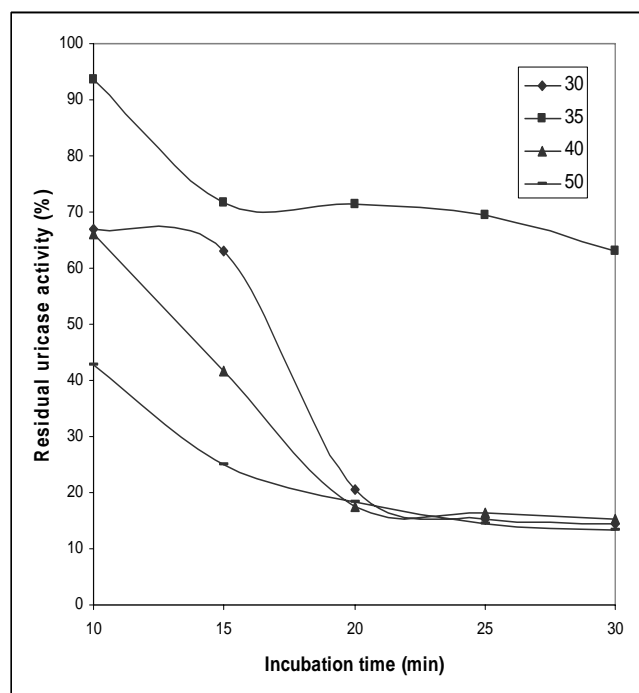


Fig. (6): Thermal stability of the purified uricase from *Gliomastix gueg* (NRC1A).

e- Effect of different metal ions on the activity of purified uricase:

This experiment was carried out in order to study the effect of the tested metal ions to the reaction mixture on the enzyme activity. Metal ions (10^{-3} M) such as Ca^{2+} , Co^{2+} , Mn^{2+} , Mg^{2+} , Fe^{2+} and Po_4^{3-} were added to the reaction mixture consisted of 2ml of a solution containing $10\mu g$ uric acid per 1ml of borate buffer 0.2M at pH 8.5 and $100\mu l$ of enzyme solution, the reaction was carried out at $25^\circ c$ for 10 min. The enzyme activity was determined as mentioned before, metal ions free reaction mixture was carried out as control. The results illustrate in Fig.(7) showed that addition some tested metal ions such as Co^{2+} , Mg^{2+} , Fe^{2+} and P_3^- reduce the enzyme activity to 69.63, 75.73, 91.70 and 78.64 U/ml, respectively when added to the reaction mixture.

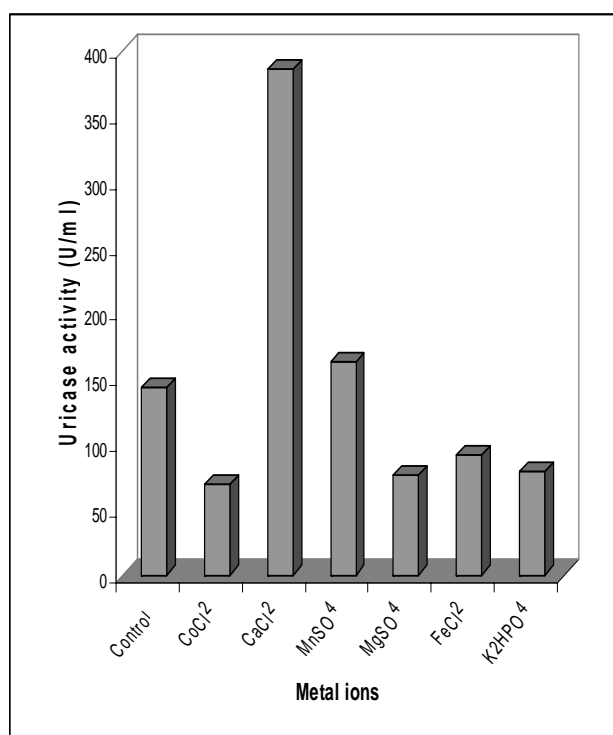


Fig.(7). Effect of different metal ions on the activity of purified uricase

On the other hand, maximum increasing of uricase activity was indicated in the presence of Ca^{2+} ions reaching 387.05 U/ml. Considerable increasing in the uricase activity is also noticed with Mn^{2+} ions. These results are in agreement with those of Saeed [14] who found that Fe^{2+} and Co^{2+}

inhibited the uricase activity by different degrees. However, metal ions such as Ca^{2+} strongly enhance the uricase activity. One possible explanation for the results that, some metal ions bind to the uricase enzyme and alter the enzymatic activity by stabilization or destabilization of the enzyme's conformation.

f- Effect of inhibitors on the activity of purified uricase:

This experiment was performed to investigate the effect of various inhibitors such as iodine, para chloromercuribenzoic acid (P.CMBA), sodium cyanide, ascorbic, folic acid, nicotinic, riboflavin, reduced glutathione, cysteine, arginine, glycine and tryptophane on the purified enzyme activity.

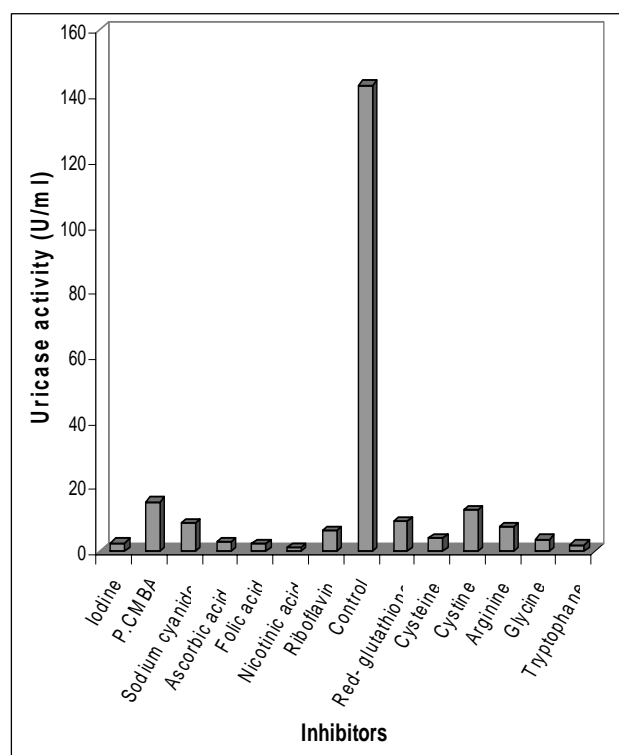


Fig. (8) Effect of inhibitors on the activity of purified uricase

All these inhibitors were applied into the reaction mixture at a concentration of 10^{-3} M, control was carried out without addition of inhibitors. Uricase activity was determined as described before. The results Fig. (8) show that all tested inhibitors inhibited the uricase activity by different degrees. However, inhibitors such as P.CMBA and cystine reduced the uricase activity to 15.26 and 12.50 U/ml.

Also, nicotinic, tryptophane, folic acid, iodine and ascorbic acid strongly decrease the uricase activity to 1.13, 2.05, 2.24, 2.42 and 2.79 U/ml.

g- Effect of substrate concentration on enzymatic activity:

This experiment was performed to investigate the effect of different substrate (uric acid) concentrations on the purified enzyme activity. The effect of different substrate concentrations was tested by incubating different substrate concentrations (10, 20, 30, 40, 50 and 60 µg/ml, respectively) with the same amount of the enzyme. The enzyme activity was plotted against substrate concentration. The results in Fig.(9) demonstrate that, 10µg of uric acid substrate proved to be high uricolytic activity (143.20 U/ml) and then uricolytic activity decrease with increasing uric acid concentration. These results are in disagreement with those of Saeed [14] who found that 20µg of uric acid (substrate) was enough to be used in the reaction mixture.

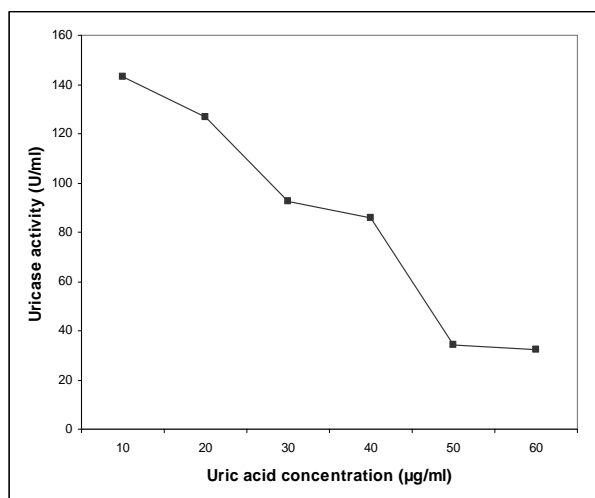


Fig. (9): Effect of different concentration of substrate (uric acid) on the activity of purified uricase.

h- Effect of enzyme concentration on enzymatic activity:

This experiment was carried out to investigate the effect of different concentrations of uricase on its activity. The purified uricase was added into the reaction mixture at concentrations of 25, 50, 75, 100, 125 and 150 µl, respectively. The relation between enzyme concentration and enzyme activity was recorded.

The results presented in Fig. (10) indicated that the enzyme activity of uricase increase with increasing the enzyme concentration up to 100µl (231.00 U/ml), then uricolytic activity decrease with increasing enzyme concentration. These results are not concomitant with those of Saeed [14] who found that 10 µl (0.2 µg) of the purified enzyme per reaction was enough to be used in the reaction mixture.

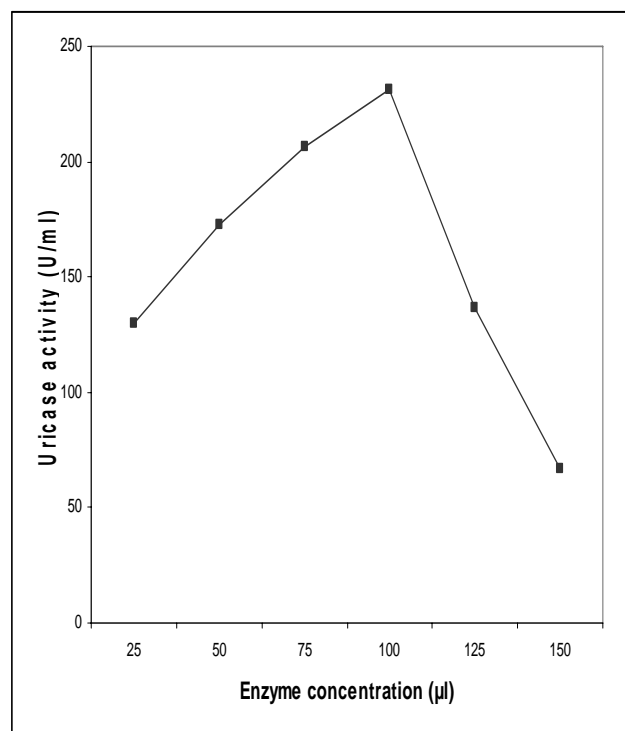


Fig. (10): Effect of different concentration of enzyme on the activity of purified uricase

Uric acid consumption by purified uricase (In vitro study):

The produced enzyme by *Gliomastix gueg* (NRC1A) was purified and tested to determine the rate of uric acid consumption. The results in Fig. (11) show that enzyme preparation was able to utilize urate as a substrate as indicated by measuring the remained uric acid in the reaction mixtures. Surprisingly, the rate of uric acid consumption increase with increasing the incubation time. Its clear from the results that potency of the purified enzyme in uric acid degradation and hence its possible use in uric acid determination in biological fluids was in ascending order by time. These results are in

agreement with those of Saeed [14] who showed that the rate of uric acid consumption was higher in the case of *P. aeruginosa* uricase than that of uricozyme.

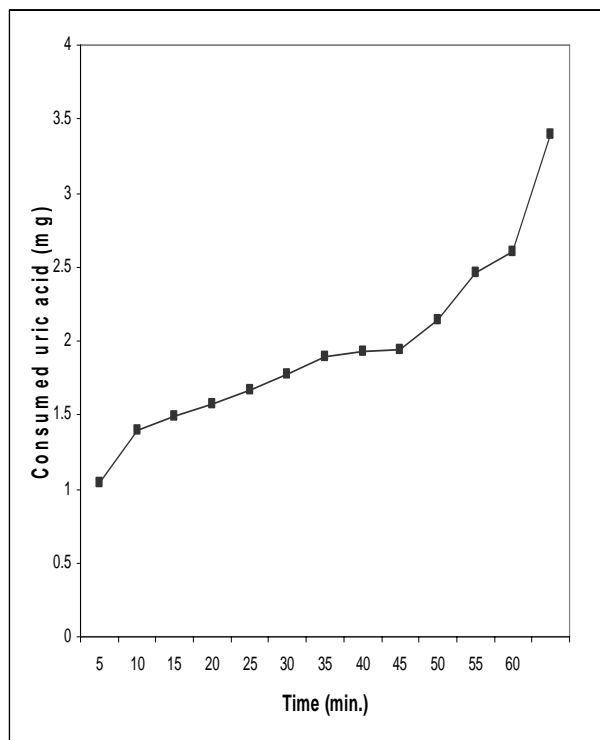


Fig.(11): Uric acid consumption by purified uricase produced from *Gliomastix gueg* (NRC1A).

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