

Studies on urate oxidase purification and optimization

Anderson Amirthanathan^{1*}

^{1*} Department of Microbiology, International University, Phnom Penh, Cambodia.

Corresponding Author

* Email: ansanmicro@yahoo.com, Tel: +855 77889097

Abstract

The urate oxidase enzyme was extracted from *Pseudomonas aeruginosa* and purified by ammonium sulphate precipitation. The molecular weight of purified uricase was determined by SDS-PAGE using marker proteins of known molecular weight. The optimum temperature, pH and the effect of various metal ions on uricase activity were evaluated. The results showed that 70% ammonium sulphate concentration proved high uricase activity by 40.0 U/ml than other concentration and cell free supernatant. The molecular weight of purified uricase enzyme was estimated to be 33 kDa by SDS-PAGE profile. The optimum temperature and pH was detected at 35°C and 8.5 for maximum uricase activity. Metal ions such as Co²⁺, Mn²⁺, Mg²⁺, Fe²⁺, Zn²⁺ and Cu²⁺ were reduced the enzyme activity to 33.17%, 92.18%, 72.47%, 32.73%, 64.32% and 90.10%, whereas Ca²⁺ enhanced the uricase activity to 126%.

Key words: Urate oxidase, purification, optimization

Introduction

Urate oxidase or uricase (urate: oxygen oxidoreductase, EC 1.7.3.3) is an enzyme participating in the purine breakdown pathway, catalyzing the oxidation of uric acid in the presence of oxygen to allantoin and hydrogen peroxide [1]. Uricase enzyme is widely present in most vertebrates but is absent in humans [2]. Higher primates (apes and humans) lack functional

uricase and excrete uric acid as the end product of purine degradation [3, 4].

.....Cont.....

Materials and Methods

Microorganism

The bacterial strain used in this study was previously isolated from poultry waste and was identified as *Pseudomonas aeruginosa* by specific biochemical tests for uricase production [5].

.....Cont.....

Uricase assay

The principle of enzyme measurement was as follows: uricase could catalyze the oxidation of uric acid into allantoin and H₂O₂, which was then measured by using a reaction system containing 4-aminoantipyrine, phenol and peroxidase as chromogen. In practical analysis, 0.1ml enzyme solution was incubated with a mixture of 0.6ml sodium borate buffer (pH 8.5, 0.1M) containing 2mM uric acid, 0.15ml 4-aminoantipyrine (30mM), 0.1ml phenol (1.5%), 0.05ml peroxidase (15U/ml) at 37 °C for 20 min [6].Cont.....

Purification of Uricase

The enzyme purification processes were performed with 0.1 M phosphate buffer at pH 7.0 (buffer A) at 4 °C. Cells from 10.0 l of media were harvested by centrifugation and washed twice with 50 mM phosphate buffer (pH 7.5), re-suspended in buffer A and disrupted by ultrasonic oscillation (120 W oscillating for 3 s with 6 s intervals, repeated 100 times).

.....Cont.....

SDS-PAGE Profile

The proteins were separated by SDS-PAGE electrophoresis and the size of polypeptide chains of given protein can be determined by comparing its electrophoretic mobility in SDS-PAGE gel with mobility marker proteins of known molecular weight [7].

Effect of pH and Temperature

To investigate the effect of pH on the activity of uricase, the enzyme was assayed at different pH; 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0, and temperature; 20, 25, 30, 35, 40, 45, 50, 55 and 60 °CCont.....

Effect of Metal ions

To study the effect of metal ions on uricase activity, the different metal ions such as Ca²⁺, Co²⁺, Mn²⁺, Mg²⁺, Fe²⁺, Zn²⁺ and Cu²⁺Cont.....

Results

Purification of Uricase

The present study was conducted to obtain the purified form of uricase and to determine its optimum conditions for maximum activity. In the first step, the uricase was obtained as a crude extract from *Pseudomonas aeruginosa* and then the uricase was purified by different concentrations of ammonium sulphate (30%, 40%, 50%, 60%, 70% and 80%). The results presented in Table 1 indicates that, 70% ammonium sulphate concentration with dialysis shown to be high uricase activity (40.0 U/ml) than 50% and 60% concentration and cell free supernatant. There is no enzyme activity was noticed with 30%, 40% and 80%.

SDS-PAGE Profile

The molecular weight of purified uricase was determined by SDS- poly acrylamide gel electrophoresis (SDS-PAGE) using standard marker proteins of known molecular weight. The

results given in Figure 1 showed that the molecular weight of uricase was estimated to be 33 kDa. Cont.....

Effect of pH and Temperature

The present study was carried out to observe the effect of different pH (6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0) and temperatures (20 - 60°C) on purified uricase activity. The result recorded in Figure 2 & 3 shows that the highest uricase activity was attained at pH 8.5 and 35°C temperature..... Cont.....

Discussion

In the present study the purified form of uricase was obtained with maximum activity using 70% ammonium sulphate. Similar result was observed from the recent study that the ammonium sulphate 70% saturation proved to be high uricolytic activity (146.84 U/ml) comparing with crude uricase and other concentrations [8]. The previous study also reported that *P. aeruginosa* uricase was purified using ammonium sulphate selective precipitation (70% concentration) and dialysis [9]..Cont.....

Conclusion

We demonstrated that the uricase enzyme by *Pseudomonas aeruginosa* was purified using 70% ammonium sulphate precipitation and dialysis. The optimum temperature and pH of purified uricase was 35°C and 8.5 to get maximum enzyme activity.Cont.....

Acknowledgements

The authors are thankful to the management for having provided the facilities to carry out this study.

References

- [1] Fraisse, L., Bonnet, M.C., Farcy J.P., Agut, C., Dersigny, D and Bayol, A. (2002). A colorimetric 96-well microtiter plate assay for determination of urate oxidase activity and its kinetic parameters. *Analytical Biochemistry*, 309: 173-179.
- [2] Schiavon, O., Caliceti, P., Ferruti, P and Veronese, F.M. (2000). Therapeutic proteins: a comparison of chemical and biological properties of uricase conjugated to linear or branched poly (ethylene glycol) and poly (Narryloylmorpholine). *II Farmaco*, 55: 264-269.
- [3] Friedman, T.B., Polanco, G.E., Appold, J.C and Mayle, J.E. (1985). On the loss of uricolytic activity during primate evolution. I. Silencing of urate oxidase in a hominoid ancestor. *Comp Biochem Physiol B*, 81(3): 653- 659.
- [4] Yeldandi, A.V., Wang, X., Alvares, K., Kumar, S., Rao, M.S and Reddy, J.K. (1990). Human urate oxidase gene: cloning and partial sequence analysis reveal a stop codon within the fifth exon. *Bioch Biophys Res Comm*, 171: 641- 645.
- [5] Anderson, A and Vijayakumar, S. (2012). Isolation and Optimization of *Pseudomonas aeruginosa* for uricase production. *Intl J Pharma Biosci*, 3 (1): 143-150.
- [6] Masaru, S. (1981). Purification and some properties of sarcosine oxidase from *Corynebacterium* sp. U-96. *J Biochem*, 89: 599–607.
- [7] Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227:680–685.
- [8] Atalla, M. Mabrouk., Eman, R. Hamed., Farag, M. M. and Nehad, E. Ahmed. (2010). Purification and Characterization of Uricase Enzyme Produced by *Gliomastix gueg*. *www.Gate2Biotech.com*, 2 (11): 1-13.

- [9] Hesham, M. Saeed., Yasser, R., Abdel-Fattah, Yousry., Gohar, M. and Mohammed, A. Elbaz. (2004). Purification and Characterization of extracellular *Pseudomonas aeruginosa* urate oxidase enzyme. *Polish Journal of Microbiology*, 53 (1): 45-52.

Figure Legends

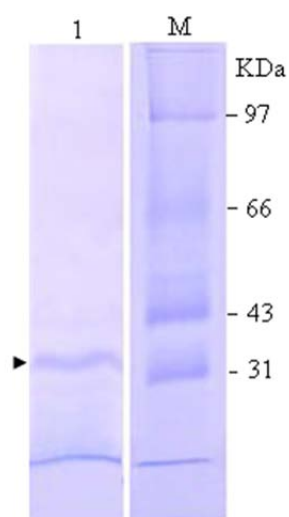


Fig.1. SDS-PAGE profile of purified uricase

Lane1: Ammonium sulphate precipitate and dialyzed sample: Arrow indicates the uricase (protein) band.

Lane M: Standard protein molecular weight marker.

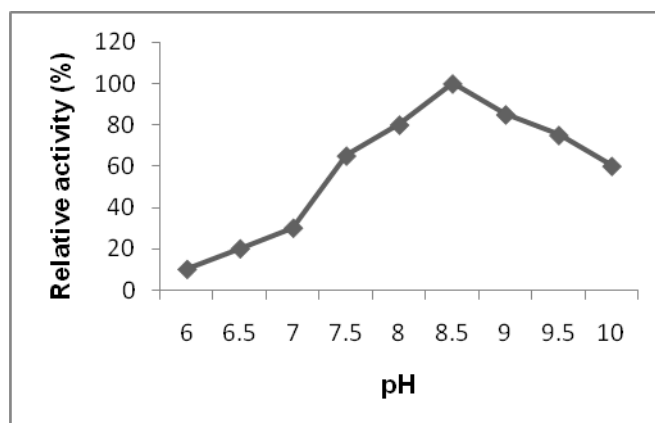


Fig.2. Effect of pH on uricase activity

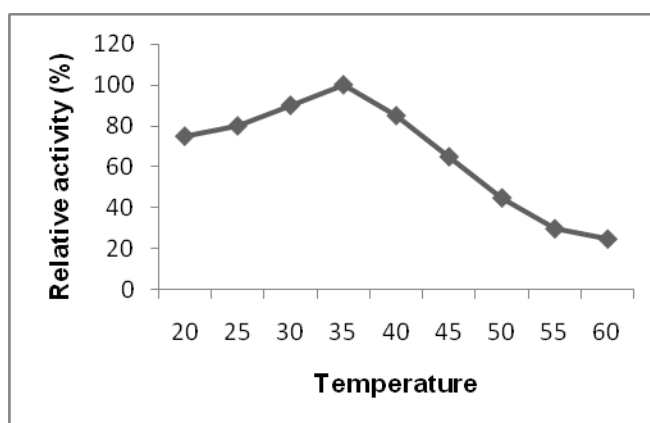


Fig.3. Effect of temperature on uricase activity

Ammonium sulphate Concentration (%)	Uricase activity U/ml
Crude	11.0
30	0
40	0
50	5.14
60	14.72
70	40.0
80	0

Table.1. Purification of uricase