Purification of uric acid lowering protein (uox) from mutated *Bacillus subtilis*

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ABSTRACT

Aim: To purify urate oxidase from mutated Basillus subtilis. **Methodology:** The uric acid lowering protein (urate oxidase) was purified by adopting variouse techniques i.e ammonium sulfate precipitation, ion exchange and gel filtration chromatography.Specific activity, Activity and protein contents in each purification stage were determined. **Results:** It was studied that mutated enzyme showed 97.56 U/mg specific activity, 42.93 U/mL activity and 0.44 mg/ml protein contents with 256.73 fold purification. The purified protein was showed molecular weight 34kDa with a single band on SDS-PAGE. **Conclusion**: it was concluded that by adopting different purification technique, the enzyme from mutated strain was purified.

Keywords: urate oxidase (uox), purification, ion exchange chromatography, gel filtration chromatography, uric acid

INTRODUCTION

Uric acid lowering protein (Uox) has active site for binding uric acid at its active site which hydrolyzed uric acids into more water soluble compound allantoin [1,2,3]. Its gene in human being is inactivated. That is the reason that some concentration of uric acid remains in the human fluid and in some pathological conditions its concentration is exceeded i.e gout, hyperuricemia, leukemia, and tumor lysis syndrome). These abnormal conditions might be controlled by using uox as a therapeutic drug. This enzyme as a therapeutic drug is preferred over other market available drugs because other drugs used to stop the synthesis of uric acid by inhibiting an enzyme xynthin oxidase which involves in its synthesis, while urate oxidase is a key enzyme in hydrolysis of uric acid into more water soluble compound which easily excretes out of the body in < 24 hours [2,4].

Before 1970s, there is a limited use of uox as a therapeutic drug. But in 1975 France introduced recombinant form of uox which was purified from Aspergillus flavus as a therapeutic drug under the trade name of uricozyme. After that uox was introduce by Italy in 1980s. Now in USA and Europe this enzyme is approved by FDA as a therapeutic drug in the treatment of gout[5,6].

This enzyme also used as diagnostic purpose after isolation and purification from different animal or microbial sources[7-11]. Uox was immobilized and also used for diagnostic purpose. This project was designed to purified enzyme from mutated Bacillus subtilus. This work is the extension of previous work..

MATERIAL AND METHODS

All chemicals and reagents were used in this research, purchased from Sigma (Aldrich-Fluka, UK; Sigma-Aldrich, USA). The stock culture was maintained and mutated as described by Meraj et al[11]. Uox was produced by using liquid state fermentation.

The assay for uric acid lowering protein was performed by adopting the procedure of Meraj et al[11]. The activity of uox was detected by following the hydrolysis of uric acid. It was observed by spectrophotometer at 293nm that concentration of uric acid was decreased when enzyme was added and it was compared with the blank solution that contains all the mixture except urate oxidase.

Sample reaction mixture	**Reference reaction mixture**
2 mL substrate (uric acid)	2 mL substrate (uric acid)
0.3 mL H_2O (distilled)	0.3 mL H_2O (distilled)
0.5 mL crude enzyme	0.2 mL potassium cyanide

Potassium cyanide was added in sample after fifteen minutes to stop the enzyme reaction. The decrease in absorbance due to enzymatic reaction was determined the activity of the enzyme. One unit of uox was equivalent to the concentration of enzyme which convert one micromole of urate per minute to allantoin at 30^{o}C..

3. RESULTS

This project is the extension of the previous work [11].

Purification of uric acid lowering protein

The protein produced from mutant strain was purified by using different techniques. First of all the crude enzyme was subjected to ammonium sulfate precipitation [20]. The supernatant (80%) of mutant derived protein was exhibited the activity 28.63 U/mL while it was 6.39 U/mL in sediment. After desalting process, the activity of the enzyme was increased 89.83 U/mL. and increase 62.90 fold purification (Table 1)

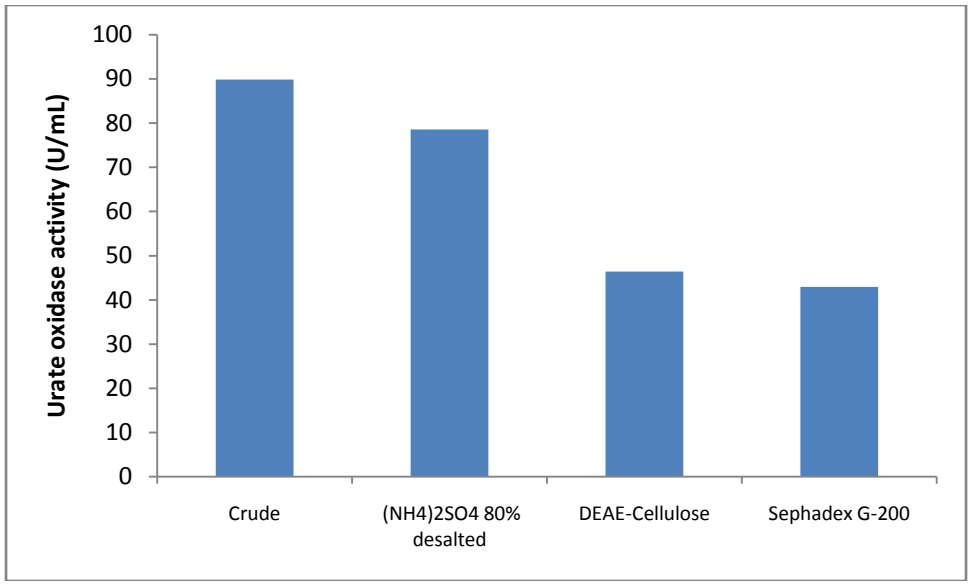

Figure 1 activity (U/mL) at various purification stages

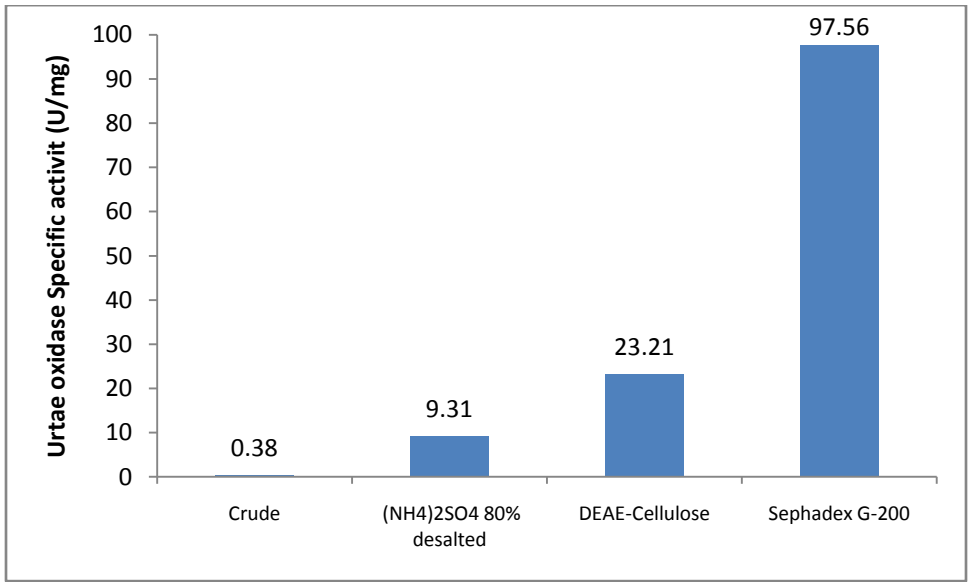

Figure 2: Urtate oxidase specific activity (U/mg) at various purification stages

Table 1: Purification summary of uox produced by mutated Bacillus subtilus

Purification stages	Activity (UmL^{-1})	Protein contents ($mg\ mL^{-1}$)	Specific activity ($U\ mg^{-1}$)	Fold purification
Crude	89.83	236.39	0.38	1
$(NH_4)_2SO_4$ 80% desalted	78.55	8.44	9.31	62.90

DEAE-Cellulose	46.42	2	23.21	156.82
Sephadex G-200	42.93	0.44	97.56	256.53

The uric acid lowering protein obtained from ammonium sulfate ppt was applied to to DEAE cellulose column for further purification[21]. After ion exchange chromatography, it was studied that this purified protein has 23.21 U mg^{-1} specific activity and 46.42 U mL^{-1} activity (Table 1). At this step, the protein was 156.82 fold purified.

The fraction number 31 showed maximum protein activity was further applied to other chromatographic column for further purification. This purified protein showed 97.56 U mg^{-1} optimum specific activity, 42.93 U mL^{-1} activity and 0.44 mg mL^{-1} protein contents (Table 1). At this stage, the purified protein showed 256.73 fold purification in fraction number 33. It was observed that during different purification stages the specific activity was enhanced which showed that most of the contaminated proteins were removed.

SDS-PAGE

Purified urate oxidase was run on SDS-PAGE (10%) with mercaptoethanol determined a single band with a mobility revealed a molecular weight of 34 kDa (Fig 1).

4. DISCUSSION

After desalting process, the activity of the enzyme was increased 89.83 U/mL. and increase 62.90 fold purification (Table 1). Similar results were observed that from previous study that $(NH_4)_2$ SO_4 precipitation (80%) showed maximum uox specific activity[16,9,21,22].

After purification stage 2 (ion exchange), It was studied that the protein contained 46.42 U mL^{-1} maximum activity and 23.21 U mg^{-1} specific activity (Table 1). Here the protein was 156.82 fold purified. Similar results were observed by various other workers, who were reported that after DEAE-Sephadex treatment, uox was 330 fold purified[22]. The said protein isolated from *Puccinia recondita* to DEAE-sephadex column and exhibited 5 fold purification and 106% yield[23]. These results exhibited a fine correlation with the previous work in that reduction in protein contents and enhance the specific activity after treated by DEAE cellulose column. Various researchers were also used chromatographic techniques to purify this protein from ox kidney, *Pseudomonas aeruginosa* and *Aspergillus flavus* by using sephdex G-200 and found specific activity 50 U mg^{-1} , 636.36 U mg^{-1} and 213.42 U mg^{-1} respectively[22,23,24].

In our study it was estimated that purified uox has molecular weight 34kDa. Previous studies observed that molecular mass of uric acid lowering protein (uox) purified from *Bacillus fastidious* by gel filtration (sephadex G-200) was observed to be 151 KDa [24]. It may be possible, during mutation; some parts of the gene deleted and show low molecular weight as compared to previous study.

5. CONCLUSION

CONSENT

All authors declare that 'written informed consent was obtained from the patient.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors.

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