Purification and Characterization of Urease Obtained from *Thiobacillus thiooxidans*

Xiaojuan Liu

Jiangsu Provincial Xuzhou Pharmaceutical Vocational College, Xuzhou, China

liuxj2000@126.com

Abstract. In this work, we described the separation of urease from *Thiobacillus thiooxidans* (*T.t*). Urease produced by *T.t* was purified to homogeneity by precipitation with 70% ethanol followed by column chromatography. The molecular weight of the purified enzyme was estimated to be 47,000 by SDS-PAGE. The enzyme exhibited a maximum activity at pH 5.6~6.1. The optimum temperature was 37℃, with stability up to 40℃. Enzyme activity was improved strongly by Ni^{2+} and was reduced markedly in the presence of Co^{2+} and Pb^{2+}. There is no relevant paper reported that found and purified urease from autotrophic bacteria in the world.

1. Introduction

Purified acid urease is quite expensive in China. There is a current need to find a technology to substitute or improve the existing processes in China. Therefore, an attempt has been made to purify the enzyme from *Thiobacillus thiooxidans (T.t)* with a view to exploring various avenues of its commercial exploitation. *T.t.* is a chemolithotrophic acidophilic bacterium that grows on elemental sulfur as energy source and is important in the microbial catalysis of sulfide oxidation. Since it oxidizes both elemental sulfur and sulfide to sulfuric acid, *T.t* plays a significant role in bioleaching of metals from sulfide ores in China. In our biological desulphurization research of *T.t*, we found that bacteria can use urea accidentally. Therefore, we have a preliminary judgment that it can produce urease. Therefore, the urease purified from *T.t*. was studied.

2. Materials and Methods

2.1 Isolation and Cultivation of microorganisms

Thiobacillus thiooxidans (T.t) was used to produce urease. *T.t* culture was performed on a substrate of nature pH containing (g/L): urea 0.1g, KH_2PO_4 1.0g, $MgSO_4$ 0.5g, powdered S_0 24g. 2g sediment obtained from last fermentation was used to inoculate into 1000mL of the liquid medium and cultivated at 37℃, 15.7 rad/s for 14 days. Sediment was obtained by centrifugation at 10000rpm for 20 min, and used for purification of urease.

2.2 Purification of urease

After 14 days of incubation, the sediment which includes most of the bacterial cells was collected. The sediment was fragmented by ultrasonic, after centrifugation (3000rpm, 30min, 4℃), ethanol was added to the supernatant to 70% saturation and the precipitate was collected by centrifugation at 1,000rpm for 20 min. The precipitate was dissolved in 5mL of 0.1M phosphate buffer (pH 5.8). The

solution was then centrifuged at 10000rpm for 20 min to remove insoluble materials, applied to a Sephadex G-50 column (1.5×30cm) equilibrated with the same buffer as used for dissolving process, and washed with the same buffer. The purity of the protein fractions after the Sephadex G-50 column was analyzed by SDS-PAGE.

2.3 Protein content determination

The protein content of samples was determined by Coomassie Blue G method of Spector, with bovine serum albumin as standard.

2.4 Enzyme Assay

The hydrolysis of urea was routinely followed by the determination of the ammonia formed. The standard assay system contained 20mL of phosphate 0.1M buffer (pH 5.8), 5mL of urea, and enzyme in a final volume of 50 mL. The enzyme was replaced by water for the blank. Incubation was carried out at 37℃ for 5 min. Ammonia formed in the reaction mixture was colorimetrically determined with phenylnitroprussiate-hypochlorite method. One unit of enzyme was defined as the amount that releases 1μmol of ammonia per min.

2.5 SDS-polyacrylamide electrophoresis

Electrophoresis in 10% polyacrylamide gels containing 0.1% sodium dodecyl sulfate were run at 20mA for 2–3h. Protein was stained with Coomassie brilliant blue R-250 and destained in a 10:15:175 (v:v) mixture of ethanol, acetic acid, and water.

3. Results and discussion

3.1 Purification of the Enzyme and Molecular Weight determination

Ethanol was added to the supernatant fragmented by ultrasonic at 70% saturation in order to collect the active fraction, and then Sephadex G-50 column chromatography was performed.

Urease was purified by Sephadex G-50 column chromatography to yield a preparation with a single protein band on SDS-PAGE (Figure 1). The purification results are summarized in Table 1. In this purification process, the urease was purified about 14-fold, and to a specific activity of 146U per mg protein with a 21% recovery in activity.

SDS-PAGE of urease purified from *Thiobacillus thiooxidans* (*T.t*) was shown in Figure 1. The first lane was molecular marker proteins: α_2-Macroglobulin (160kd), β-Galactosidase (112kd), Lactoferrin (83kd), Pyruvate Kinase (59kd), Fumarase (37kd), Lactic Dehydrogenase (31kd). The molecular weight of urease was estimated to be 47,000 by SDS-PAGE.

Table 1. Summary of purification of urease from T.t

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Fold purification	Yield (%)
Supernatant	4.365	44.85	10.27	1	100
70% ethanol	1.458	31.06	21.30	2.07	69.25
Sephadex G-50	0.066	9.64	146.06	14.22	21.49

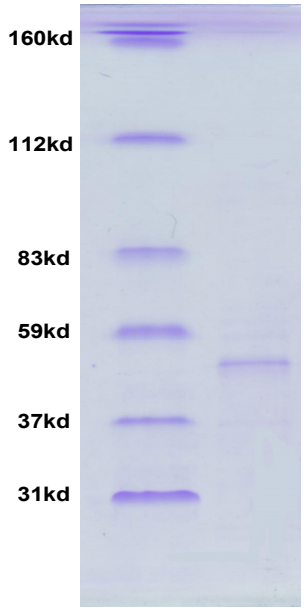

Figure 1. SDS-PAGE of urease purified from *T.t*. Lane 1, molecular marker proteins: α_2-Macroglobulin (160,000), β-Galactosidase (112,000), Lactoferrin (83,000), Pyruvate Kinase (59,000), Fumarase (37,000), Lactic Dehydrogenase (31,000)

3.2 Absorption Spectrum

The solution of purified urease was colourless, and exhibited absorption maxima at 280nm, with a slight shoulder at 300nm (Figure 2). Absorption in the visible region was not found.

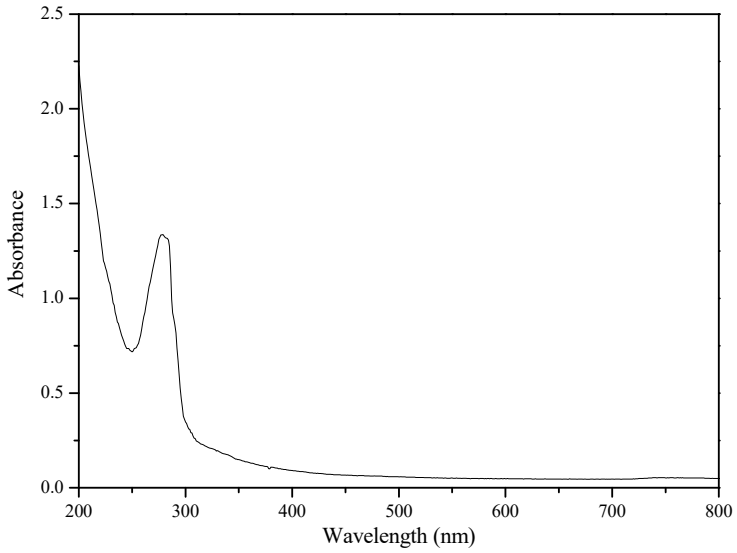

Figure 2. Absorption spectrum of urease from T.t

3.3 Effects of pH and Temperature on Enzyme Activity

Enzyme activity and stability were examined at various pH. Optimum urease activities were observed in a pH range of 5.5–6.5 and more than 80% of activity was lost below pH 5 and at pH 8.5. This urease exhibited a maximum activity at pH 5.8 (Figure 3).

The optimum temperature for the enzyme was determined at various temperatures at pH 5.8. It

exhibited maximum activity at 37℃, with activity almost constant in the range from 37 to 40℃ (Figure 4). The enzyme was stable up to 37℃ for 30 min but lost activity completely at 60℃ for 30 min.

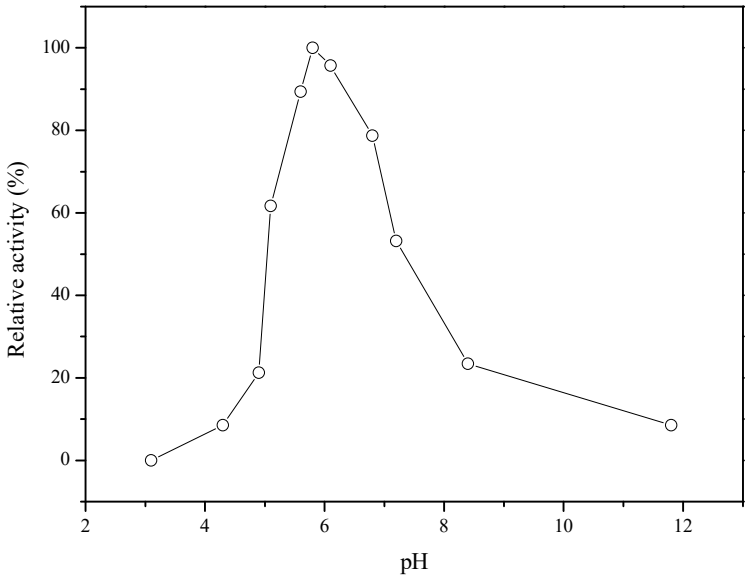

Figure 3. Effects of pH on enzyme activity

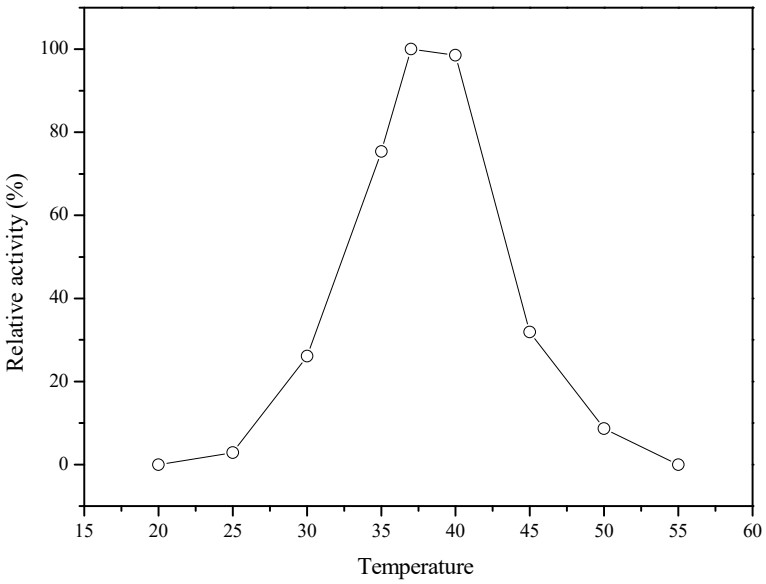

Figure 4. Effects of temperature on enzyme activity

3.4 Effects of Metal Ions

To determine the effect of metal ions on urease activity, various ions (Ag^{+}, Ba^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) were added to the enzyme solution at a final concentration of 1mM. The activity of urease was inhibited almost completely by Ni^{2+} and weakly by Co^{2+} and Pb^{2+} (Table 2).

Table 2. Effects of metal ions on enzyme activity

Metal	**Relative**

ion	Activity (%)
None	100
Ag^{3+}	99.8
Ba^{2+}	100
Ca^{2+}	99.6
Co^{2+}	77.1
Cu^{2+}	100
Fe^{2+}	98.9
Hg^{2+}	63.2
Mg^{2+}	100
Mn^{2+}	100
Ni^{2+}	108
Pb^{2+}	100
Zn^{2+}	97.7

4. Conclusion

In the research of biological desulphurization on *T.t*, we found that T.t could use urea as the exclusive nitrogenous source. Therefore, we have a preliminary judgment that urea as an inducement that stimulates *T.t* producing urease. Then urease catalyzes the hydrolysis of the urea to ammonia and carbon dioxide. Finally, ammonia used directly as nitrogenous source by *T.t*. This preliminary judgment has been initially proved in our research. Urease obtained was induced by urea. This conclusion is from two parallel experiments carried on *T.t*: the one we offered NH_4^+ as the exclusive nitrogenous source, and the other one, we offered urea. The result was that in the former test there was on urease, but in the latter one we detected urease. In this article, we described the purification of urease from *T.t*. Urease produced by *T.t* was purified to homogeneity by precipitation with 70% ethanol followed by column chromatography, and a study made of its characteristics. The molecular weight of the purified enzyme was estimated to be 47,000 by SDS-PAGE. The enzyme exhibited a maximum activity at pH5.6~6.1. The optimum temperature was 37℃, with stability up to 40℃. Enzyme activity was inhibited strongly by Ni^{2+} and was reduced markedly in the presence of Co^{2+} and Pb^{2+}.

Thiobacillus thiooxidans (T.t), as a kind of autotrophic bacteria, its industrial productions are much simpler than those of heterotrophic bacteria because it does not need sterilization equipments and so on. Therefore, purifying urease from *T.t* has great commercial potential in the production process.

Acknowledgements

This work was financially sponsored by Qing Lan Project of Jiangsu Province(2017).

References

[1] Claudio M.and Claudio C 2000 *Biol Fertil Soils.* **32** 415
[2] Cristian F. G., Wassermann C. R., Carlini 2004 *Plant Science.* **167** 241
[3] Elmar R., Athanasios M., Alexander M. H 2000 *Journal of Chromatography B.* **737** 203
[4] Hausinger R. P. 1987 *Microbiol.* **51** 22
[5] Hiroshi K., Kenji I., Hideyuki M., Yumi N., Tatsuo T., Hidehiko T 2000 *Journal of Bioscience and Bioengineering.* **90** 459
[6] Hsuan L. L., Bor Y. C., Yann W. L., Yang C. C 2004 *Chemical Engineering Journal.* **97** 195
[7] Hsuan L. L.., Yann W. L., Yang C. C 2004 *Process Biochemistry.* **39** 1953