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Effect of Catalase on Biocatalytic Synthesis of Pyruvate by Enzymes from *Pseudomonas sp.*

Jing Song GU*, Yuan Xiu WANG, Qiang JIAO

School of Chemistry and Chemical Engineering, Jinan University, Jinan 250022

Abstract: Pyruvate was produced from *DL*-lactate by a kind of green-chemical biocatalyst — cell-free extract from bacterial strain *Pseudomonas sp.* SM-6. Catalase in cell-free extract, which could stabilize the pyruvate formed by lactate oxidase, played an important role in pyruvate preparation. The effect of catalase in conversion process was evaluated.

Keywords: Pyruvate, catalase, lactate oxidase, *Pseudomonas*.

Compared with other small non-chiral building blocks, pyruvate is relatively expensive. Pyruvate production by enzymatic method from *DL*-lactate was a fairly competitive and attractive process because the starting material is cheap and the process is pollution-free. Lactate oxidase catalyzed the direct formation of pyruvate from lactate without requirement of NAD^+ or $NADP^+$ as a cofactor, which made its prospect of application broad[1, 2].

$$\text{Lactate} + O_2 \leftrightarrow \text{pyruvate} + H_2O_2 \text{ (lactate oxidase)} \qquad \text{(Eq.1)}$$

Pseudomonas sp. SM-6 has been described as a new bacterial resource for lactate oxidase[2,3]. However, the biocatalyst for pyruvate preparation extracted from *Pseudomonas sp.* SM-6 strain, contained not only lactate oxidase but also other kinds of enzyme. In this study, we tried to find the role of catalase in cell-free extract so as to further improve the catalytic ratio for pyruvate production.

Exprimental

Pseudomonas sp. SM-6 was cultivated for 24-48 hrs. The cells were harvested by centrifugation (10000 r/min, 15 min, 4°C), then disrupted by ultrasonic oscillator (Sonic & Material Inc., Danbury CT. USA) for 5 min at 0°C. The cell extracts were further centrifuged (12000 r/min, 15 min, 4°C), the supernatant was collected and used as an enzyme source. Crude enzyme protein was assayed by method of Bradford test[4]. The lactate concentrations were measured by SBA-40C lactate analyzer (The Academy of

* E-mail: gujingsong@sdu.edu.cn

Science in Shandong Province, China). Pyruvate estimations were carried out spectrophotometrically as the 2, 4-dinitrophenylhydrazone derivative[5]. Lactate oxidase activity was determined according to literature procedure[2]. Catalase activity was quantified by Beers & Sizers' method[6].

Results and Discussion

Comparison of catalase and lactate oxidase activity in Pseudomonas sp. SM-6 cell-free extract

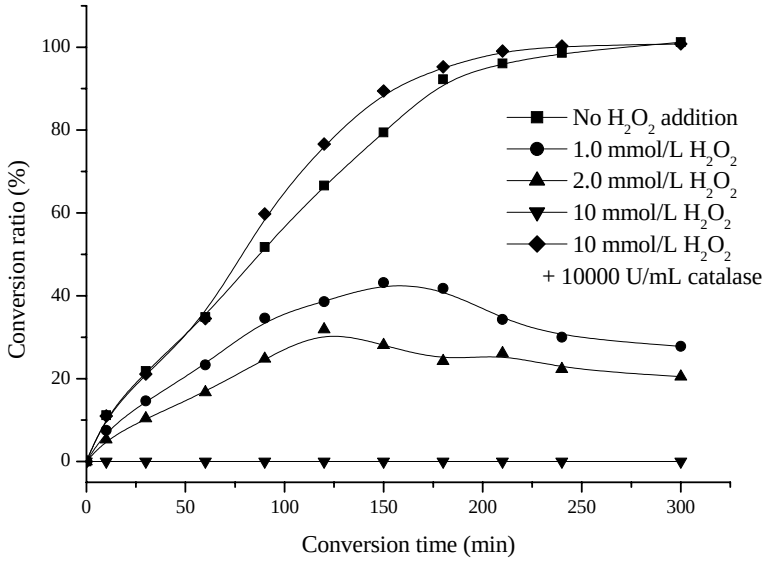
As shown in **Table 1**, catalase activity in SM-6 cell-free extract was much higher than that of lactate oxidase. So the by-product hydrogen peroxide formed by lactate oxidase (Eq.1) was quickly further catabolized through catalase pathway[7].

$$H_2O_2 + H_2O_2 \leftrightarrow O_2 + 2H_2O \text{ (catalase)} \qquad \text{(Eq.2)}$$

Table 1 Comparison of catalase and lactate oxidase activity in *Pseudomonas sp*. SM-6 cell-free extract

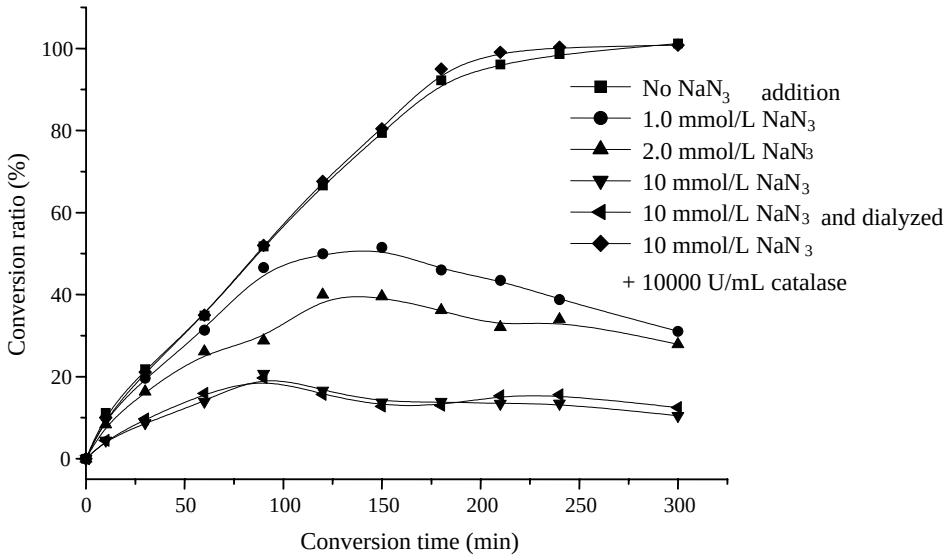
Strain	*Pseudomonas sp.* SM-6
protein (mg/mL)	0.420
Lactate oxidase activity (U/mL)	0.941
Catalase activity (U/mL)	82.5

Figure 1 Effect of hydrogen peroxide addition on conversion by *Pseudomonas sp*. SM-6 cell-free extract



Reaction was carried in 10 mL containing 70 mg/L of crude enzyme protein and 33 mmol/L phosphate in pH 7.4, at 37°C. Initial *DL*-lactate concentration was 5.5 mmol/L

Figure 2 Effect of sodium azide addition on conversion by *Pseudomonas sp*. SM-6 cell-free extract



Reaction was carried in 10 mL containing 70 mg/L of crude enzyme protein and 33 mmol/L phosphate in pH 7.4, at 37°C. Initial *DL*-lactate concentration was 5.5 mmol/L

Decrease in concentration of hydrogen peroxide made a successful pyruvate accumulation. Thus the formed pyruvate would not be affected by hydrogen peroxide.

Effect of hydrogen peroxide addition on conversion by Pseudomonas sp. SM-6 cell-free extract

As shown in **Figure 1**, cell-free extract from SM-6 containing 70 mg/L of protein could convert 5.5 mmol/L of *DL*-lactate to pyruvate with yield of 92% in 3 h. After 5 h, it could transform almost all *DL*-lactate (5.5 mmol/L) into pyruvate. But the conversion ratio was apparently affected after hydrogen peroxide was added into catalytic reaction mixture. When the concentration of hydrogen peroxide in catalytic reaction mixture reached 2.0 mmol/L, the pyruvate conversion ratio dropped by more than 50%. And when the concentration of hydrogen peroxide was increased to 10 mmol/L, none of pyruvate could be detected in catalytic reaction mixture. As one of the product of lactate oxidase, hydrogen peroxide could inhibit LOD activity and decrease conversion[1]. Furthermore, relatively high concentration of hydrogen peroxide decomposed pyruvate to acetate, carbon dioxide and water, followed by lowering the production yield[8].

$$\text{pyruvate} + H_2O_2 \leftrightarrow \text{acetate} + CO_2 + H_2O \qquad \text{(Eq.3)}$$

Conversion not only recovered but also reached a higher level after much more catalase (10000 U/mL, Sigma, USA) was added into catalytic reaction mixture.

Effect of catalase inhibitor on conversion by Pseudomonas sp. SM-6 cell-free extract

Sodium azide is a kind of typical inhibitor of catalase[7]. According to the results shown in **Figure 2**, pyruvate conversion ratio dropped apparently due to catalase activity decrease caused by sodium azide. Decrement of catalase led to hydrogen peroxide accumulation in the catalytic reaction mixture (Equation 2), and further affected the conversion ratio. Inhibitor could not be removed through dialysis, but the conversion could be recovered by catalase addition (10000 U/mL, Sigma, USA), too.

Conclusion

Cell-free extract from *Pseudomonas sp.* SM-6 is a potential biocatalyst for pyruvate preparation, for it not only contained lactate oxidase but also catalase. Catalase, as well as lactate oxidase, played an important role in pyruvate conversion process. Catalase decomposed hydrogen peroxide formed by lactate oxidase and limit the nonenzymatic oxidation of pyruvate to acetate, carbonate, thereby ensuring the yield of pyruvate. Decrease of catalase apparently affected the pyruvate conversion.

References

1. K. Maeda-Yorita, K. Aki, V. Massey, *Biochim.,* **1995**, *77*, 631.
2. J. S. Gu, P. Xu, Y. B. Qu, *Chin. J. Appl. Environ. Biol.*, **2001**, *7*, 617.
3. J. S. Gu, P. Xu, Y. B. Qu, *Chin. Chem. Lett*., **2002**, *13*, 1213.
4. M. M. Bradford, *Anal. Biochem.* , **1976**, *72*, 248.
5. C. T. Walsh, A.Schonbrunn, R. H. Abeles, *J. Biol. Chem.*, **1971**, *246*, 6855.
6. R. F. Beers, I. W. Sizers, *J. Biol. Chem.*, **1952**, *195*, 133.
7. M. Dixon, E. C. Webb, *Enzymes*, 3rd Edition, Longman Group Ltd, London, **1979**, pp220, pp369, pp647.
8. W. Adam, M. Lazarus, B. Boss, *J. Org. Chem.*, **1997**, *62***,** 7841.

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