

重组具有改良特性的 D-氨基酸氧化酶

S.V Khoronenkova¹, and V.I. Tishkov^{1,2}

1 Department of Chemical Enzymology, Chemistry Faculty, M. V. Lomonosov Moscow State University, Lenin's Hills, Moscow 119992, Russian Federation

2 Innovations and High Technologies MSU Ltd., Moscow 109559, Russian Federation

摘要: 在大肠杆菌细胞中表达三角酵母 D-氨基酸氧化酶, 并对重组酶的性质进行了研究。制备的单一突变体与野生型酶相比, 具有 2.4 倍的热稳定性或底物特异性变化光谱。结果显示突变的 TvDAAO 在氧化头孢菌素中催化效果优于野生型酶。并将一个突变的重组 TvDAAO 制备成结晶, 并解析了 2.8 Å 分辨率下的晶体结构。

关键词: D-氨基酸氧化酶, 三角酵母, 定向诱变, X 射线结构

Recombinant D-amino Acid Oxidase with Improved Properties

S.V Khoronenkova¹, and V.I. Tishkov^{1,2}

1 Department of Chemical Enzymology, Chemistry Faculty, M. V. Lomonosov Moscow State University, Lenin's Hills, Moscow 119992, Russian Federation

2 Innovations and High Technologies MSU Ltd., Moscow 109559, Russian Federation

Abstract: D-amino acid oxidase from *Trigonopsis variabilis* (TvDAAO) was overproduced in *Escherichia coli* cells and properties of the recombinant enzyme were studied. Single point mutants of the enzyme with 2.4-fold higher thermal stability or changed spectra of substrate specificity compared to wild-type enzyme were prepared. It was shown that mutant TvDAAO has higher catalytic efficiency in cephalosporin C oxidation in comparison with wild-type enzyme. One mutant of recombinant TvDAAO was crystallized and its structure was solved with resolution 2.8 Å.

Keywords: D-amino acid oxidase, *Trigonopsis variabilis*, directed mutagenesis, X-ray structure

REFERENCES

- [1] Pollegioni L, Piubelli L, Sacchi S, *et al.* Physiological functions of D-amino acid oxidases: from yeast to humans. *Cell Mol Life Sci*, 2007, **64**: 1373–1394.
- [2] Tishkov VI, Khoronenkova SV. D-amino acid oxidase: structure, catalytic mechanism, and practical application. *Biochemistry (Mosc.)*, 2005, **70**: 51–67.
- [3] Corvin A, Donohoe G, McGhee K, *et al.* D-amino acid oxidase (DAO) genotype and mood symptomatology in schizophrenia. *Neurosci Lett*, 2007, **426**: 97–100.
- [4] Conlon HD, Baqai J, Baker K, *et al.* Two-step immobilized enzyme conversion of cephalosporin C to 7-aminocephalosporanic acid. *Biotechnol Bioeng*, 1995, **46**: 510–513.
- [5] Inaba Y, Mizukami K, Hamada-Sato N, *et al.* Development of a D-alanine sensor for the monitoring of a fermentation using the improved selectivity by the combination of D-amino acid oxidase and pyruvate oxidase. *Biosens Bioelectron*, 2003, **19**: 423–431.
- [6] Nakajima N, Conrad D, Sumi H, *et al.* Continuous conversion to optically pure L-methionine from

Received: October 17, 2008; **Accepted:** November 25, 2008

Corresponding author: V.I. Tishkov. Tel: +7-495-9393208; Fax: +7-495-9393208; E-mail: vitishkov@gmail.com

- D-enantiomer contaminated preparations by an immobilized enzyme membrane reactor. *J Ferm Bioeng*, 1990, **70**: 322–325.
- [7] Beard TM, Turner NJ. Deracemisation and stereoinversion of α -amino acids using D-amino acid oxidase and hydride reducing agents. *Chem Commun (Camb.)*, 2002, **3**: 246–247.
- [8] Pollegioni L, Molla G, Sacchi S, *et al.* Properties and applications of microbial D-amino acid oxidases: current state and perspectives. *Appl Microbiol Biotechnol*, 2008, **78**: 1–16.
- [9] Pollegioni L, Caldinelli L, Molla G, *et al.* Catalytic properties of D-amino acid oxidase in cephalosporin C bioconversion: a comparison between proteins from different sources. *Biotechnol Prog*, 2004, **20**, 467–473.
- [10] Gabler M, Hense M, Fischer L. Detection and substrate selectivity of new microbial D-amino acid oxidases. *Enz Microb Technol*, 2000, **27**: 605–611.
- [11] Davydova EE, Tishkov VI. Cloning of D-amino acid oxidase gene from yeast *Trigonopsis variabilis*. *Mosc Univ Chem Bull*, 2002, **43**: 353–355.
- [12] Pollegioni L, Fukui K, Massey V. Studies on the kinetic mechanism of pig kidney D-amino acid oxidase by site-directed mutagenesis of tyrosine 224 and tyrosine 228. *J Biol Chem*, 1994, **269**: 31666–31673.
- [13] Molla G, Vegezzi C, Pilone MS, *et al.* Overexpression in *Escherichia coli* of a recombinant chimeric *Rhodotorula gracilis* D-amino acid oxidase. *Protein Expr Purif*, 1998, **14**: 289–294.
- [14] Geueke B, Weckbecker A, Hummel W. Overproduction and characterization of a recombinant D-amino acid oxidase from *Arthrobacter protophormiae*. *Appl Microbiol Biotechnol*, 2007, **74**: 1240–1247.
- [15] Yu J, Li DY, Zhang YJ, *et al.* High expression of *Trigonopsis variabilis* D-amino acid oxidase in *Pichia pastoris*. *J Mol Catal B: Enz*, 2002, **18**: 291–297.
- [16] Savin SS, Chernyshov IV, Tishkov VI, *et al.* Substrate specificity of D-amino acid oxidase from yeast *Trigonopsis variabilis* expressed in *E. coli* cells. *Mosc Univ Chem Bull*, 2006, **47**: 25–30.
- [17] Sacchi S, Lorenzi S, Molla G, *et al.* Engineering the substrate specificity of D-amino-acid oxidase. *J Biol Chem*, 2002, **277**: 27510–27516.

The full-text article is available online at www.sciencedirect.com

