Catalytic and Structural Properties of Carp D-Amino Acid Oxidase

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1 Introduction.

D-Amino acid oxidase (DAO, EC 1.4.3.3) catalyzes the enantioselective oxidation of a variety of D-amino acids to the corresponding α-keto acids. DAO has been known to distribute widely in nature from microbes to mammals. The physiological functions of DAO, however, remain unclear because its substrates, D-amino acids, are not abundant in vertebrates. Possible functions proposed so far include the catabolic means of bacterial cell wall components, regulator of D-serine, an agonist of *N*-methyl-D-aspartate receptor in mammalian brain, and metabolizing agents of exogenous and/or endogenous free D-amino acids. Recently, we cloned a gene encoding DAO from carp for the first time in animals other than mammals [1]. In view of the enigmatic nature and the industrial potential of DAO, more enzymes from different sources are required to pave the way for further research on biological functions and applications of DAO. Thus, we purified and characterized recombinant carp hepatopancreas DAO (chDAO).

2 Materials and Methods.

The expression vector containing carp DAO cDNA was constructed with pET 11c vector and transformed into a host *E. coli* strain AD494(DE3)pLysS. DAO expressed in *E. coli* was purified with DEAE-Toyopearl, Phenyl-Toyopearl, and HiPrep^{TM} 16/60 Sephacryl^{TM} High Resolution gel filtration columns. Three-dimensional model was constructed by using primary sequence of chDAO with ProModII supplied by Swiss-Model [2].

3 Result and discussion.

Recombinant chDAO was purified to 5.6-fold with a yield of 50%. chDAO had a specific activity of 293 unit/mg protein. It showed high activity against D-alanine with a *K*m of 0.227 (mM) and *K*cat of 190 (S^{-1}) (Table 1). Whereas, *K*cat values for pork kidney (pkDAO) and *Rhodotorula gracilis* (RgDAO) are about 10 (S^{-1}) and 300 (S^{-1}), respectively. The optimum temperature and pH were 35°C and 8.5, respectively. This enzyme exhibited a good thermal and pH stability. It was completely inhibited by Ag^{+} and Hg^{+}. The inhibition by creatinine, *p*-chloromercuribenzoate, and benzoate was competitive with a *K*i of 5.1, 6.0, and 12.5 mM, respectively. Three-dimensional (3D) model of chDAO was analogous to that of pkDAO [3] and RgDAO [4] (Figure 1). Two main topological differences were observed from pkDAO as well as RgDAO. One is the presence of shorter active site loop (9 residues in chDAO *versus* 13 in pkDAO). The active site loop contains an important residue Tyr224 probably involved in a broad range of substrates/products fixation and interaction with substrate α-amino group. In yeast this active site loop is not present, however, Tyr238 (corresponding to Tyr224) found at a similar position plays the same role. Another is the absence of a long C-terminal loop found in RgDAO (6 residues in chDAO and 4 in pkDAO *verses* 21 in RgDAO). This long C-

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terminal loop allows “head to tail” dimer mode that is more stable than “head to head” one found in mammals and carp. The conformational change in large size active site loop controls the overall rate of turnover of the mammalian enzyme, where product release is rate-limiting. Comparison of the 3D structures of chDAO with pkDAO and RgDAO suggests that evolutive pressure has led to the conformational change from microbes to mammals DAOs that share the same chemical process, but use different kinetic efficiency for catalysis.

Table 1: Kinetic parameters of chDAO for some representative D-amino acid oxidase

Substrate	Relative activity (%)	*V* max (U/mg)	*K* m (mM)	*K* cat (s^{-1})	*K* cat/*K* m (s^{-1}/mM)
D-Alanine	100	292	0.227	189.8	836.1
D-Valine	89.9	262	0.263	170.3	647.5
D-Proline	75.1	219	1.13	142.35	126.0
D-Phenylalanine	63.0	183	0.357	118.95	333.2

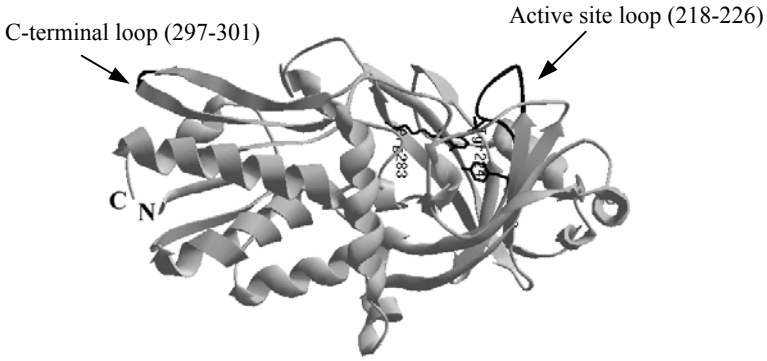

Figure 1. Ribbon representation of chDAO structure. Arg283, Tyr224, and Tyr228 are thought to be key catalytic residues in pkDAO [3]. Active site loop and C-terminal loop are shown in black.

References

[1] Sarower, M.G., Okada, S. and Abe, H. 2003. Molecular characterization of D-amino acid oxidase from common carp *Cyprinus carpio* and its induction with exogenous free D-alanine. *Archives of Biochemistry and Biophysics* 420:121-129.

[2] Peitsch, M.C. 1996. ProMod and Swiss-model: Internet-based tools for automated comparative protein modeling. *Biochemistry Society Transaction* 24:274-279.

[3] Mattevi, A., Vanoni, M.A., Todone, F., Rizzi, M., Teplyakov, A., Coda, A., Bolognesi, M. and Curti, B. 1996. Crystal structure of D-amino acid oxidase: a case of active site mirror-image convergent evolution with flavocytochrome b2. *Proceedings of the National Academy of Sciences USA* 93:7496–7501.

[4] Pollegioni, L, Diederichs, K., Molla1, G., Umhau, S., Welte, W., Ghisla, S. and Pilone, M.S. 2002. Yeast D-amino acid oxidase: structural basis of its catalytic properties *Journal of Molecular Biology* 324:535–546.