ENGINEERING OF HALOALKANE DEHALOGENASE ENANTIOSELECTIVITY TOWARDS B-BROMOALKANES: OPEN-SOLVATED VERSUS OCCLUDED-DESOLVATED ACTIVE SITES

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Enzymatic catalysis is widely used for preparing optically pure chemicals. Natural catalysts have to be often optimized to exhibit sufficient enantioselectivity towards industrially attractive non-natural substrates. Understanding the molecular basis of enzyme–substrate interactions involved in enantiodiscrimination is essential for rational design of selective catalysts. Haloalkane dehalogenases (EC 3.8.1.5) can convert a broad range of halogenated aliphatic compounds to their corresponding alcohols via S_N2 mechanism [1]. The very first haloalkane dehalogenase exhibiting high enantioselectivity towards β-brominated alkanes (E-values of up to 174) was DbjA from Bradyrhizobium japonicum USDA110 [2]. This enzyme has a wide open solvent-accessible active site and its enantioselectivity towards β-brominated alkanes is modulated by a surface loop unique to DbjA [2]. Assuming that the active site geometry is crucial for substrate recognition, it was proposed that DbjA's enantioselectivity could be transferred to closely related, but non-selective DhaA from Rhodococcus rhodochrous NCIMB13064 [1] by active site transplantation [3]. The unique loop fragment from DbjA together with additional 8-point substitutions was inserted to DhaA. Although the crystal structure of resulting variant DhaA12 exhibited identical geometry of the active site and the access tunnel as DbjA, it did not reach identical level of hydration and flexibility and lacked enantioselectivity towards β-bromoalkanes (E-value = 18) [3]. Interestingly, the variant DhaA31 constructed independently with a goal to enhance enzyme activity towards anthropogenic compound 1,2,3-trichlopropane [4], exhibited high enantioselectivity towards 2-bromopentane (E-value = 179) [5] as DbjA (E-value = 174) [2, 3]. DhaA31 contains five mutations, I135F, C176Y, V245F, L246I and Y273F, located in a main and a slot tunnel. Four of five mutations are large and aromatic residues narrowing two access tunnels and occluding the enzyme active site [4]. The level of DhaA31 active site hydration, so important for DbjA's enantioselectivity [2, 3] is low, suggesting a different structural basis of enantioselectivity towards 2-bromopentane. A systematic study on the molecular basis of enantioselectivity in DbjA, DhaA, and DhaA31 using thermodynamic and kinetic analyses, site-directed mutagenesis, and molecular modeling was carried out. DhaA31 enantioselectivity arises from the hydrophobic substrate's interactions with the occluded and desolvated active site [5], while DbjA enantioselectivity results from water-mediated interactions of 2-bromopentane with the active site's hydrophobic wall [2]. Our data imply that enantioselectivity of haloalkane dehalogenases can be achieved by both occluded-desolvated active site and open-solvated active site. The engineering of "DbjA-like" enantioselectivity by modification of the active site hydration remains challenging.

References:

1. Koudelakova, T., et al. 2013. Biotechnol. J. 8: 32–45.
2. Prokop, Z., et al. 2010. Angew. Chem. Int. Ed., 49: 6111-6115.
3. Sykora, J., et al. 2014. Nat. Chem. Biol., 10: 428-430.
4. Pavlova, M., et al. 2009. Nat. Chem. Biol., 5: 727-733.
Liskova, V., et al. 2017. Angew. Chem. Int. Ed., DOI: 10.1002/anie.201611193.