

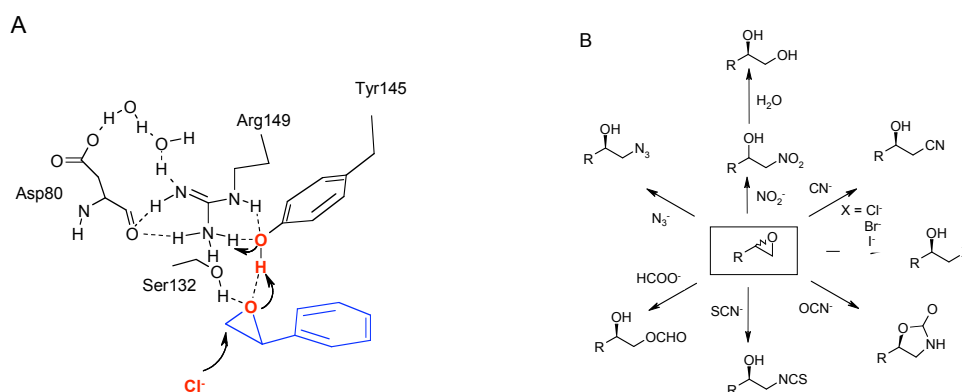
Halohydrin dehalogenases: catalytic mechanism, engineering and biocatalytic applications

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Halohydrin dehalogenases catalyze the conversion of *vicinal* haloalcohols to epoxides and are important for the bacterial degradation of certain halogenated alcohols (e.g. 1,3-dichloro-2-propanol) and compounds that are degraded via haloalcohols (epichlorohydrin, 1,2-dibromoethane). We have investigated the structure, catalytic mechanism, and possible biocatalytic applications of these enzymes [1]. Structurally and mechanistically, halohydrin dehalogenases are similar to members of the short-chain dehydrogenase reductase superfamily of proteins, but instead of a nicotinamide cofactor binding site, there is a halide binding site. Based on the structure and kinetic properties of the halohydrin dehalogenase from *Agrobacterium radiobacter* (HheC), improved variants have been constructed with higher activity, enhanced enantioselectivity, and reduced sensitivity to proteolytic and oxidative inactivation [2].

The promiscuity of the halide binding site allows a range of anionic nucleophiles to be accepted in the reverse reaction, which is epoxide-ring opening. This includes cyanide, azide, formate, cyanate, nitrite and halides. Various enantiopure products can be formed this way.



A: Ring opening reaction catalyzed by halohydrin dehalogenase. B: promiscuity of the enzyme in nucleophile acceptance, allowing the production of a range of enantiopure β -substituted secondary or tertiary alcohols.

The rate of dehalogenation reactions could be enhanced by increasing the rate of halide export. Enantioselectivity was influenced by mutations of tryptophan residues that line the active site and that undergo a conformational change upon substrate- or halide binding. Mutations that enhance the rate of cyanide-mediated cleavage of epoxides were also discovered. Some of the mutations overlap those detected in a directed evolution study [3].

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2. Tang L, Torres Pazmiño DE, Fraaije MW, de Jong RM, Dijkstra BW, Janssen DB. 2005. Improved catalytic properties of halohydrin dehalogenase by modification of the halide-binding site. *Biochemistry* 44:6609-18.
3. Fox RJ, Davis SC, Mundorff EC, Newman LM, *et al.* 2007. Improving catalytic function by ProSAR-driven enzyme evolution. *Nat Biotechnol* 25:338-44.