Halohydrin dehalogenase: an new enantioselective biocatalyst with unusually broad specificity

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Halohydrin dehalogenases are key enzymes in the bacterial degradation of vicinal halopropanols and structurally related nematocides [1]. They catalyze the reversible conversion of a vicinal haloalcohol such as 2,3-dichloro-1-propanol to its corresponding epoxide by the intramolecular nucleophilic substitution of the halogen by the vicinal hydroxyl group, thereby releasing a proton and a halide ion. Substrates include both chlorinated and brominated C_2 and C_3 alcohols such as 2-chloroethanol, 1,3-dichloro-2-propanol and 2,3-dichloro-1-propanol and their brominated analogues, but also aromatic halohydrins such as 1-chloro-2-phenylethanol. The observation that the dehalogenation of chiral halohydrins can proceed with high enantioselectivity, has attracted the broad interest in these enzymes [2]. Furthermore, the enzyme has the ability to use several different nucleophiles in the reverse reaction, the enantioselective ring-opening of epoxides, which include azide and cyanide [3]. These properties give this enzyme a high potential to be used as a biocatalyst in the regio- and enantioselective synthesis of chiral epoxides, haloalcohols, azidoalcohols and cyanoalcohols.

Crystals of *A. radiobacter* halohydrin dehalogenase C (HheC) were obtained from hanging drop experiments at room temperature using drops of 2 µl of protein and 2 µl of reservoir solution suspended over 1 ml of reservoir solution [4]. A reservoir solution containing 50 - 70% saturated ammoniumsulfate solution (saturated at room temperature) in 100 m*M* bis-Tris (bis[2-hydroxyethyl]imino-tris[hydroxymethyl]methane) buffer of pH 6.5 to 7.1, resulted in the formation of multiple bipyramidal crystals of dimensions up to 0.4 x 0.3 x 0.3 mm^3, grown within two weeks. Multiple wavelength anomalous dispersion experiments exploiting the anomalous signal of bromide ions, firmly bound in a halide binding site of the enzyme, led to the elucidation of its structure (Fig 1).

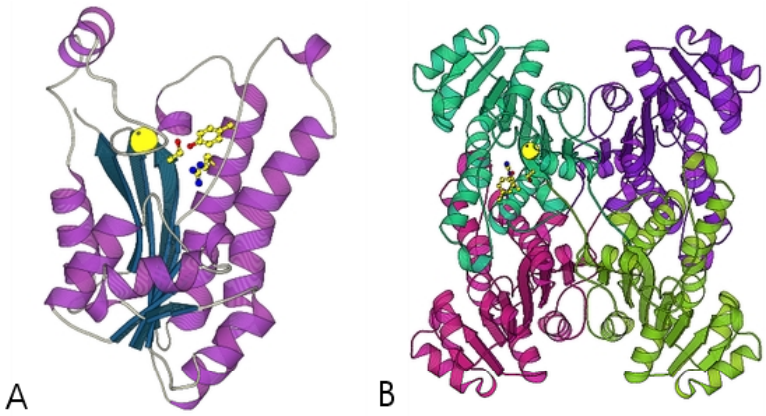

Figure 1. The monomeric (A) and tetrameric (B) structure of halohydrin dehalogenase HheC.

At the EMBL beamline X31 at the DORIS storage ring of the DESY synchrotron, cocrystals of HheC with 10 mM sodium azide and 20 mM of racemic styreneoxide and 10 mM sodium cyanate and 20 mM of racemic styreneoxide were collected to respectively 2.5 Å and 2.3 Å resolution. Azide ions are used by the enzyme in an enantioselective ring-opening of the epoxide to form the corresponding azidoalcohol. Performing this reaction in the protein solution, before preparation of the crystallization setup, could lead to an enzyme:product complex. Cyanate ions are good inhibitors of the azidoalcohol producing reaction and are believed to bind in a similar way as azide in the active site of the enzyme. Cyanate, however, does not perform the nucleophilic attack on the epoxide ring and therefore could mimic the state of the enzyme prior to the nucleophilic attack of azide on the epoxide ring.

The results of the measurements are shown in Figure 2. The data of the cocrystal of HheC with 10 mM sodium azide and 20 mM of racemic styreneoxide was of bad quality and only 80% complete and does not give a clear view of the content of the active site (not shown). The data of the cocrystal of HheC with 10 mM sodium cyanate and 20 mM of racemic styreneoxide, however, showed clear density for a bound *R* enantiomer of styreneoxide and for what seems to be a cyanate ion in close interaction with the epoxide. As a reference, a picture of a complex structure of styreneoxide and a chloride ion that was obtained earlier is added. Currently, this dataset is being refined.

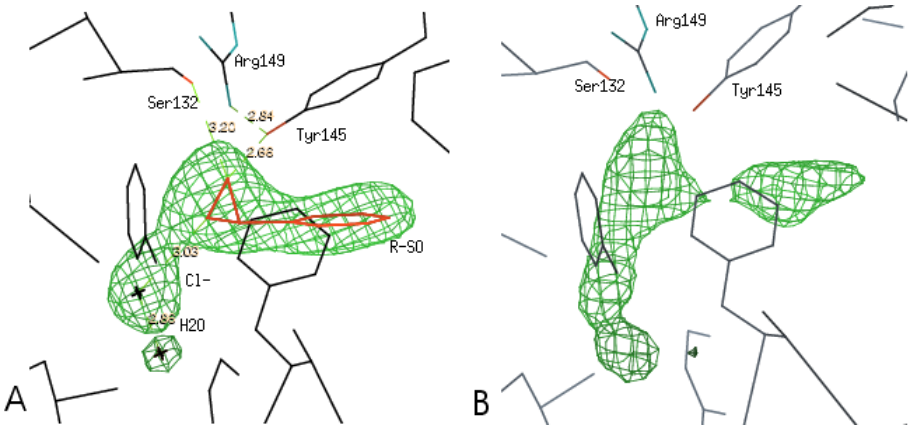

Figure 2. A Fo-Fc omit map of the contents of the active site of HheC (the styreneoxide molecule bound to the active site serine and tyrosine, a chloride ion and a water molecule were omitted to obtain this map) contoured at 2.5 σ (A). A Fo-Fc difference map of the contents of the active site of HheC contoured at 2.5 σ (phases were obtained by rigid body refinement using the native structure of HheC), which clearly shows the bound substrate and nucleophile (B).

Altogether, the enzyme:substrate complex structures give a good explanation of the ability of the enzyme to use several different nucleophiles in the enantioselective ring opening of epoxides. This information adds to the understanding of the catalytic mechanism conducted by halohydrin dehalogenases and is currently used in the improvement of the catalytic properties by knowledge-based design in combination with site-directed mutagenesis.

References

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