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## Degradation of halogenated compounds: structures and catalytic mechanisms of dehalogenases

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From the beginning of the past century, halogenated hydrocarbons have been extensively applied in industry and agriculture. Decades after the start of their widespread use, evidence started to accumulate that some of these xenobiotic halogenated compounds are persistent and highly toxic, stimulating investigations how they could be degraded. It appeared that specific bacterial enzymes exist, dehalogenases, which can degrade halogenated compounds. These enzymes make use of a variety of distinctly different catalytic mechanisms to cleave carbon-halogen bonds.

X-ray structures of haloalkane dehalogenases, haloacid dehalogenases, and 4-chlorobenzoyl-CoA dehalogenase demonstrated the power of substitution mechanisms that proceed via a covalent aspartyl intermediate.

Structural characterizations of haloalcohol dehalogenases revealed the details of another elegant catalytic strategy, exploiting the presence of a vicinal hydroxyl group in the substrate.

Finally, 3-chloroacrylic acid dehalogenases function in the bacterial degradation of 1,3-dichloropropene, a compound used in agriculture to kill plant-parasitic nematodes. Crystal structures of these enzymes showed that they function as hydratases to remove the halogen atom. Glu-52 is positioned to function as the water-activating base for the addition of a hydroxyl group to the C-3 atom of 3-chloroacrylate, while the nearby Pro-1 is positioned to provide a proton to C-2. Two arginine residues,  $\alpha$ Arg-8 and  $\alpha$ Arg-11, interact with the C-1 carboxylate groups, thereby polarizing the  $\alpha,\beta$ -unsaturated acids. The resulting product is an unstable halohydrin, 3-chloro-3-hydroxypropionate, which decomposes into the products malonate semialdehyde and HCl.

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## Substrate distortion by a lichenase highlights the different conformational itineraries harnessed by related glycoside hydrolases

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Hydrolysis of the glycosidic bond is one of the most critical processes in nature and has considerable technical importance. Glycoside hydrolases have been shown to be extremely proficient at the acceleration of this reaction by increasing rates by a factor of  $10^{17}$ , this makes them among the most effective of enzymes. This effectiveness is reflected in the tight binding of the oxocarbenium transition state. Determination of the conformation of the substrate whilst in this transition state is of importance not only for improved understanding of the action of these enzymes but also for the design of specific and powerful enzyme inhibitors. Members of glycoside hydrolase family 26 are predominately  $\beta$ 1-4 mannanases, however a *Clostridium thermocellum* lichenase shows different activity. We present here the determination of the crystal structure of this enzyme in complex with various inhibitors which reveal its conformational itinerary and demonstrate the differences between family 26 enzymes active on glucose rather than mannose configured substrates. In particular we have trapped the covalent intermediate by use of a difluoro-derived compound. This work allows us to draw conclusions as to the structure of the transition states along the reaction pathway and the mechanism of substrate recognition.

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