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**FOR THE RECORD**

# Crystallization and preliminary X-ray analysis of L-2-haloacid dehalogenase from *Xanthobacter autotrophicus* GJ10

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**Abstract:** Haloacid dehalogenases are enzymes that cleave carbon–chlorine or carbon–bromine bonds of 2-haloalkanoates. X-ray-quality crystals of L-2-haloacid dehalogenase from the 1,2-dichloroethane-degrading bacterium *Xanthobacter autotrophicus* GJ10 have been grown at room temperature from 20% PEG 8000, 200 mM sodium formate at pH 6.8–7.0, using macroseeding techniques. The crystals, which diffract in the X-ray beam up to 2.0 Å resolution, belong to the spacegroup $C222_1$. Cell parameters are $a = 58.8$ Å, $b = 93.1$ Å, $c = 84.2$ Å. A native data set to 2.3 Å has been collected, with a completeness of 97% and an $R_{sym}$ of 6.0%.

**Keywords:** L-2-haloacid dehalogenase; protein crystals; 2-chloroacetate degradation; *Xanthobacter autotrophicus* GJ10; X-ray crystallography

The bacterium *Xanthobacter autotrophicus* is able to grow on short-chain haloalkanes as its sole source of carbon and energy (Janssen et al., 1985). Its natural substrate, 1,2-dichloroethane, is degraded via 2-chloroethanol, chloroacetaldehyde, and chloroacetate to glycolate in four consecutive enzymatic reactions before it enters the central metabolic routes. The halogen atoms are cleaved off in the first and fourth step by two different dehalogenases that occur in the bacterium. In the first step, a haloalkane dehalogenase catalyzes the conversion of the substrate to 2-chloroethanol and chloride. The three-dimensional structure of this enzyme has been determined by Franken et al. (1991), and Verschueren et al. (1993) have elucidated its catalytic mechanism using X-ray crystallography.

A 2-haloacid dehalogenase is involved in the fourth degradation step. It catalyzes the conversion of chloroacetic acid to glycolate and chloride. Some 20 2-haloacid dehalogenases (E.C. 3.8.1.2) have been found in various sources (Fetzner & Lingens, 1994). They have been classified into five different groups according to their substrate specificity and stereospecific action: two groups that are active with either the L- or the D-form of 2-monochloropropionic acid (2-MCPA), yielding products with an inverted configuration at the chiral carbon atom. Two other groups act on both stereomers, one with inversion of configuration and the other with retention of configuration. The fifth group, consisting of the haloacetate dehalogenases, is identified by its inactivity toward substrates longer than haloacetates. High amino acid sequence identities are observed among haloacid dehalogenases within the separate groups (Barth et al., 1992; Kawasaki et al., 1994). In general, no clear homology is apparent between the haloacid dehalogenases from different classes.

The 2-haloacid dehalogenase from *X. autotrophicus* belongs to the group of L-specific dehalogenases. The *dhlB* gene encoding for it was cloned and sequenced (Van der Ploeg et al., 1991). The protein consists of a single polypeptide chain of 253 amino acids and has a molecular weight of 27,558 Da. The amino acid sequence is more than 40% identical to six other L-specific 2-haloacid dehalogenases from different sources (Kawasaki et al., 1994; Liu et al., 1994), but it shows no homology to the haloalkane dehalogenase from *X. autotrophicus* (Van der Ploeg et al., 1991). The enzyme shows maximum activity at pH 9.5. Its pI is 4.2, as was determined from an isoelectric focusing gel.

Because dehalogenases can act as detoxifying enzymes toward environmentally harmful compounds such as chlorinated and brominated aliphatic compounds (McConnell et al., 1975), they form a fascinating target for research. Additionally, the stereospecificity of the haloacid dehalogenase from *X. autotrophicus* could make it applicable to the production of enantiomerically pure 2-hydroxyalkanoic acids, which are important as starting materials in the chemical industry. Two possible mechanisms for the dehalogenase reaction of the enzyme have been suggested, both with inversion of configuration (Goldman et al., 1968; Van der Ploeg et al., 1991). The first is a one-step nucleophilic attack on the C2-carbon of the haloacid by an activated water molecule. The second possibility is the attack by a carboxylate group

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from the enzyme, yielding a covalently bound ester intermediate, followed by hydrolysis of the ester. In both suggested mechanisms, the carboxylate group of the substrate is held in position by a positively charged residue, thus causing stereospecific action. From site-directed mutagenesis, Kurihara et al. (1995) identified seven amino acid residues that possibly play indispensable roles in the catalytic reaction of the L-2-haloacid dehalogenase from *Pseudomonas* sp. YL. To gain more insight into the factors that determine the substrate specificity and stereospecific catalysis and to obtain structural evidence for one of the possible reaction mechanisms, we extended our crystallographic studies on dehalogenating enzymes to the L-2-haloacid dehalogenase.

L-2-Haloacid dehalogenase was prepared as described (Van der Ploeg et al., 1991). Crystals were grown at room temperature by vapor diffusion in hanging drops (3 $\mu$L of protein and 3 $\mu$L of precipitant) suspended over a 1-mL reservoir of precipitant. Previous attempts had yielded crystals from two different precipitants, 36–40% ammonium sulfate at pH 6.4–7.6, and 30% (w/v) polyethylene glycol (PEG) 8000 at pH 9.0. These crystals were either not suitable for X-ray diffraction experiments or were difficult to reproduce. However, new Sparse Matrix trials (adapted from Jancarik & Kim, 1991) gave two new starting conditions: 27% (w/v) PEG 8000 and 100 mM sodium formate as precipitant in a 100 mM bis-Tris + Tris buffer (bis[2-hydroxyethyl]iminotris[hydroxymethyl]methane + 2-amino-2-(hydroxymethyl)-1,3-propanediol), at pH 6.8. This resulted in an intergrown rod (0.05-mm thickness) within a few weeks. Using 5.4 M sodium formate as a precipitant and 100 mM bis-Tris buffer, pH 7.0, gave numerous thin rods. Improvement of the first condition in combination with macroseeding yielded crystals suitable for X-ray analysis.

Rod-shaped crystals (maximum size $0.6 * 0.2 * 0.2$ mm$^3$) are now obtained within a few weeks by seeding a 6-$\mu$L drop containing 2.5 mg/mL protein, 15% PEG 8000, 200 mM sodium formate, and 100 mM bis-Tris buffer, pH 6.8–7.0. The well contains 1 mL 20% PEG 8000, 200 mM sodium formate, and 100 mM bis-Tris buffer, pH 6.8–7.0.

A native data set to 2.3 Å has been collected at the EMBL Outstation at the DESY synchrotron in Hamburg on beamline X31, equipped with an 18-cm MAR image plate area detector. A wavelength of 0.91 Å was applied. All data were collected at room temperature from one crystal ($0.3 * 0.15 * 0.15$ mm$^3$). Processing was done with MARXDS software (Kabsch, 1988).

The crystal belongs to spacegroup $C222_1$, with cell axes $a$ = 58.8 Å, $b$ = 93.1 Å, $c$ = 84.2 Å. This corresponds to a unit cell volume of $461 * 10^3$ Å$^3$. The volume per unit mass, $V_M$ (Matthews, 1968), is 2.08 Å$^3$/Da, with one molecule in the asymmetric unit. This is within the range of 1.7–3.5 found to be typical for proteins. The solvent content is 43%, assuming a specific volume of 0.74 cm$^3$/g for the protein molecule. Crystals diffract to a maximum resolution of 2.0 Å for the first frames. The data set contains 10,198 unique reflections (49,346 observed) in the resolution range of 26–2.3 Å, with an overall $R_{sym}$ on intensities of 6.0% (22.7% in the shell 2.4–2.3 Å). The completeness is 97.0% (overall) and 97.9% in the highest resolution shell. A search for heavy atom derivatives is in progress, aimed at the full three-dimensional structure determination of this haloacid dehalogenase.

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