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Reaction Mechanism of DL-2-Haloacid Dehalogenase from *Pseudomonas* sp. 113: Hydrolytic Dehalogenation Not Involving Enzyme-Substrate Ester Intermediate

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DL-2-Haloacid dehalogenase from *Pseudomonas* sp. 113 (DL-DEX 113) catalyzes the hydrolytic dehalogenation of D- and L-2-haloalkanoic acids, producing the corresponding L- and D-2-hydroxyalkanoic acids, respectively. L-2-Haloacid dehalogenase, haloalkane dehalogenase, and 4-chlorobenzoyl-CoA dehalogenase, which catalyze the hydrolysis of various organohalogen compounds, have an active site carboxylate group that attacks the substrate carbon atom bound to the halogen atom, leading to the formation of an ester intermediate. This is subsequently hydrolyzed, resulting in the incorporation of an oxygen atom of the solvent water molecule into the carboxylate group of the enzyme. In the present study, we analyzed the reaction mechanism of DL-DEX 113. When a single turnover reaction of DL-DEX 113 was carried out with a large excess of the enzyme in H\textsubscript{2}O\textsuperscript{18}O with either D- or L-2-chloropropionate, the major product was found to be \textsuperscript{18}O-labeled lactate. After a multiple turnover reaction in H\textsubscript{2}O\textsuperscript{18}O, the enzyme was digested with proteases, and the molecular masses of the peptide fragments were measured. No peptide fragments contained \textsuperscript{18}O. These results indicate that the H\textsubscript{2}O\textsuperscript{18}O of the solvent directly attacks the \textalpha-carbon of 2-haloalkanoic acid to displace the halogen atom. This is the first example of an enzymatic hydrolytic dehalogenation that proceeds without formation of an ester intermediate.

**Keywords**: 2-Haloacid dehalogenase/ Ionspray mass spectrometry/ Reaction mechanism

Halidohydrolases catalyze hydrolytic cleavage of carbon-halogen bonds of various organohalogen compounds. 2-Haloacid dehalogenases (EC 3.8.1.2), haloacetate dehalogenases (EC 3.8.1.3), haloalkane dehalogenases (EC 3.8.1.5), and 4-chlorobenzoyl-CoA dehalogenases (EC 3.8.1.6) are included in this group of enzymes. 2-Haloacid dehalogenases are further classified into the following three groups: 1) L-2-Haloacid dehalogenase (L-DEX) catalyzes hydrolysis of L-2-haloalkanoic acids to produce the corresponding D-2-hydroxyalkanoic acids. 2) D-2-Haloacid dehalogenase (D-DEX) catalyzes the conversion of D-2-haloalkanoic acids into L-2-hydroxyalkanoic acids. 3) DL-2-Haloacid dehalogenase (DL-DEX) dehalogenates both D- and L-2-haloalkanoic acids.
and the enzyme.

A multiple turnover reaction of DL-DEX 113 was carried out in H\textsuperscript{18}O with D- or L-2-chloropropionate as a substrate. After completion of the reaction, the enzyme was digested with TPCK-treated trypsin, and the resulting peptide fragments were separated on a reversed phase column interfaced with an ionspray mass spectrometer as a detector. If the reaction proceeds through the mechanism in Fig. 1 (B), \textsuperscript{18}O should not be detected in the proteolytic fragments. The molecular masses of all peptides were virtually indistinguishable from the predicted ones whether the reaction was conducted with D- or L-2-chloropropionate. Since peptides containing amino acid residues 1, 106-107, 135-142, 181-183, 229-238, 250-254, 284-285, and 299-300 were not found in the trypsin-digested sample, we also analyzed lysyl endopeptidase-digested enzyme by the same method. Peptides 120-142, 232-285, and 299-306 were identified, and their molecular masses were virtually indistinguishable from the predicted ones.

In the above experiment, the molecular masses of the peptides containing amino acid residues 1, 106-107, 181-183 and 229-231 could not be measured. Therefore, we could not exclude the possibility that \textsuperscript{18}O was incorporated into Asp or Glu in these peptides. However, among these peptides, only peptide 181-183 contains an acidic residue, Asp181. We replaced Asp181 with Ala, Arg, and Glu by site-directed mutagenesis to clarify whether Asp181 is involved in the catalytic reaction shown in Fig. 1 (A). The activities of these mutant enzymes were similar to that of the wild-type enzyme, indicating that Asp181 is not essential for the catalysis.

All the above results are consistent with the general base mechanism shown in Fig. 1 (B), but not with the mechanism shown in Fig. 1 (A). This applies to the dehalogenations of both enantiomers of 2-haloalkanoic acids because the results obtained for both enantiomers were virtually the same. We previously reported that DL-DEX 113 has a single and common catalytic site for both D- and L-enantiomers based on a site-directed mutagenesis experiment and kinetic analysis [2]. In the present study, we analyzed the reaction mechanism of DL-DEX 113 by means of \textsuperscript{18}O incorporation experiments [3].

We conducted the single turnover reaction of DL-DEX 113 in H\textsuperscript{18}O with D- or L-2-chloropropionate as a substrate, using an excess amount of the enzyme. We found that a majority of the lactate produced was labeled with \textsuperscript{18}O. This makes a clear contrast with the results on the L-DEX YL reaction, which proceeds through the mechanism involving an ester intermediate (Fig. 1 (A)). Only 10% of the D-lactate produced from L-2-chloropropionate by L-DEX YL was labeled with \textsuperscript{18}O. This suggests that in the DL-DEX 113 reaction an oxygen atom of the solvent water was directly incorporated into the product. While supporting the mechanism shown in Fig. 1 (B), this is not compatible with the mechanism in Fig. 1 (A), in which an oxygen atom of the solvent water is first incorporated into the enzyme.

![Figure 1. Reaction mechanisms of dehalogenases](image)

**References**