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Bacterial 2-haloacid dehalogenases: structures and reaction mechanisms

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Abstract

Microbial dehalogenases have been attracting a great deal of attention because of their possible application to fine chemical synthesis and bioremediation of halo compound-polluted environment. Dehalogenases employ various different mechanisms to cleave the carbon–halogen bond. 2-Haloacid dehalogenases catalyze the hydrolytic dehalogenation of 2-haloalkanoic acids to produce the corresponding 2-hydroxyalkanoic acids. The reaction mechanism of L-2-haloacid dehalogenase from *Pseudomonas* sp. YL has been clarified by ^{18}O incorporation experiment, site-directed mutagenesis and X-ray crystallographic analysis. The carboxylate group of Asp10 performs a nucleophilic attack on the α-carbon atom of the substrate to displace the halogen atom and produce the ester intermediate, which is subsequently hydrolyzed to produce the corresponding D-2-hydroxyalkanoic acid and regenerate the Asp10 residue. The reaction catalyzed by fluoroacetate dehalogenase from *Moraxella* sp. B similarly proceeds in two steps: the carboxylate group of Asp105 performs a nucleophilic attack on the substrate α-carbon atom to form an ester intermediate, and the intermediate is hydrolyzed by a water molecule activated by His272. In contrast with these two enzymes, a water molecule directly attacks the substrate to displace the halogen atom and produce 2-hydroxyalkanoic acid in the reaction catalyzed by DL-2-haloacid dehalogenase from *Pseudomonas* sp. 113.

Keywords: L-2-Haloacid dehalogenase; DL-2-Haloacid dehalogenase; Fluoroacetate dehalogenase; 2-Haloalkanoic acid; 2-Hydroxyalkanoic acid

1. Introduction

Optically active 2-hydroxyalkanoic acids are useful as starting materials for the synthesis of various pharmaceuticals and agrochemicals. Bacterial 2-haloacid dehalogenases catalyze the hydrolytic dehalogenation of 2-haloalkanoic acids, and are applicable to the production of optically active 2-hydroxyalkanoic acids [1,2]. Dehalogenases are also expected to play a role in bioremediation of halo compound-polluted environment because they detoxify various organohalogen compounds [3].

2-Haloacid dehalogenases are classified into three groups based on their substrate specificities (Fig. 1). L-2-Haloacid dehalogenase catalyzes the dehalogenation of L-2-haloalkanoic acids, whereas D-2-haloacid dehalogenase specifically acts on D-2-haloalkanoic acids. DL-

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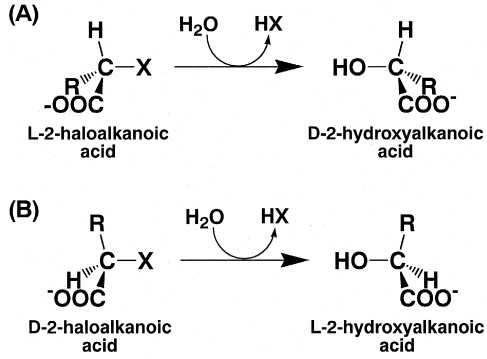

Fig. 1. Reactions catalyzed by 2-haloacid dehalogenases. L-2-Haloacid dehalogenase catalyzes the reaction shown in (A), whereas D-2-haloacid dehalogenase catalyzes the reaction (B). DL-2-Haloacid dehalogenase catalyzes both (A) and (B). R indicates an alkyl group.

2-Haloacid dehalogenase acts on both enantiomers of the substrates. All these reactions proceed with the inversion of the C_2-configuration of the substrates. Haloacetate dehalogenases catalyze similar reactions: hydrolysis of haloacetates to produce glycolate. Haloacetate dehalogenases are classified into two types. One acts on fluoroacetate, and the other does not. The former enzyme is called fluoroacetate dehalogenase. In this review, we describe properties and reaction mechanisms of L-2-haloacid dehalogenase, DL-2-haloacid dehalogenase, and fluoroacetate dehalogenase to compare with one another.

2. L-2-Haloacid dehalogenase

2.1. Properties of L-2-haloacid dehalogenase

L-2-Haloacid dehalogenase has been isolated from various bacterial strains including *Pseudomonas* sp. YL [4,5], *Pseudomonas putida* No. 109 [6], and *Xanthobacter autotrophicus* sp. GJ10 [7]. The enzyme specifically acts on L-enantiomers of 2-haloalkanoic acids, and the reaction proceeds with inversion of C_2-configuration of the substrates. Thus, the enzyme is useful for the production of D-2-hydroxyalkanoic acids.

Pseudomonas sp. YL produces thermostable L-2-haloacid dehalogenase (L-DEX YL) inducibly synthesized by 2-chloropropionate [5]. The enzyme was purified to homogeneity and was shown to be composed of two subunits with an identical molecular weight of 28,000. L-DEX YL acts not only on short carbon chain length 2-haloalkanoic acids such as chloroacetate and iodoacetate in aqueous solution, but on long carbon chain length 2-haloalkanoic acids such as 2-bromohexadecanoate in *n*-heptane (Table 1). The enzyme is also active in water-miscible organic solvents such as dimethyl sulfoxide: 3 M dimethyl sulfoxide does not inactivate the enzyme after incubation at 30°C for 5 min. L-DEX YL is thermostable: it retains its full activity upon heating at 60°C for 30 min. The pH and temperature optima for dehalogenation of L-2-chloropropionate were 9.5 and 65°C, respectively. L-2-Haloacid dehalogenases from other bacteria have several of these properties in common: their molecular weights are between 25,000 and 28,000, they show the maximum reactivities in the pH range of 9–11, and their amino acid sequences are similar to each other. High thermostability, high temperature optimum, and low substrate specificity are characteristics of L-DEX YL. The stereospecificity of L-DEX YL is not affected at all by organic solvents. This property makes the enzyme useful for the production of various D-2-hydroxyalkanoic acids from their halo analogs because

Table 1
Dehalogenation of 2-haloalkanoic acids catalyzed by L-DEX YL in *n*-heptane
The activity toward L-2-chloropropionate in *n*-heptane is 3.3% of that in water.

Substrate	Relative activity (%)
L-2-Chloropropionate	100
DL-2-Chlorohexanoate	13
DL-2-Chloroheptanoate	17
DL-2-Bromooctanoate	11
DL-2-Bromodecanoate	24
DL-2-Bromododecanoate	4
DL-2-Bromotetradecanoate	16
DL-2-Bromohexadecanoate	19

2-haloalkanoic acids, in particular, 2-bromoalkanoic acids, are generally unstable in aqueous solution resulting in the production of racemic 2-hydroxyalkanoic acids, but are more stable and soluble in organic solvents.

2.2. Primary structure of L-2-haloacid dehalogenase

The nucleotide sequence of the gene encoding L-DEX YL has been determined [8]. The open reading frame consists of 696 nucleotides corresponding to 232 amino acid residues. The protein molecular weight was estimated to be 26,179. The amino acid sequence predicted is similar to those of L-2-haloacid dehalogenases from cells of other bacterial strains and haloacetate dehalogenase H-2 from *Moraxella* sp. B (36–70% identity). However, these dehalogenases show a very low level of similarity to other halidohydrolases including fluoroacetate dehalogenase from *Moraxella* sp. B, haloalkane dehalogenase from *X. autotrophicus* GJ10, and DL-2-haloacid dehalogenase from *Pseudomonas* sp. 113 in their amino acid sequences.

The gene was efficiently expressed in the recombinant *Escherichia coli* cells, and the amount of L-DEX YL corresponded to about 49% of the total soluble proteins. This thermostable enzyme can be easily purified from the *E. coli* cell extract by heat treatment and ion-exchange column chromatography [5,9].

2.3. Reaction mechanism of L-2-haloacid dehalogenase

The roles of all the 36 highly conserved charged and polar amino acid residues of L-DEX YL were examined [10]. Each amino acid residue was replaced by another residue by site-directed mutagenesis as follows: Asp by Asn, Glu by Gln, Arg by Lys, and vice versa, Ser and Thr by Ala, Tyr and Trp by Phe, Met by Leu and His by Asn. The replacement of Asp10, Thr14, Arg41, Lys151, Tyr157, Ser175, Asn177, Asp180, and Ser118 led to a significant loss in the enzyme activity or an increase in K_m value for the substrate, showing their involvement in catalysis (Fig. 2).

Two reaction mechanisms were proposed for L-2-haloacid dehalogenase (Fig. 3). According to the Fig. 3A mechanism, a carboxylate group of the enzyme attacks the α-carbon atom of the substrate to release the halogen atom, resulting in the formation of an ester intermediate. In the second step of the reaction, the ester intermediate is hydrolyzed to produce D-2-hydroxyalkanoic acid and regenerate the active-site carboxylate group. In contrast, a water molecule

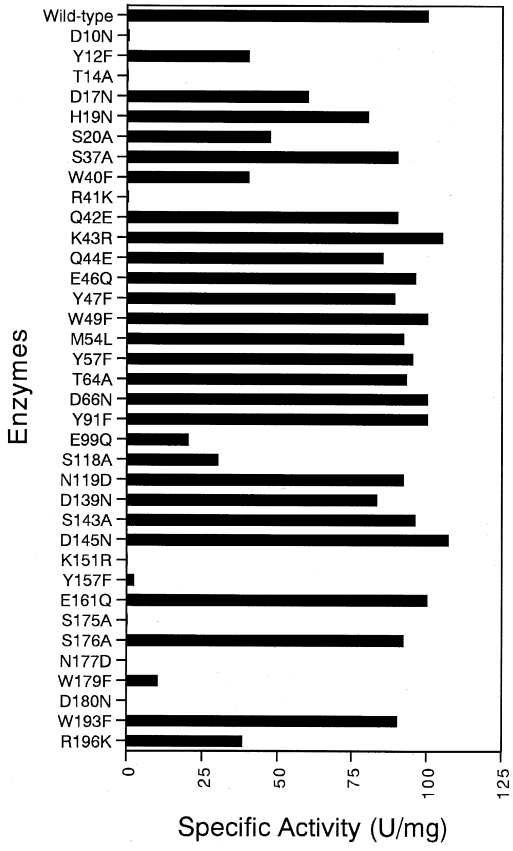

Fig. 2. Activities of wild-type and mutant L-DEX YL and L-2-chloropropionate. The specific activities measured with 25 mM L-2-chloropropionate are shown. The activities of all these mutant enzymes, except for S118A, for 1 mM substrate were almost the same as those for 25 mM substrate. The activity of S118A for 1 mM substrate was not detectable, indicating its high K_m value.

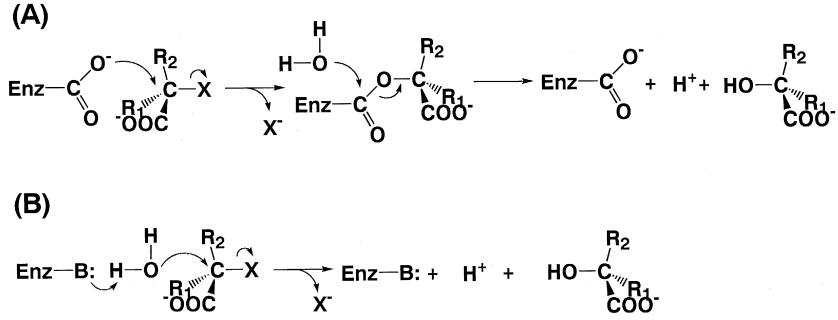

Fig. 3. Two proposed mechanisms for 2-haloacid dehalogenases and haloacetate dehalogenases. (A) Nucleophilic attack by a carboxylate group of an acidic amino acid residue followed by hydrolysis of the ester intermediate. (B) A general base catalytic mechanism. R_1 and R_2 indicate either an alkyl group or a hydrogen atom.

directly attacks the substrate to displace the halogen atom in the mechanism in Fig. 3B.

The reaction mechanism of L-DEX YL has been analyzed by ^{18}O incorporation experiment [11]. When the multiple-turnover enzyme reaction was carried out in $H_2{}^{18}O$ with L-2-chloropropionate as a substrate, lactate produced was labeled with ^{18}O. However, when the single-turnover enzyme reaction was performed by use of a large excess of the enzyme, the product was not labeled. This suggests that an oxygen atom of the solvent water is first incorporated into the enzyme, and then transferred to the product. This agrees with the Fig. 3A mechanism, but is not compatible with the Fig. 3B mechanism.

To determine the residue providing the carboxylate group for the catalysis, an ^{18}O labeling experiment was conducted [11]. After the multiple-turnover reaction in $H_2{}^{18}O$, the enzyme was digested with lysyl endopeptidase, and the molecular masses of the peptide fragments formed were measured by ionspray mass spectrometry. A hexapeptide, Gly6-Lys11, was shown to be labeled by two ^{18}O atoms. Tandem mass spectrometric analysis of this peptide revealed that Asp10 was labeled with two ^{18}O atoms. These results indicate that Asp10 acts as a nucleophile that attacks the α-carbon of the substrate leading to the formation of an ester intermediate. The site-directed mutagenesis experiment described above shows that the replacement of Asp10 leads to a significant loss in the enzyme activity, indicating that Asp10 is a catalytic residue.

Evidence for the formation of an ester intermediate was obtained by paracatalytic inactivation of L-DEX YL by hydroxylamine as well [12]. Ionspray mass spectrometrical analysis of the enzyme revealed that the molecular mass of the enzyme increased by 73 and 87 Da when the enzyme was paracatalytically inactivated by hydroxylamine in the presence of chloroacetate and L-2-chloropropionate, respectively. The increment was due to the modification of Asp10, which was most likely converted into an aspartate β-hydroxamate carboxyalkyl ester residue. This implies that hydroxylamine attacked the carbonyl carbon atom of the ester intermediate, supporting the view that the ester intermediate is produced in the reaction.

Reactions catalyzed by haloalkane dehalogenase [13–15] and 4-chlorobenzoyl-CoA dehalogenase [16–18] also proceed in two steps through formation of an ester bond between the enzyme and the substrate. These two classes of enzymes have an aspartate residue as a catalytic nucleophile. Thus, they are similar to L-DEX YL in this respect.

2.4. Three-dimensional structure of L-2-haloacid dehalogenase

The crystal structure of L-DEX YL has been determined by a multiple isomorphous replace-

ment method and refined at 2.5 Å resolution to a crystallographic *R*-factor of 19.5% [19]. The enzyme has a core domain of α/β structure, which is topologically different from those of the α/β hydrolase fold family proteins, and a subdomain with a four-helix-bundle structure. L-2-Haloacid dehalogenase from *X. autotrophicus* GJ10 was shown to have a similar overall structure [20]. These structures are not similar to those of haloalkane dehalogenase [21] and 4-chlorobenzoyl-CoA dehalogenase [17]. Thr14, Arg41, Ser118, Lys151, Tyr157, Ser175, Asn177, and Asp180 of L-DEX YL, which were shown to be essential for catalysis by site-directed mutagenesis, are located in the vicinity of Asp10, an active site nucleophilic residue [19].

The roles of these residues were estimated by study of the crystal structure of the enzyme complexed with various substrates and substrate analogs including chloroacetate, L-2-chlorobutyrate, L-2-chloro-3-methylbutyrate, L-2-chloro-4-methylvalerate, and L-2-chloropropionamide [22,23]. S175A mutant enzyme provided good crystals, and was used for this experiment. In each case, Asp10 was esterified with the dechlorinated moiety of the substrate. These structures are regarded as a reaction intermediate. The substrate moieties in all but the chloroacetate intermediate had a D-configuration at the C_2 atom.

Arg41 is the only basic residue located at the entrance of the active site of the enzyme. This residue probably serves as a recognition site for the substrate carboxyl group when the enzyme takes L-2-haloalkanoic acid into the active site (Fig. 4). Once the substrate reaches the active site, Ser118 probably serves as a binding residue for the substrate carboxyl group because this residue is located near the carboxyl group of the substrate moiety in the ester intermediate structure.

The hydrophobic pocket, which is primarily composed of the Tyr12, Gln42, Leu45, Phe60, Lys151, Asn177, and Trp179 side chains, exists around the alkyl group of the substrate moiety. This pocket may play an important role in stabilizing the alkyl group of the substrate moiety through hydrophobic interactions, and may also play a role in determining the stereospecificity of the enzyme. The possibility of a D-substrate binding to the enzyme was examined by exchanging the positions of the hydrogen atom and the alkyl group that are attached to the C_2 atom of the substrate moiety. It turns out that no alkyl groups could be accommodated properly in the place of hydrogen due to steric hindrance by the main-chain and side-chain atoms of Leu11, Tyr12, and their neighbors. Thus, the hydrophobic pocket probably determines the stereospecificity of the enzyme by selectively accommodating the alkyl side chain of L-2-haloalkanoic acid.

The guanidino group of Arg41 is a candidate for the acceptor of the halide ion released from the substrate, because this residue is the only functional residue that is in a suitable position in all ester intermediates: the $O^{\delta 2}$ atom of Asp10, the C_2 atom of the substrate, and the $N^{\eta 1}$ atom of Arg41 line up approximately on a straight line (Fig. 4). This presents a striking contrast to the case of haloalkane dehalogenase from *X. autotrophicus* GJ10. Haloalkane dehalogenase employs two tryptophane residues to accept the halide ion released from the substrate [13].

A water molecule, which is absent in the substrate-free enzyme, is found in the vicinities of the carboxyl carbon of Asp10 and the side chains of Asp180, Asn177, and Ala175 (the residue replacing Ser175 of the wild-type enzyme) in each complexed enzyme [22]. This water molecule most likely hydrolyzes the ester intermediate in the second step of the reaction, and the deprotonated carboxylate of Asp180 probably serves as the general base to activate the water molecule hydrolyzing the ester intermediate (Fig. 4). No histidine residue was found in the vicinity of Asp10, indicating that histidine residue is not involved in hydrolysis of the ester intermediate. Haloalkane dehalogenase [15] and fluoroacetate dehalogenase (described below) have a histidine residue as a catalytic base

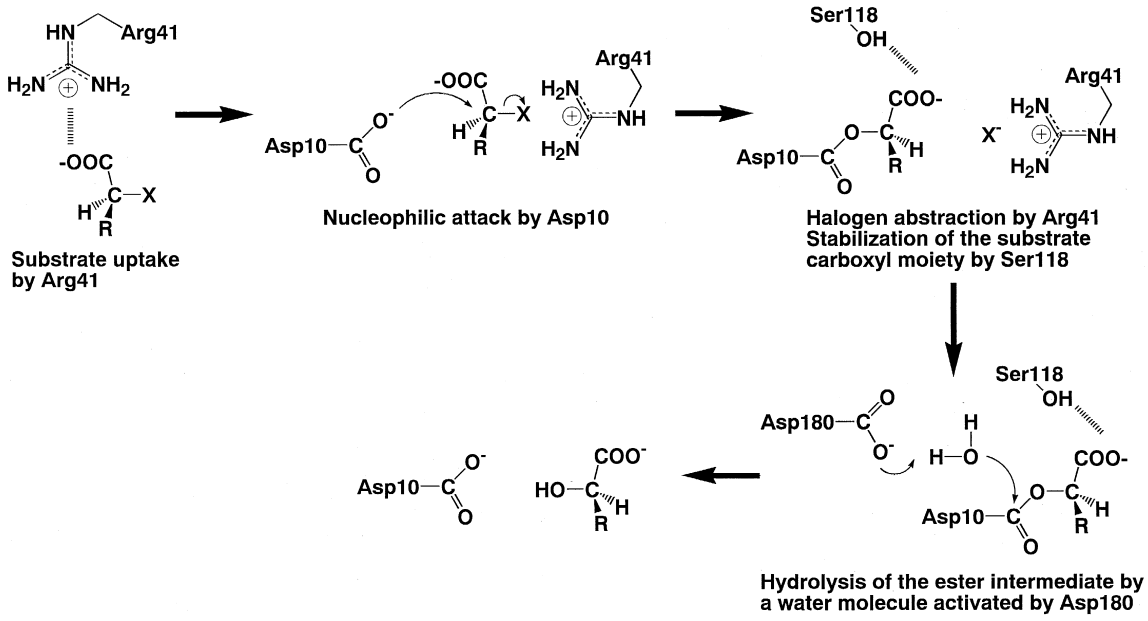

Fig. 4. Reaction mechanism of L-DEX YL.

to hydrolyze the ester intermediate, and are different from L-2-haloacid dehalogenase in this respect.

3. Fluoroacetate dehalogenase

Fluoroacetate is one of the most toxic compounds for mammals. Some plants in Australia, Africa, and Central America are known to synthesize high concentrations of fluoroacetate from fluoride [24]. In Australia, rumen bacteria genetically modified to produce recombinant fluoroacetate dehalogenase have been applied to the detoxification of poisonous plants containing high concentrations of fluoroacetate to prepare for cases in which domestic animals ingest these plants [25].

Fluoroacetate dehalogenase catalyzes the hydrolytic dehalogenation of fluoroacetate and other haloacetates to produce glycolate and a halide ion. The dissociation energy of the C–F bond of aliphatic fluoro compounds is among the highest found in bonds of natural products. Fluoroacetate dehalogenase is the only known enzyme that catalyzes the cleavage of C–F bond. Surprisingly, the enzyme shows the highest activity toward fluoroacetate. The relative activities for haloacetates are as follows: fluoroacetate, 480; chloroacetate, 100; bromoacetate, 60; and iodoacetate, 2.6.

The gene encoding fluoroacetate dehalogenase from *Moraxella* sp. B was isolated and sequenced [26]. The open reading frame consists of 882 nucleotides corresponding to 294 amino acid residues. The protein molecular weight was estimated to be 33,307. The amino acid sequence is similar to that of haloalkane dehalogenase from *X. autotrophicus* GJ10 (18% identity) [27] especially in the regions around Asp105 and His272, which correspond to the active site nucleophile Asp124 and the base catalyst His289 of the haloalkane dehalogenase, respectively. Despite this sequence similarity, fluoroacetate dehalogenase is quite different from haloalkane dehalogenase in its substrate specificity. The most notable difference is that the former enzyme catalyzes the cleavage of C–F bond, whereas the latter cannot cleave the bond. Another difference is that the latter en-

zyme does not act on haloacetates as a substrate.

The reaction mechanism of fluoroacetate dehalogenase was analyzed by ^{18}O labeling experiment to determine which mechanism in Fig. 3 the enzyme reaction proceeds through [28]. After multiple turnovers of the reaction in $H_2{}^{18}O$, the enzyme was digested with trypsin, and the molecular masses of the peptide fragments formed were measured by ionspray mass spectrometry. Two ^{18}O atoms were shown to be incorporated into the octapeptide, Phe99–Arg106. Tandem mass spectrometric analysis of this peptide revealed that Asp105 was labeled with two ^{18}O atoms. These results indicate that Asp105 acts as a nucleophile to attack the α-carbon of the substrate, leading to the formation of an ester intermediate, which is subsequently hydrolyzed to produce glycolate and restore the Asp105 residue (Fig. 5). This two-step mechanism involving the formation of an ester intermediate is similar to that of L-DEX YL described above, as well as that of haloalkane dehalogenase and 4-chlorobenzoyl-CoA dehalogenase.

H272N mutant enzyme showed no activity with either fluoroacetate or chloroacetate [28]. Ionspray mass spectrometry revealed that the mutant enzyme was covalently alkylated with the substrate. The reaction of the H272N mutant enzyme with [^{14}C]chloroacetate showed the incorporation of radioactivity into the enzyme. These suggest that His272 is not involved in the formation of the ester intermediate, but is essential for hydrolysis of the intermediate. His272 probably acts as a base catalyst for the hydrolysis of the covalent ester intermediate (Fig. 5).

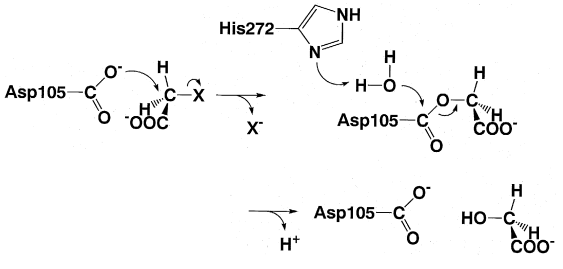

Fig. 5. Reaction mechanism of fluoroacetate dehalogenase.

Fluoroacetate dehalogenase is thought to belong to the α/β hydrolase fold family including haloalkane dehalogenase, epoxide hydrolase, and various other hydrolases [29], based on its sequence similarity to these enzymes. All the enzymes in this fold family have a catalytic triad, whose nucleophilic residue is located in an extremely sharp (γ-like) turn between a β-strand and an α-helix of the enzyme. The observations described above indicate that Asp105 and His272 of fluoroacetate dehalogenase are the constituents of the catalytic triad.

Phe128 of haloalkane dehalogenase from *X. autotrophicus* GJ10 is a binding site for the Cl-2 atom, which is not removed by the enzyme, of the substrate 1,2-dichloroethane [13]. The amino acid sequence around this residue is conserved in fluoroacetate dehalogenase, but Phe128 is replaced by Arg109 in the latter enzyme. Because guanidino group of an arginine residue is the most common binding site for the carboxyl group of substrates, Arg109 of fluoroacetate dehalogenase most likely acts as the binding site for the carboxyl group of haloacetates.

Trp125 and Trp175 of haloalkane dehalogenase from *X. autotrophicus* GJ10 accepts a halide ion released from the substrate [13]. These residues are conserved as Arg106 and Trp151 in fluoroacetate dehalogenase. Arg106 and Trp151 probably function as a halide ion acceptor in fluoroacetate dehalogenase. However, detailed mechanism for fluoride ion abstraction remains to be elucidated.

4. DL-2-Haloacid dehalogenase

DL-2-Haloacid dehalogenase from *Pseudomonas* sp. 113 (DL-DEX 113) catalyzes the hydrolytic dehalogenation of both D- and L-2-haloalkanoic acids to produce the corresponding L- and D-2-hydroxyalkanoic acids, respectively [30]. DL-DEX 113 is unique in respect that it

acts on the chiral carbons of both enantiomers. Racemases also act on the chiral carbons of both enantiomers of substrates, but are markedly different from DL-DEX 113 in that racemases catalyze stereochemical but not chemical conversion of substrates. DL-DEX 113 is similar to L-2-haloacid dehalogenase and D-2-haloacid dehalogenase in that they catalyze the hydrolytic dehalogenation of 2-haloalkanoic acids with inversion of the C_2-configuration of the substrates.

One might expect that DL-DEX 113 is composed of two domains, one of which is similar to that of D-2-haloacid dehalogenase and the other is similar to that of L-2-haloacid dehalogenase, in order to dehalogenate both enantiomers of the substrates. To examine this possibility, the gene encoding DL-DEX 113 has been isolated and sequenced [31]. The open reading frame consists of 921 bp corresponding to 307 amino acid residues. The protein molecular weight was estimated to be 34,242. The amino acid sequence of DL-DEX 113 was similar to that of D-2-haloacid dehalogenase from *P. putida* AJ1 that specifically acts on D-2-haloalkanoic acids [32]: 23.5% residues are completely conserved between these two enzymes. In contrast, no sequence similarity was found with L-2-haloacid dehalogenases. Accordingly, DL-DEX 113 resembles only D-2-haloacid dehalogenase, and the active site structure of DL-DEX 113 is probably similar solely to that of D-2-haloacid dehalogenase.

To find out catalytically essential residues, 26 charged and polar amino acid residues of DL-DEX 113 that are conserved between DL-DEX 113 and D-2-haloacid dehalogenase were replaced by another residue [31]. Thr65, Glu69 and Asp194 were found to be essential for the dehalogenation of not only D-2-haloalkanoic acids but L-2-haloalkanoic acids. The activities of each of all the 26 mutant enzymes toward D- and L-2-chloropropionate were almost equal to each other. Moreover, D-2-chloropropionate competitively inhibits the enzymatic dehalogenation of L-2-chloropropionate, and vice versa. Based on these results, it is proposed that both enantiomers share a common active site of DL-DEX 113.

Two possible mechanisms can be delineated for the reaction catalyzed by DL-DEX 113 as it was the case for L-DEX YL and fluoroacetate dehalogenase described above (Fig. 3). The reaction mechanism of DL-DEX 113 was analyzed by ^{18}O-labeling experiment and site-directed mutagenesis [33]. When a single turnover reaction of DL-DEX 113 was carried out with a large excess of the enzyme in $H_2{}^{18}O$ with a 10-time-smaller amount of the substrate, either D- or L-2-chloropropionate, the major product was found to be ^{18}O-labeled lactate by ionspray mass spectrometry. After a multiple turnover reaction in $H_2{}^{18}O$, the enzyme was digested with trypsin or lysyl endopeptidase, and the molecular masses of the peptide fragments were measured with an ionspray mass spectrometer. No peptide fragments were labeled with ^{18}O. These results indicate that the $H_2{}^{18}O$ of the solvent directly attacks the α-carbon of 2-haloalkanoic acid to displace the halogen atom. Site-directed mutagenesis experiments showed that Glu69 and Asp194 are essential for the catalysis of DL-DEX 113. These residues may function as a catalytic base to activate a water molecule that attacks the α-carbon atom of the substrate (Fig. 6).

Every halidohydrolase studied so far (L-2-haloacid dehalogenase, fluoroacetate dehalogenase, haloalkane dehalogenase, and 4-chlorobenzoyl-CoA dehalogenase) except for DL-DEX 113 has an active site carboxylate group that attacks the substrate carbon atom bound to the halogen atom, leading to the formation of an ester intermediate, which is subsequently hydro-

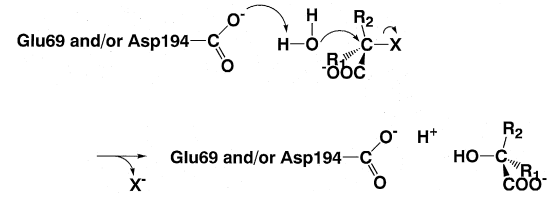

Fig. 6. Reaction mechanism of DL-DEX 113.

lyzed. Thus, DL-DEX 113 is unique in respect that its reaction proceeds without formation of an ester intermediate.

5. Conclusions

This review describes structures, properties, and reaction mechanisms of bacterial dehalogenases catalyzing hydrolytic dehalogenation of 2-haloalkanoic acids. These enzymes employ two different mechanisms to catalyze the reactions. The reaction catalyzed by one group of the enzymes (L-2-haloacid dehalogenase and fluoroacetate dehalogenase) proceeds through the mechanism depicted in Fig. 3A, which involves a carboxylate group of the enzyme acting as a nucleophile to produce an ester intermediate. In the reaction catalyzed by DL-2-haloacid dehalogenase, a water molecule directly attacks the substrate to displace the halogen atom (Fig. 3B). Enzymes described in this review and other dehalogenases such as haloalkane dehalogenase are useful in chemo-, regio-, and enantioselective transformations of organohalogen compounds. Detailed information on structures and functions of these enzymes will enable us to create modified dehalogenases with desired catalytic properties.

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