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trans-3-Chloroacrylic Acid Dehalogenase from Pseudomonas pavonaceae 170 Shares Structural and Mechanistic Similarities with 4-Oxalocrotonate Tautomerase

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The genes (caaDJ and caaD2) encoding the trans-3-chloroacrylic acid dehalogenase (CaaD) of the 1,3-dichloropropene-utilizing bacterium Pseudomonas pavonaceae 170 were cloned and heterologously expressed in Escherichia coli and Pseudomonas sp. strain GJ1. CaaD is a protein of 50 kDa that is composed of α-subunits of 75 amino acid residues and β-subunits of 70 residues. It catalyzes the hydrolytic cleavage of the β-vinylic carbon-chlorine bond in trans-3-chloroacrylic acid with a turnover number of 6.4 s⁻¹. On the basis of sequence similarity, oligomeric structure, and subunit size, CaaD appears to be related to 4-oxalocrotonate tautomerase (4-OT). This tautomerase consists of six identical subunits of 62 amino acid residues and catalyzes the isomerization of 2-oxo-4-hexene-1,6-dioate, via hydroxymuconate, to yield 2-oxo-3-hexene-1,6-dioate. In view of the oligomeric architecture of 4-OT, a trimer of homodimers, CaaD is postulated to be a hexameric protein that functions as a trimer of αβ-dimers. The sequence conservation between CaaD and 4-OT and site-directed mutagenesis experiments suggested that Pro-1 of the β-subunit and Arg-11 of the α-subunit are active-site residues in CaaD. Pro-1 could act as the proton acceptor/donor, and Arg-11 is probably involved in carboxylate binding. Based on these findings, a novel dehalogenation mechanism is proposed for the CaaD-catalyzed reaction which does not involve the formation of a covalent enzyme-substrate intermediate.

Isomer-specific 3-chloroacrylic acid dehalogenases catalyze the hydrolytic cleavage of the β-vinylic carbon-chlorine bond in either cis- or trans-3-chloroacrylic acid to yield malonic acid semialdehyde and HCl. These enzymes are produced by both gram-positive and gram-negative bacteria, including Pseudomonas pavonaceae 170 (27), Pseudomonas cepacia CAA1 (11), and the coryneform bacterial strains FG41 (47) and CAA2 (11), enabling these organisms to use one or both isomers of the xenobiotic compound 3-chloroacrylic acid for growth. The dehalogenases from strain FG41 were purified to homogeneity, and trans-3-chloroacrylic acid dehalogenase (CaaD) was found to be a 50-kDa enzyme composed of different subunits of 8.7 and 7.4 kDa, whereas the cis-3-chloroacrylic acid dehalogenase was an enzyme composed of two or three identical 16-kDa subunits (47). Although large fragments of these dehalogenating enzymes were sequenced, no significant sequence similarities with other protein sequences were found when the different databases were searched in 1992 (47).

Whereas most hydrolytic dehalogenase that are active with halogenated aliphatic compounds (so-called halohydrolases), such as haloalkane dehalogenases (26, 30, 50), haloacetate dehalogenases (15–17), and 2-haloacid dehalogenases (21, 25, 31), are only able to displace halogens bound to sp²-hybridized carbon atoms, 3-chloroacrylic acid dehalogenases are unique in that they can cleave the much more stable vinylic carbon-halogen bond, in which the halogen is bound to an sp²-hybridized carbon atom. Cleavage of the latter can also occur with 4-chlorobenzyol-coenzyme A dehalogenases, but in that case activation of the substrate (4-chlorobenzoate) to its coenzyme A derivative is needed (2, 5, 52). 3-Chloroacrylic acid dehalogenases are, to our knowledge, the only enzymes known to dehalogenate substrates with unactivated vinylic halogens.

Nothing is known about the catalytic mechanism of 3-chloroacrylic acid dehalogenases. To obtain insight on the structure, mechanism, and ancestry of these enzymes, we sequenced the genes encoding CaaD of P. pavonaceae 170 and characterized the expressed protein. The results indicate that the dehalogenase has both structural and mechanistic similarities to 4-oxalocrotonate tautomerase (4-OT), an enzyme involved in the bacterial catabolism of catechol to metabolites in the Krebs cycle.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The characteristics of P. pavonaceae 170, formerly known as Pseudomonas cichorii 170, have been given elsewhere (27, 48). Escherichia coli JM101 (53) and plasmid pBluescript SK⁻ (Stratagene) were used for subcloning experiments. E. coli HB101(pRK600) (8) was the helper strain used for mobilizing pLAFR3-derived cosmid and pDSK519-derived plasmids in triparental matings with Pseudomonas sp. strain GJ1 (13). Cosmid pLAFR3 and plasmid pDSK519 are mobilizable broad-host-range vectors (18, 35).

E. coli BL21(DE3) was used in combination with the T7 expression system (pET5a system; Promega) for overexpression of the dehalogenase and the mutant enzymes (40).

Cells for general cloning and expression were cultivated at 30°C in Luria-Bertani (LB) medium (33). When required, Difco agar (15 g/liter) was added to the medium. Antibiotics were added in the following amounts: ampicillin, 100 μg/ml; tetracycline, 12.5 μg/ml; chloramphenicol, 50 μg/ml; and kanamycin, 50 μg/ml.

General methods. Techniques for restriction enzyme digestion, ligation, transformation, and other standard molecular biology manipulations were based on methods described by Sambrook et al. (33). Triparental matings were carried out.

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† Present address: Department of Microbiology, University of Groningen, 9751 NN Haren, The Netherlands.
as described elsewhere (14). DNA sequencing was performed at the BioMedical Technology Centre (Groningen, The Netherlands) using a Pharmacia ALF- Express automatic sequencing machine according to the instructions provided with the Amersham Thermo Sequenase cycle-sequencing kit. The base sequence was confirmed using an NdI restriction site (in bold) and the codon for the desired mutation (in italics). The αF39a, αF39Y, and βP1A mutants were generated by overlap extension PCR. Primers A and D were used as the external PCR primers. For the αF39a mutant, the internal PCR primers were oligonucleotides 5'-GA GCCGCCAGAGAGACTGTTTGGATT-3' (mutated codon in italics) and 5'-AAATTGTTCTCGCGGGCTC-3' (primer E). For the αF39Y mutant, the internal PCR primers were oligonucleotides 5'-TCAGTAAATAG AGAGATAATCATGCCTC-3' (mutated codon in italics) and 5'-CATGT TACCTCTCATTACCTAGT-3'.

PCR was carried out as described above, and PCR products were purified using the Qiagen PCR purification kit or the Qiaex II gel extraction kit. The restriction sites NdI and BamHI that were introduced during the amplification reactions were used to clone the PCR products into plasmid pET5a for overexpression of the dehalogenase mutants. The cloned dehalogenase genes were sequenced in order to verify the mutations.

Preparation of crude extracts. CaaD and the mutant enzymes were expressed in E. coli BL21(DE3) using the pET system. Fresh BL21(DE3) transformants containing the desired plasmid were collected from a plate by resuspending them in 1 ml of LB medium and used to inoculate 100 ml of LB-ampicillin medium to a starting optical density at 600 nm of 0.1. After overnight growth at 30°C, cells were harvested by centrifugation (10 min at 10,000 × g), washed with 1 volume of 50 mM Tris-sulfate buffer (pH 8.2), and disrupted at 4°C in an appropriate amount of this buffer by sonication (10 s per ml of suspension at a 70-W output in a Vibra cell sonicator). A crude extract was obtained by centrifugation (45 min at 16,000 × g).

Purification of the dehalogenase. For isolation of CaaD of P. pavo- nauseae 170, a single colony of strain 170 was used to inoculate 100 ml of LB medium. After overnight growth at 30°C, the culture was used to inoculate 1 liter of LB medium. This culture was grown at 30°C until the early stationary-growth phase. Cells were harvested by centrifugation (10 min at 10,000 × g, washed with 1 volume of TEMAG buffer (10 mM Tris-SO₄, 1 mM EDTA, 1 mM ammonium sulfate in TEMAG). Fractions that showed the highest dehalogenase activity (1 h at 50,000 rpm in a type 70 Ti rotor (Beckman). The supernatant was applied to a DEAE-cellulose column which had previously been equilibrated with TEMAG buffer. The column was washed with 1 volume of TEMAG buffer, and the proteins were eluted with a linear gradient of 0 to 50 mM ammonium sulfate in TEMAG buffer. Fractions that showed the highest dehalogenase activity with 0.02% sodium azide, 10% glycerol (pH 6.5)). The dialyzed was loaded onto a hydroxylapatite column which had previously been equilibrated with PEMA buffer (20 mM potassium phosphate, 1 mM EDTA, 1 mM β-mercaptoethanol, 20°C. CaaD was purified to homogeneity by a modification of a published procedure (47). In a typical experiment, cells of a 1-liter culture were thawed and suspended in 20 ml of TEMAG buffer. The cells were disrupted by sonication, after which unbroken cells and debris were removed by centrifugation for 1 h at 50,000 rpm in a type 70 Ti rotor (Beckman). The supernatant was applied to a DEAE-cellulose column which had previously been equilibrated with TEMAG buffer. The column was washed with 1 volume of TEMAG buffer, and the proteins were eluted with a linear gradient of 0 to 5 M ammonium sulfate in TEMAG buffer. Fractions that showed the highest dehalogenase activity with 0.02% sodium azide, 10% glycerol (pH 6.5)). The dialyzed was loaded onto a hydroxylapatite column which had previously been equilibrated with PEMA buffer (20 mM potassium phosphate, 1 mM EDTA, 1 mM β-mercaptoethanol, 20°C. CaaD was purified to homogeneity by a modification of a published procedure (47). In a typical experiment, cells of a 1-liter culture were thawed and suspended in 20 ml of TEMAG buffer. The cells were disrupted by sonication, after which unbroken cells and debris were removed by centrifugation for 1 h at 50,000 rpm in a type 70 Ti rotor (Beckman). The supernatant was applied to a DEAE-cellulose column which had previously been equilibrated with TEMAG buffer. The column was washed with 1 volume of TEMAG buffer, and the proteins were eluted with a linear gradient of 0 to 50 mM potassium phosphate in TEMAG. Fractions with the highest CaaD activity were analyzed by SDS-PAGE, and those that contained purified enzyme were pooled and dialyzed against TEMAG buffer. The enzyme was stored at 4 or −20°C.

Nucleotide sequence accession number. The nucleotide sequence of the dehalogenase gene region has been deposited in the GenBank database under accession number AJ290446.

RESULTS

Cloning and characterization of the genes encoding CaaD. The trans-specific 3-chloroacrylic acid dehalogenase of P. pavo-
the dehalogenase in strain GJ1 in a soluble and active form up to a concentration equivalent to 13% of the total soluble cellular protein (Table 1). The dehalogenase was isolated from strain GJ1(pDSKcaaD) with a yield of 20 mg of pure protein, as judged by SDS-PAGE, from a 1-liter culture. The purified enzyme could be stored for several months in TEMAG buffer at 4 or −20°C without significant loss of activity.

The native molecular mass of the dehalogenase was estimated by gel filtration chromatography to be 50 kDa. A comparison of this value to the subunit molecular masses (8.47 and 7.64 kDa) determined from the primary amino acid sequences suggests that the native dehalogenase is a hexameric protein, probably consisting of three α and three β subunits (α3β3).

The dehalogenase catalyzed the liberation of halide from trans-3-chloroacrylic acid (specific activity, 18 U/mg of protein) and trans-3-bromocrotonic acid (48 U/mg), but showed no detectable dehalogenase activity (<0.005 U/mg) toward cis-3-chloroacrylic acid, indicating that the enzyme is completely iso-}

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To isolate the genes encoding CaaD, individual clones of a previously constructed cosmid library of P. pavonaceae 170 (29) were screened for dehalogenase activity by monitoring halide production upon incubation with trans-3-chloroacrylic acid. Out of 2,500 E. coli HB101 clones tested, 1 clone expressing CaaD was found. The recombinant cosmid (pPS41) encoding the dehalogenase was isolated, and the localization of the dehalogenase genes was determined by screening subclones for dehalogenase activity.

Nucleotide sequencing of a 2.6-kb SalI subclone of pPS41 revealed several open reading frames. The two open reading frames encoding CaaD (designated caaD1 and caaD2) were identified by using the N-terminal amino acid sequences determined for both subunits of the enzyme isolated from P. pavonaceae 170. These N-terminal amino acid sequences are identical to those predicted from the DNA sequence if the trans

Sequence similarity with 4-oxalocrotonase tautomerases/isomerases. Database searches identified seven related proteins as having significant sequence similarity with CaaD (Table 2). Two are well-studied enzymes involved in the bacterial catabolism of catechol to metabolites in the Krebs cycle, the 4-OT from Pseudomonas putida mt-2 (39) and the 73% identical isozyme from Pseudomonas sp. strain CF600 (41). Both are hexameric proteins that consist of identical subunits of 62 amino acid residues (4) and catalyze the isomerization of 2-oxo-4-hexene-1,6-dioate, via hydroxymunonate, to yield 2-oxo-3-hexene-1,6-dioate (51). The other five proteins that were retrieved from the similarity search have not been studied, but based on sequence similarity to 4-OT, they might be classified as putative 4-oxalocrotonate tautomerases/isomerases. Pairwise identities among the seven identified 4-OT homologues range from 35 to 92%.

Alignments optimizing identity between the amino acid sequences of the two dehalogenase subunits and the seven 4-OT homologues are shown in Fig. 1. Pairwise identities between the α-subunit (CaaD1) of the dehalogenase and the 4-OT sequences fall between 23 and 35% (Table 2), with the highest sequence similarity in the N-terminal region, particularly in the region boxed in Fig. 1A. Pairwise identities between the β-subunit (CaaD2) and the 4-OT sequences are lower and range from 16 to 25% (Table 2).

The sequence alignment in Fig. 1 suggest catalytic residues for CaaD that are in the same position in the alignment as the identified catalytic residues of 4-OT. Affinity labeling (36), kinetic analysis (37), chemical synthesis (9), nuclear magnetic resonance (38, 39), site-directed mutagenesis (6), and crystal-
lographic studies (41) identified the amino-terminal proline as the catalytic base in the 4-OT-catalyzed tautomerization reaction. This N-terminal proline is invariant among all identified 4-OT homologues (Fig. 1). Both dehalogenase subunits also possess an N-terminal proline, indicating that one of these prolines may serve as the catalytic base in the CaaD-catalyzed dehalogenation reaction. The second catalytic residue of 4-OT, Arg-11, which is absolutely conserved among the 4-OT homologues (Fig. 1), was proposed to interact with the 6-carboxylate of the substrate (2-oxo-4-hexene-1,6-dioate) to facilitate both substrate binding and catalysis (10, 41). The sequence similarity indicates that Arg-11 is present only in the α-subunit of the dehalogenase and may perform an analogous role by interacting with the carboxylate group of trans-3-chloroacrylic acid. The third catalytic residue of 4-OT, Arg-39, which was proposed to interact with the 1-carboxylate and the 2-keto group of the substrate to promote carbonyl polarization and catalysis (10, 41), is not conserved in the dehalogenase sequence. This seems plausible since the dehalogenase substrate contains only one carboxylate group.

Characterization of dehalogenase mutants. In the homohexameric (α₆) 4-OT molecule, Pro-1 is important for tautomerization.

### Table 2. 4-OT sequences similar to CaaD

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein length (residues)</th>
<th>Organism</th>
<th>% Identity to α-Subunit</th>
<th>% Identity to β-Subunit</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>xylH</td>
<td>62</td>
<td><em>Pseudomonas putida</em> mt-2</td>
<td>26</td>
<td>25</td>
<td>Q01468</td>
</tr>
<tr>
<td>dmpI</td>
<td>62</td>
<td><em>Pseudomonas sp.</em> strain CF600</td>
<td>26</td>
<td>22</td>
<td>P49172</td>
</tr>
<tr>
<td>ywbB</td>
<td>61</td>
<td><em>Bacillus subtilis</em> 168</td>
<td>35</td>
<td>16</td>
<td>CAB02512</td>
</tr>
<tr>
<td>nahl</td>
<td>62</td>
<td><em>Pseudomonas stutzeri</em> AN10</td>
<td>26</td>
<td>24</td>
<td>AAD02155</td>
</tr>
<tr>
<td>xyH</td>
<td>79</td>
<td><em>Sphingomonas aromaticivorans</em> F199</td>
<td>25</td>
<td>22</td>
<td>AAD03991</td>
</tr>
<tr>
<td>phnL</td>
<td>76</td>
<td><em>Pseudomonas sp.</em> strain DJ77</td>
<td>23</td>
<td>24</td>
<td>AAD03865</td>
</tr>
<tr>
<td>nahl</td>
<td>62</td>
<td><em>Pseudomonas putida</em> G7</td>
<td>26</td>
<td>22</td>
<td>AAD13221</td>
</tr>
</tbody>
</table>

*Pairwise identities between the α- or β-subunit of the dehalogenase and the 4-OT sequences were calculated by using ClustalW.

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**Fig. 1.** Alignments of the amino acid sequences of the α-subunit (CaaD1) and β-subunit (CaaD2) of CaaD with the seven 4-OT sequences (A and B) and with the amino-terminal sequences of the subunits of the CaaD isolated from strain FG41 (C). Residues conserved throughout all sequences are indicated by an asterisk. Dashes represent residues absent in other sequences. The catalytically important residues are marked with + and shown in boldface. The region of highest sequence identity among CaaD1 and the 4-OT sequences is boxed. Ppa, *P. pavonaceae*; Bs, *B. subtilis*; Psp, *Pseudomonas* sp.; Pp, *P. putida*; Ps, *P. stutzeri*; Sa, *S. aromaticivorans*; Csp, coryneform bacterial strain.
In many reactions involving carbon-halogen bond cleavage, the carbon-halogen bond is weakened by functional groups that interact with the halogen substituent (26, 32, 49, 50). In the α-subunit of CaaD, Phe-39 is in the same position in the alignment as the catalytically important Arg-39 of 4-OT, suggesting that Phe-39 may be one of the residues that promote carbon-halogen bond cleavage by interacting with the chlorine atom of the dehalogenase substrate. To test if Phe-39 is catalytically important, this residue was mutated to alanine and tyrosine. The αF39A and αF39Y mutants were still able to catalyze halide release from both dehalogenase substrates, although 5- to 10-fold slower than the wild-type enzyme (Table 3), indicating that Phe-39 is not essential for dehalogenase activity.

### DISCUSSION

The enzyme CaaD is produced by the soil bacterium *P. putida* 170 as part of a degradative pathway for the xenobiotic nematocide *trans-1,3-dichloropropene* (27). This hydrolytic dehalogenase, of which the properties are reported in this work, has no sequence similarity with other halohydrolases but appears to be related to the family of 4-OTs. No other bacterial 3-chloroacrylic acid dehalogenase genes have been cloned, but the N-terminal sequences of the α and β subunits of the CaaD isolated from the gram-positive coryneform bacterial strain FG41 (47) have extensive similarity with the N-terminal parts of the α and β subunits of CaaD, respectively (Fig. 1C), suggesting that these two proteins have a common evolutionary origin and are mechanistically similar. As might be expected, the two CaaD sequences are more related to each other than to the 4-OTs.

The primary amino acid sequence of 4-OT shows no apparent similarity with those of the mammalian enzymes D-dopachrome tautomerase (DDT) (42) and macrophage migration inhibitory factor (MIF) (43), nor with that of the bacterial enzyme 5-carboxymethyl-2-hydroxymuconate isomerase (CHMI) (41), but remarkably, these four proteins have a common structural architecture (24, 42). DDT, MIF, and CHMI have an almost identical subunit topology, with two βαβ motifs related by pseudo-twofold symmetry and trimeric β-sheet packing (24, 41, 42). While CHMI, MIF, and DDT are functional as homotrimmers, 4-OT is a hexamer of identical monomers. The 4-OT subunit is composed of only 62 residues and is dimerized by twofold symmetry to form a structure similar to that of the CHMI, MIF, and DDT monomer. Therefore, 4-OT is a trimer of homodimers that shows 32 symmetry; its overall hexameric structure is very similar to the trimeric structure of CHMI, MIF, and DDT (41–43). An interesting difference between the four structures is that, because of the higher symmetry of the 4-OT hexamer, there are potentially six active sites in 4-OT, yet only three are conserved in CHMI, MIF, and DDT (41, 42).

One of the characteristics of this superfamily of 4-OT-related proteins is that its members possess an amino-terminal proline that is located at the bottom of a hydrophobic pocket. CHMI and 4-OT utilize this proline as a catalytic base in their isomerization reactions (6, 9, 36–39, 41). Pro-1 of MIF is required for its D-dopachrome tautomerase and phenylpyruvate tautomerase activities (22, 34). The N-terminal proline of DDT is proposed to serve as the catalytic base in the DDT-

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**TABLE 3. Activities of wild-type and mutant CaaD for *trans*-3-chloroacrylic acid (CAA) and *trans*-3-bromoacrylic acid (BAA)**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Dehalogenase sp act a (mU/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAA</td>
</tr>
<tr>
<td>Wild type</td>
<td>1,500</td>
</tr>
<tr>
<td>αP1A</td>
<td>1,260</td>
</tr>
<tr>
<td>βP1A</td>
<td>&lt;5</td>
</tr>
<tr>
<td>αR11A</td>
<td>&lt;5</td>
</tr>
<tr>
<td>αR11K</td>
<td>110</td>
</tr>
<tr>
<td>αF39A</td>
<td>350</td>
</tr>
<tr>
<td>αF39Y</td>
<td>240</td>
</tr>
</tbody>
</table>

a Activities were measured in cell extracts containing similar amounts of dehalogenase. Halide production with 5 mM substrate was determined at 30°C and pH 8.2.
FIG. 2. Comparative reaction scheme for 4-OT and CaaD. The primed residues come from other subunits. (A) Reaction catalyzed by 4-OT (adopted from references 10 and 41). (B) Two proposed reaction schemes for CaaD, one involving the formation of 3-chloro-3-hydroxypropanoic acid (route 1) and the other involving the formation of a carbanion intermediate (route 2). Pro-1 is shown as the catalytic base to activate a water molecule that attacks the substrate. Arg-11 is implicated in substrate binding.
catalyzed tautomerization reaction (42). The Pro-1 residue is conserved among all known homologues of 4-OT (Fig. 1), MIF (22, 44), and DDT (42) and is also conserved in both subunits of CaaD (Fig. 1). In the CaaD isolated from strain FG41, however, the amino-terminal proline is present only in the subunit that aligns with the β-subunit of CaaD (Fig. 1C). This suggests that Pro-1 of the β-subunit may serve as a catalytic base that activates a water molecule to attack the acidic residue, which may be the protonated proline or another amino acid. Loss of a proton from the hydroxyl at C-3 would produce the malonic acid semialdehyde, which may be facilitated by water or another proton acceptor. Hydration of monofluoromalate by fumarase also yielded an unstable intermediate, α-fluorohydride (α-fluoromalate), which subsequently decomposes to oxaloacetate and HF (23).

Because CaaD catalyzes a dehalogenation reaction, it is anticipated that functional groups involved in halogen/halide binding are required in addition to Pro-1 and Arg-11. We speculate that Phe-39 in the α-subunit of CaaD, which is in the same position in the alignment as Arg-39 in 4-OT (Fig. 1), may interact with the chlorine atom of the substrate to promote carbon-halogen bond cleavage. Indeed, aromatic ring systems are known to be partially positively charged in the plane of the ring (3). Phenylalanine residues were also proposed to contribute to halogen/halide binding in haloalkane dehalogenase (DhIA) and L-2-haloacid dehalogenase (DhIB) from Xanthobacter autotrophicus GJ10 (7, 32). However, the F39A and F39Y mutants of CaaD still had some residual activity, indicating that this residue is not essential. The presence of other functional groups interacting with the halogen atom of the substrate could explain why these mutants retained some activity. Indeed, in DhIA and DhIB, the halogen/halide-binding site is formed by more than one residue (20, 32, 49).

Screening of the cosmId library of P. pavonaceae 170 did not reveal clones that expressed the cis-3-chloroacrylic acid dehalogenase. Thus far, the only sequence information available for cis-specific 3-chloroacrylic acid dehalogenases is the N-terminal sequence of the enzyme isolated from the coryneform bacterial strain FG41 (47). This enzyme is probably a trimeric protein of 16.2-kDa subunits, and a comparison of its amino-terminal sequence with those of the CaaDs from strains FG41 and 170 revealed no overall similarity but showed that Pro-1 and Arg-11 are conserved (data not shown). Therefore, both cis- and trans-specific 3-chloroacrylic acid dehalogenases may catalyze the dehalogenation of their respective 3-chloroacrylic acid isomers through the mechanism shown in Fig. 2. This mechanism is different from that of most other hydrolytic dehalogenases in that it does not involve the formation of a covalent enzyme-substrate intermediate.

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