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Purification, characterization, and gene cloning of a novel fluoroacetate dehalogenase from *Burkholderia* sp. FA1

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Dedicated to Professor Dr. Kenji Soda in honor of his 70th birthday

Abstract

Fluoroacetate dehalogenase catalyzes the hydrolytic defluorination of fluoroacetate to produce glycolate. The enzyme is unique in that it catalyzes the cleavage of the highly stable carbon–fluorine bond in an aliphatic compound. The bacterial isolate FA1, which was identified as *Burkholderia*, grew on fluoroacetate as the sole carbon source to produce fluoroacetate dehalogenase (FAc-DEX FA1). The enzyme was purified to homogeneity and characterized. The molecular weights were estimated to be 79,000 and 34,000 by gel filtration and SDS-polyacrylamide gel electrophoresis (PAGE), respectively, suggesting that the enzyme is a dimer. The purified enzyme was specific to haloacetates, and fluoroacetate was the best substrate. The activities toward chloroacetate and bromoacetate were less than 5% of the activity toward fluoroacetate. The K_m and V_{max} values for the hydrolysis of fluoroacetate were 5.1 mM and 11 μmol per minute milligram, respectively. The gene coding for the enzyme was isolated, and the nucleotide sequence was determined. The open reading frame consisted of 912 nucleotides, corresponding to 304 amino acid residues. Although FAc-DEX FA1 showed high sequence similarity to fluoroacetate dehalogenase from *Moraxella* sp. B (FAc-DEX H1) (61% identity), the substrate specificity of FAc-DEX FA1 was significantly different from that of FAc-DEX H1: FAc-DEX FA1 was more specific to fluoroacetate than FAc-DEX H1.

Keywords: Fluoroacetate dehalogenase; Defluorination; Haloacetate; *Burkholderia* sp.; Haloalkane dehalogenase

1. Introduction

Various halogenated compounds have been synthesized and widely used as herbicides, insecticides, plastics, and solvents, but many of them are toxic and cause serious environmental pollution. Microbial dehalogenases detoxify harmful halogenated compounds by cleaving their carbon–halogen bonds [1–4]. These enzymes are attracting a great deal of attention from the viewpoint of environmental technology [5]. Dehalogenases are also useful in organic synthesis when organohalogen compounds are used as the reactants [6,7]. Various dehalogenases, such as L-2-haloacid dehalogenases from *Pseudomonas* sp. YL [8,9] and *Xanthobacter autotrophicus* GJ10 [10,11], haloalkane dehalogenases from *X. autotrophicus* GJ10 [12,13], *Rhodococcus* sp. [14], and *Sphingomonas paucimobilis* UT26 [15], and 4-chlorobenzoyl-CoA dehalogenase from *Pseudomonas* sp. CBS-3 [16], have been well characterized based on their crystal

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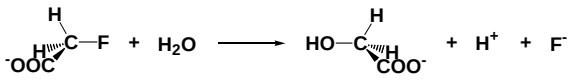

Fig. 1. The reaction catalyzed by fluoroacetate dehalogenase.

structures. However, none of them catalyzes the hydrolytic cleavage of a carbon–fluorine bond because the dissociation energy of a carbon–fluorine bond in aliphatic compounds is much higher than that in other carbon–halogen bonds. The only exception is fluoroacetate dehalogenase, which catalyzes the hydrolytic defluorination of fluoroacetate (Fig. 1). The enzyme has been demonstrated in a few *Pseudomonas* and *Moraxella* strains and characterized [17–19], and the primary structure of the enzyme from *Moraxella* sp. B (FAc-DEX H1) has been determined [20].

We analyzed the reaction mechanism of FAc-DEX H1 and identified two catalytically essential amino acid residues: Asp105 nucleophilically attacks the α-carbon atom of fluoroacetate to release the fluoride ion, resulting in the formation of the ester intermediate, and the intermediate is subsequently hydrolyzed by a water molecule activated by His272 to produce glycolate [21]. The reaction mechanism of FAc-DEX H1 resembles those of L-2-haloacid dehalogenase [22] and haloalkane dehalogenase [12] in that the catalytic nucleophile of the enzyme attacks the carbon atom of the substrate to release the halide ion. It still remains unclear why only fluoroacetate dehalogenase catalyzes the cleavage of the carbon–fluorine bond. Crystallographic analysis of the enzyme would be necessary to understand the requirements for the defluorination activity. However, so far, all the attempts to obtain a FAc-DEX H1 crystal suitable for X-ray analysis have been unsuccessful. Moreover, it is impossible to predict the catalytically important amino acid residues based on the conservation of the residues among various fluoroacetate dehalogenases because FAc-DEX H1 is the only fluoroacetate dehalogenase whose primary structure has been determined [20].

In the present study, we have isolated a novel fluoroacetate dehalogenase (FAc-DEX FA1) from the fluoroacetate-utilizable bacterium, *Burkholderia* sp. FA1, characterized the enzyme, and determined its primary structure. The enzyme showed high sequence similarity with FAc-DEX H1. However, FAc-DEX FA1 was different from FAc-DEX H1 in its substrate specificity: FAc-DEX FA1 was more specific to fluoroacetate than FAc-DEX H1.

2. Experimental

2.1. Isolation of bacteria

The medium for the screening of microorganisms producing fluoroacetate dehalogenase was composed of 0.2% sodium fluoroacetate, 0.1% ammonium sulfate, 0.1% dipotassium hydrogenphosphate, 0.05% potassium dihydrogenphosphate, 0.01% magnesium sulfate, and 0.01% yeast extract. The pH was adjusted to 7.0. Soil samples were suspended in water (10% (w/v)). The suspensions (2 ml) were incubated at 28 °C with reciprocal shaking. After 2 days, 100 μl of the culture showing the microbial growth was transferred to 2 ml of the fresh medium. The transfer was repeated several times, and the culture was spread on an agar plate. Single colonies were picked up, and the isolates were maintained on the same medium.

2.2. Identification of bacteria

To identify the bacteria assimilating fluoroacetate, the nucleotide sequence of 16S rDNA was determined. The mixture (25 μl) for PCR contained 10 mM Tris–HCl (pH 8.3), 50 mM potassium chloride, 1.5 mM magnesium chloride, 250 μM of each dNTP, 0.4 μM of each primer (5′-AGAGTTTGATCCTGGCTCAG-3′ and 5′-ACGGCTACCTTGTTACGACTT-3′), 1.25 units of Ex Taq DNA polymerase (Takara, Kyoto, Japan), and 15 ng of template DNA isolated from the bacteria by the method reported [23]. The reaction conditions were as follows: 1 min (95 °C), 1 min (55 °C), and 2 min (72 °C), for 30 cycles. The DNA fragment of about 1.5 kbp was amplified, separated by agarose gel electrophoresis, and used for direct DNA sequencing. DNA sequencing was performed with an Applied Biosystems DNA Sequencer 377B with a Dye Terminator sequencing kit.

2.3. Enzyme and protein assays

The defluorination activity of fluoroacetate dehalogenase was determined by measuring the fluoride

ions released from fluorinated compounds according to the method of Foster [24]. In this assay, fluoride ions quench the reddish orange color developed by mixing iron(III) chloride and ammonium thiocyanate. The reaction mixture (100 μl) contained 2 μmol of substrate, 10 μmol of Tris–nitrate (pH 9.5), and the enzyme. After incubation at 30 °C for 15 min, the reaction was terminated by addition of 10 μl of 1.0 M nitric acid. Fluoroacetate dehalogenase was routinely assayed by the determination of glycolate ions produced from fluoroacetate or chloroacetate by the method of Dagley and Rodgers [25], or by the determination of halide ions released from chloroacetate or bromoacetate by the method of Iwasaki et al. [26]. The standard assay mixture (100 μl) contained 2 μmol of haloacetate, 10 μmol of Tris–sulfate (pH 9.5), and the enzyme. After incubation at 30 °C for 10 min, the reaction was terminated by addition of 10 μl of 1.5 M sulfuric acid. One unit of the enzyme was defined as the amount of the enzyme that catalyzes the dehalogenation of 1 μmol of haloacetate per minute. To examine the effect of pH on the enzyme activity, the activity was measured in the standard assay mixtures with the following buffers: a 100 mM potassium phosphate buffer (pH 7–8), a 100 mM Tris–sulfate buffer (pH 8–10), and a 100 mM glycine–sodium hydroxide buffer (pH 10–11). Protein concentration was determined with a Bio-Rad protein assay kit and with bovine serum albumin as a standard.

2.4. Enzyme purification

All operations were performed at 4 °C, and 50 mM potassium phosphate (pH 7.5) was used as the standard buffer unless otherwise stated. The bacterial cells were grown aerobically at 28 °C for 2 days in the same medium as that for the screening of bacteria. The cells harvested from a 5 l culture were suspended in 15 ml of the standard buffer and disrupted with a Biomic 7500 Ultrasonic Processor (Seiko, Tokyo) for 15 min. The cell debris was removed by centrifugation. The supernatant was fractionated with ammonium sulfate. A fraction of 30–65% saturation was dissolved in the standard buffer and then applied to a Butyl-Toyopearl 650 M column (3 cm × 8 cm) (TOSOH, Tokyo, Japan). The column was washed with 150 ml of the standard buffer supplemented with 30% saturated ammonium sulfate, and the enzyme was eluted with a linear gradient of 30–0% saturated ammonium sulfate in the standard buffer with a total volume of 400 ml. The active fractions, dialyzed against the standard buffer, were applied to a DEAE-Toyopearl 650 M column (3 cm × 7 cm) (TOSOH, Tokyo, Japan) equilibrated with the same buffer. After washing the column with 150 ml of the standard buffer, the enzyme was eluted with a linear gradient of a 50–100 mM potassium phosphate buffer (pH 7.5) with a total volume of 400 ml. The active fractions were pooled and used as a purified enzyme.

2.5. Determination of the molecular weight

The molecular weight of the enzyme was determined by gel filtration with a Superose 12 HR 10/30 column (1 cm × 30 cm). The molecular weight markers used were as follows: cytochrome c (12,400), myokinase (32,000), enolase (67,000), lactate dehydrogenase (142,000), and glutamate dehydrogenase (290,000) (Oriental Yeast Co. Ltd., Osaka, Japan). SDS-polyacrylamide gel (PAGE) electrophoresis was carried out with the following marker proteins: phosphorylase b (molecular weight 94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α-lactalbumin (14,400) (Pharmacia Fine Chemicals, Uppsala, Sweden).

2.6. N-terminal and internal amino acid sequencing

FAc-DEX FA1 was digested with trypsin (the final concentration of trypsin, 20 μg/ml; the ratio of trypsin to FAc-DEX FA1, 1:50) in a 50 mM Tris–HCl buffer (pH 8.0) by overnight incubation at 37 °C. The peptides obtained were purified by HPLC with a 4.6 mm × 250 mm C18 packed column (Shiseido, Tokyo, Japan). The column was washed with 20% acetonitrile for 5 min, and the peptides were eluted with a 20–100% acetonitrile gradient containing 0.1% trifluoroacetic acid for 30 min. The flow rate was 500 μl/min, and the eluate was monitored at 210 nm to collect the peptides. The N-terminal amino acid sequences of these peptides and intact FAc-DEX FA1 were determined with a fully automated protein sequencer Shimadzu PPSQ-10 (Kyoto, Japan).

Table 1
Synthetic oligonucleotide primers used for cloning of the FAc-DEX FA1 gene

Name	Sequence
FA-1	5′-ATGTTYGARGGNTTYGARMG-3′
FA-8	5′-GGRAANCGRTCNACRAARAARTG-3′
FA-N1	5′-CATCAATTCACGCTGGTCGGATGCCATTGC-3′
FA-N2	5′-GAAGGTTCTGCGGAAAACCGTGGAG-3′
FA-C1	5′-TTGGTGTTTTCCGGTTCTGCCGGCCTGATG-3′
FA-C2	5′-GGCGAACATGCGTTTCGCATCGCTG-3′
FA-ON1	5′-GCGTAATACGACTCACTATAGGGAG-3′
FA-OC1	5′-ACGCATGACGCGCGCCGCGGCCAAT-3′

R = A or G; Y = C or T; M = A or C; N = A, T, C, or G.

2.7. Nucleotide sequencing of the gene

All the primers used for the amplification of the FAc-DEX FA1 gene are listed in Table 1. The genomic DNA of *Burkholderia* sp. FA1 was isolated with QIAGEN Genomic-tip (Tokyo, Japan). The DNA fragment coding for a part of FAc-DEX FA1 was amplified by PCR. The reaction mixture (50 μl) contained 10 mM Tris–HCl (pH 8.3), 50 mM potassium chloride, 2.0 mM magnesium chloride, 200 μM of each dNTP, 1.0 μM of each primer (FA-1 and -8, the degenerate primers coding for the N-terminal and internal amino acid sequences of the enzyme), 2.5 units Ex Taq DNA polymerase, and 500 ng of template DNA. The reaction conditions were as follows: 30 s (94 °C), 30 s (40 °C), and 1 min (72 °C), for 30 cycles. The DNA fragment of about 800 bp was amplified and purified by agarose gel electrophoresis for direct DNA sequencing. DNA sequencing was performed with a PE Biosystems ABI PRISM 310 Genetic Analyzer with a dye-labeled terminator sequencing kit. The nucleotide sequence determined was used for designing the primers, FA-N1, FA-N2, FA-C1, and FA-C2.

The DNA fragments coding for the N-terminal and C-terminal regions were amplified with a TaKaRa PCR in vitro Cloning Kit. The *Sau*3AI cassette, FA-N1, and FA-N2 were used to obtain the DNA fragment coding for the N-terminal region. The *Sau*3AI cassette, FA-C1, and FA-C2 were used to obtain the DNA fragment encoding the C-terminal region. The nucleotide sequences of the amplified DNA fragments were determined and used for designing the primers FA-ON1 and FA-OC1.

The DNA fragment covering the entire FAc-DEX FA1 gene was obtained by PCR as follows. The reaction mixture (50 μl) contained 10 mM Tris–HCl (pH 8.3), 50 mM potassium chloride, 2.5 mM magnesium chloride, 200 μM of each dNTP, 0.2 μM of each primer (FA-ON1 and FA-OC1), 2.5 units of La Taq DNA polymerase (Takara, Kyoto, Japan), and 100 ng of genomic DNA of *Burkholderia* sp. FA1. The reaction conditions were as follows: 30 s (94 °C), 30 s (55 °C), and 3 min (72 °C), for 30 cycles. The amplified DNA fragment of about 1.1 kbp was purified by agarose gel electrophoresis and used for direct DNA sequencing.

The sequences of FAc-DEX H1 and haloalkane dehalogenase from *X. autotrophicus* GJ10 (DhlA) were aligned as described previously [27]. The FAc-DEX FA1 sequence was aligned with these sequences with the program MegAlign (DNASTAR Inc., Madison, WI) by the Clustal method.

3. Results

3.1. Isolation of fluoroacetate-assimilating bacterium

A bacterial strain utilizing fluoroacetate as the sole carbon source was isolated from garden soil of Uji, Kyoto, Japan. The partial nucleotide sequence of 16S rDNA of this bacterium showed the highest similarity to those of the bacteria in the genus *Burkholderia*, especially to that of *Burkholderia glathei* (98% identity). Accordingly, we named the bacterium *Burkholderia* sp. FA1. The crude extract of this bacterium showed fluoroacetate dehalogenase activity when it was cultivated in the standard medium containing fluoroacetate. No fluoroacetate dehalogenase activity was found when the cells were grown in the LB medium, suggesting that the enzyme is inducibly synthesized in the presence of fluoroacetate in the cultivation medium.

3.2. Characterization of fluoroacetate dehalogenase from Burkholderia sp. FA1

Fluoroacetate dehalogenase was purified 130-fold with a 41% yield from the crude extract of *Burkholderia* sp. FA1 (Table 2). The final preparation showed

Table 2
Purification of FAc-DEX FA1

Step	Total activity (units)	Total protein (mg)	Specific activity (units/mg)	Purification (fold)	Yield (%)
Crude extract	27	380	0.071	1	100
Ammonium sulfate	22	190	0.12	1.7	81
Butyl-Toyopearl	27	38	0.71	10	100
DEAE-Toyopearl	11	1.2	9.2	130	41

The enzyme was purified as described in Section 2. The dehalogenase activities were determined by measuring the glycolate ions released from 20 mM fluoroacetate.

a single major protein band on SDS-polyacrylamide gel electrophoresis (Fig. 2). The specific activity of the purified enzyme was 9.2 units/mg. The molecular weights of the enzyme determined by gel filtration and SDS-polyacrylamide gel electrophoresis were about 79,000 and about 34,000, respectively, suggesting that the enzyme is composed of two identical subunits. The enzyme had a maximum activity at pH 9.5 (Fig. 3). With fluoroacetate as the substrate, the enzyme followed the Michaelis–Menten kinetics, and the K_m and V_{max} values were determined to be 5.1 mM and 11 units/mg, respectively. The substrate specificity of the enzyme is shown in Fig. 4. The enzyme was specific to haloacetates, and fluoroacetate was the best substrate for FAc-DEX FA1. Although fluoroacetate served as the best substrate also for FAc-DEX H1 [21], FAc-DEX FA1 was more specific to fluoroacetate than FAc-DEX H1: the activities of FAc-DEX H1 toward chloroacetate and bromoacetate were 21 and 13% of that toward fluoroacetate, respectively, whereas the activities of FAc-DEX FA1 toward chloroacetate and bromoacetate were 3.9 and 3.4% of that toward fluoroacetate, respectively.

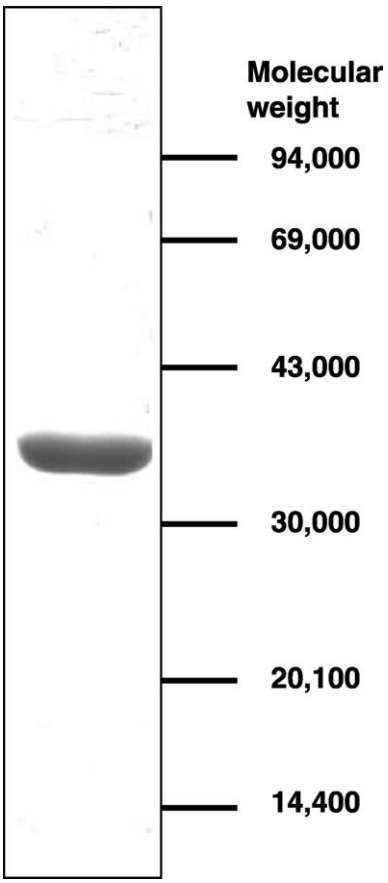

Fig. 2. SDS-polyacrylamide gel electrophoresis of FAc-DEX FA1. The positions of molecular weight markers are indicated.

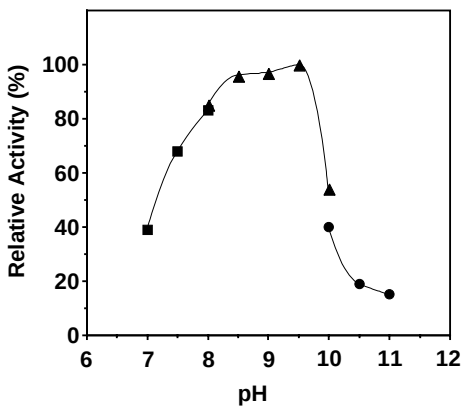

Fig. 3. The effect of pH on the enzyme activity. The activity of FAc-DEX FA1 was measured with a 100 mM potassium phosphate buffer (pH 7–8, filled squares), a 100 mM Tris–sulfate buffer (pH 8–10, filled triangles), and a 100 mM glycine–sodium hydroxide buffer (pH 10–11, filled circles).

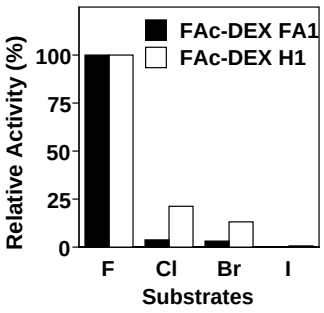

Fig. 4. Comparison of the substrate specificities of FAc-DEX FA1 and FAc-DEX H1. The activities of FAc-DEX FA1 (filled bar) and FAc-DEX H1 (open bar) toward fluoroacetate (F), chloroacetate (Cl), bromoacetate (Br), and iodoacetate (I) are shown. The activities were determined by measuring the amount of glycolate ions produced. The following substrates were inert for both enzymes: difluoroacetate, trifluoroacetate, and DL-2-chloropropionate.

3.3. Primary structure of fluoroacetate dehalogenase from Burkholderia sp. FA1

The N-terminal and internal amino acid sequences of FAc-DEX FA1 were determined as described in Section 2 (Table 3), and the oligonucleotide primers for isolation of the FAc-DEX FA1 gene were designed based on these sequences (Table 1). The gene was isolated as described in Section 2, and the nucleotide sequence was determined (Fig. 5). All the peptide sequences shown in Table 3 were found in the deduced amino acid sequence (Fig. 5). The calculated molecular weight of the gene product (34,082) was in good agreement with the value estimated by SDS-polyacrylamide gel electrophoresis of the purified enzyme. FAc-DEX FA1 shares 61% sequence identity with FAc-DEX H1 [20] and 18% sequence identity with DhlA [28] (Fig. 6). The catalytically essential residues of FAc-DEX H1, Asp105 and His272, were conserved in FAc-DEX FA1 as Asp104 and His271, respectively.

Table 3
The N-terminal and internal amino acid sequences of FAc-DEX FA1

Position	Sequence
N-terminal	MFEGFERRLVDVGDVTINCVVGGS
Internal-1	FASLPGGHFFVDRFPDDTAR
Internal-2	AYWHWYFLQQPAPYPEK
Internal-3	VAPLLANEYTVVCADLR
Internal-4	VIGADPDTFYEGCLF

4. Discussion

We have isolated a soil bacterium that effectively assimilates fluoroacetate as a carbon source. The strain, *Burkholderia* sp. FA1, inducibly produced fluoroacetate dehalogenase named FAc-DEX FA1 when grown on fluoroacetate. FAc-DEX FA1 was specific to haloacetates: 2-chloropropionate did not serve as the substrate (Fig. 4). Among different haloacetates, the enzyme showed the highest activity toward fluoroacetate although the dissociation energy of the carbon–fluorine bond is much higher than those of the carbon–chlorine, carbon–bromine, and carbon–iodine bonds. The bulkiness of the chlorine, bromine, and iodine atoms probably prevents the substrates containing these atoms from binding to the active site of the enzyme.

FAc-DEX FA1 is similar to FAc-DEX H1 [21] in several respects. Both of them are specific toward haloacetates, and fluoroacetate is the best substrate. Both enzymes show a maximum activity at pH 9.5. Moreover, their primary structures are highly similar to each other: 61% of the amino acid residues are identical. Nevertheless, FAc-DEX FA1 differs from FAc-DEX H1 in its substrate specificity. Fig. 4 shows that FAc-DEX FA1 is more specific to fluoroacetate than FAc-DEX H1. Perhaps the accessibility to the active site of FAc-DEX FA1 is more restricted than that to the active site of FAc-DEX H1, but this should be verified by structural analysis of these enzymes.

We have previously shown that Asp105 and His272 of FAc-DEX H1 function as the catalytic nucleophile and the catalytic base, respectively [21]. These residues are conserved in FAc-DEX FA1 as Asp104 and His271. Homology modeling of the three-dimensional structure of FAc-DEX H1, based on the crystal structure of DhlA, suggested that the active site of FAc-DEX H1 is mainly composed of hydrophobic and basic amino acid residues: Phe35, His104, Arg106, Tyr148, Trp151, Tyr213, Gln248, and Phe273 were found in the vicinity of Asp105 and His272 [27]. All these residues, except for Gln248, are conserved in FAc-DEX FA1. Gln248 is replaced by Ser247 in FAc-DEX FA1. It would be interesting

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   1    ATGTTTGAAGGATTCGAGCGACGCCTTGTCGATGTCGGTGACGTAACGATCAACTGCGTC   60
   1    MetPheGluGlyPheGluArgArgLeuValAspValGlyAspValThrIleAsnCysVal   20

  61    GTAGGCGGTAGCGGTCCGGCGCTTCTATTGCTCCACGGTTTTCCGCAGAACCTTCACATG  120
  21    ValGlyGlySerGlyProAlaLeuLeuLeuLeuHisGlyPheProGlnAsnLeuHisMet   40

 121    TGGGCCCGCGTCGCCCCCTTGCTCGCAAACGAGTACACCGTCGTCTGTGCGGATCTGCGG  180
  41    TrpAlaArgValAlaProLeuLeuAlaAsnGluTyrThrValValCysAlaAspLeuArg   60
              3

 181    GGCTACGGCGGTTCGTCGAAGCCGGTGGGCGCCCCCGACCATGCCAACTACTCCTTTCGT  240
  61    GlyTyrGlyGlySerSerLysProValGlyAlaProAspHisAlaAsnTyrSerPheArg   80

 241    GCAATGGCATCCGACCAGCGTGAATTGATGCGAACGCTGGGGTTCGAGCGGTTTCACCTG  300
  81    AlaMetAlaSerAspGlnArgGluLeuMetArgThrLeuGlyPheGluArgPheHisLeu  100

 301    GTCGGACACGATCGGGGCGGAAGAACGGGGCATCGCATGGCGCTGGACCACCCCGATAGC  360
 101    ValGlyHisAspArgGlyGlyArgThrGlyHisArgMetAlaLeuAspHisProAspSer  120

 361    GTGCTCTCGCTCGCCGTTCTGGACATCATCCCAACCTATGTGATGTTCGAAGAAGTCGAC  420
 121    ValLeuSerLeuAlaValLeuAspIleIleProThrTyrValMetPheGluGluValAsp  140

 421    CGTTTCGTGGCACGGGCCTACTGGCATTGGTATTTTCTTCAGCAGCCTGCGCCTTATCCC  480
 141    ArgPheValAlaArgAlaTyrTrpHisTrpTyrPheLeuGlnGlnProAlaProTyrPro  160
                      2

 481    GAGAAGGTCATTGGCGCCGATCCCGACACCTTTTACGAAGGCTGTCTCTTCGGCTGGGGC  540
 161    GluLysValIleGlyAlaAspProAspThrPheTyrGluGlyCysLeuPheGlyTrpGly  180
            4

 541    GCGACGGGTGCTGATGGCTTCGACCCCGAGCAACTGGAAGAGTATCGAAAACAATGGCGC  600
 181    AlaThrGlyAlaAspGlyPheAspProGluGlnLeuGluGluTyrArgLysGlnTrpArg  200

 601    GATCCCGCAGCAATTCACGGCAGTTGCTGCGACTACCGGGCCGGCGGGACGATCGACTTC  660
 201    AspProAlaAlaIleHisGlySerCysCysAspTyrArgAlaGlyGlyThrIleAspPhe  220

 661    GAACTCGACCACGGGGACCTGGGCCGGCAGGTCCAGTGCCCTGCCTTGGTGTTTTCCGGT  720
 221    GluLeuAspHisGlyAspLeuGlyArgGlnValGlnCysProAlaLeuValPheSerGly  240

 721    TCTGCCGGCCTGATGCACTCTCTCTTCGAGATGCAGGTCGTCTGGGCGCCGCGACTGGCG  780
 241    SerAlaGlyLeuMetHisSerLeuPheGluMetGlnValValTrpAlaProArgLeuAla  260

 781    AACATGCGTTTCGCATCGCTGCCGGGTGGGCACTTCTTCGTCGATCGGTTCCCCGACGAC  840
 261    AsnMetArgPheAlaSerLeuProGlyGlyHisPhePheValAspArgPheProAspAsp  280
              1

 841    ACGGCACGCATCCTTCGCGAGTTTCTAAGTGATGCGCGCAGCGGTATCCAGCAGACCGAG  900
 281    ThrAlaArgIleLeuArgGluPheLeuSerAspAlaArgSerGlyIleGlnGlnThrGlu  300

 901    CGAAGAGAATCATGA                                               915
 301    ArgArgGluSer***                                               304
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Fig. 5. Nucleotide sequence of the FAc-DEX FA1 gene and deduced amino acid sequence. The underlined amino acid sequences are identical to those determined with the purified enzyme (Table 3). The numbers assigned for the internal sequences correspond to those in Table 3.

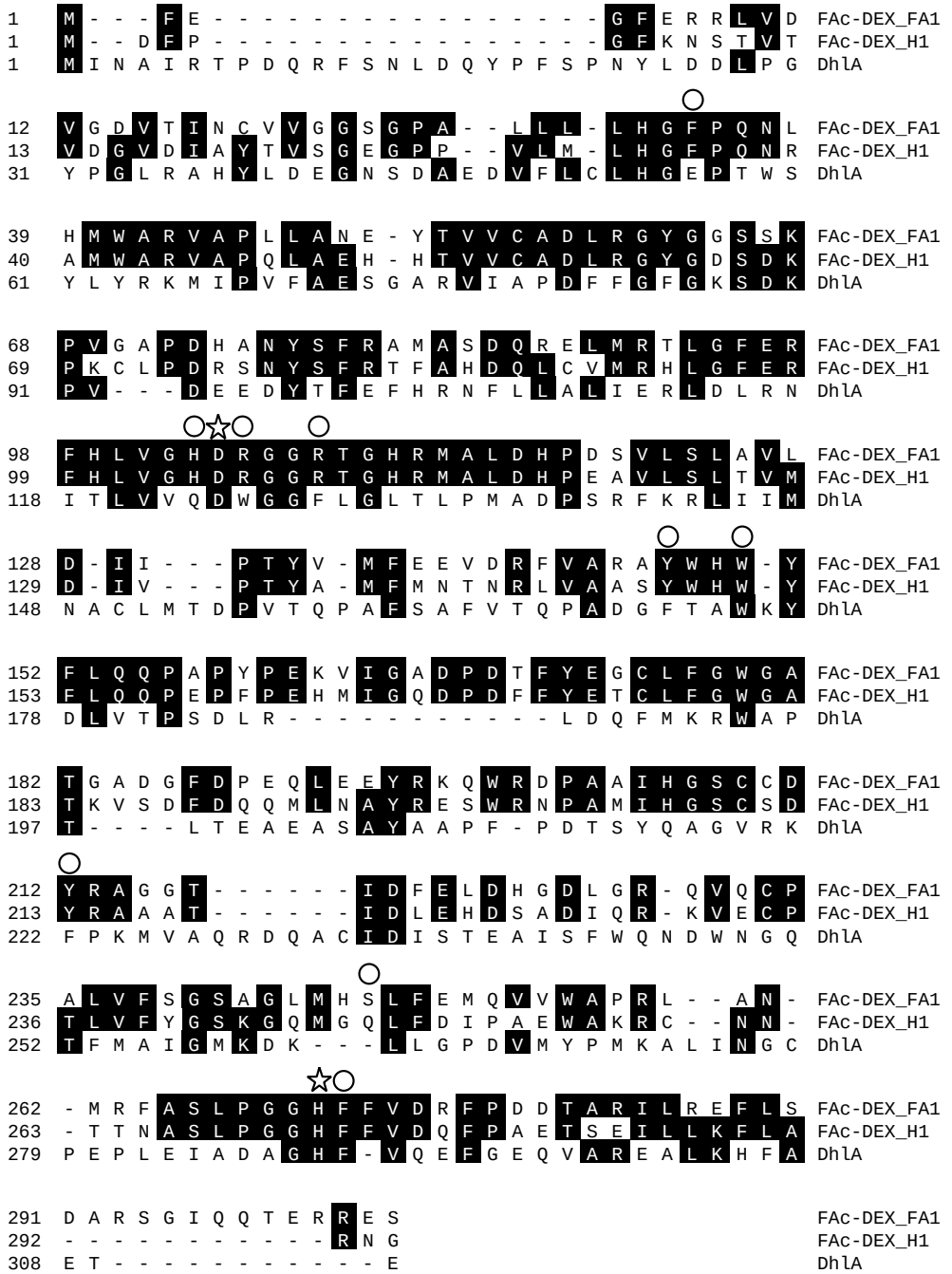

Fig. 6. Sequence alignment of FAc-DEX FA1, FAc-DEX H1, and DhlA. The conserved residues are shown in the black boxes. The stars indicate the catalytic nucleophiles and the catalytic bases. The circles indicate the residues found in the active site by crystallographic analysis (DhlA) [12] and the residues supposed to be present in the active site based on the homology modeling of the three-dimensional structures (FAc-DEX H1 and FAc-DEX FA1) [27].

to see whether this substitution is related to the different substrate specificities of these enzymes.

The crystallographic analysis of DhlA revealed that Phe128 and Phe172 interact with the Cl_2 atom of 1,2-dichloroethane (Cl_1 is the atom released from the substrate by DhlA, and the Cl_2 atom remains on the substrate) [12]. The positions corresponding to these residues are occupied by Arg and Tyr, respectively, in FAc-DEX FA1 and FAc-DEX H1. These Arg and Tyr residues may be important for the recognition of the carboxylate group of fluoroacetate.

Trp125 and Trp175 of DhlA are the residues that accept the halide ion released from the substrate [12]. Although Trp175 is not conserved among different types of haloalkane dehalogenases, Trp125 is completely conserved [29]. Interestingly, Trp125 is replaced by Arg in FAc-DEX FA1 and FAc-DEX H1. Arg105 of FAc-DEX FA1 and Arg106 of FAc-DEX H1 probably play an essential role in abstracting the fluorine atom of fluoroacetate.

More detailed structural and functional analyses of FAc-DEX FA1, FAc-DEX H1, and other dehalogenases would clarify what structural elements determine the substrate specificity of these enzymes, and such information may enable us to modify the substrate specificity of dehalogenases to make them more useful in production of various chemicals from organohalogen compounds and bioremediation of environments contaminated with organohalogen pollutants.

Acknowledgements

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