Purification and Characterization of 4-Chlorobenzoyl CoA Dehalogenase from *Arthrobacter* sp. Strain 4-CB1†

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ABSTRACT: 4-Chlorobenzoyl coenzyme A dehalogenase was purified to homogeneity from *Arthrobacter* sp. strain 4-CB1 (previously known as *Acinetobacter* sp. strain 4-CB1), a bacterium isolated from PCB-contaminated soil. Purification was accomplished by four chromatographic steps, including a novel affinity chromatography step. 4-Chlorobenzoyl CoA dehalogenase is a homotetramer of 33-kDa subunits with an isoelectric point of 6.1. The enzyme is stable between pH 6.5 and 10. The optimum pH for k_{cat} is pH 8. The enzyme is able to dehalogenate substrates bearing fluorine, chlorine, bromine and iodine in the 4-position, although the rate of dehalogenation of 4-fluorobenzoyl CoA is quite slow. The enzyme is specific for dehalogenation at the 4-position, as 3-chloro- and 2-chlorobenzoyl CoA are not dehalogenated. The N-terminal sequence of the *Arthrobacter* sp. strain 4-CB1 dehalogenase is almost identical to that of the 4-chlorobenzoyl CoA dehalogenase from *Arthrobacter* sp. strain SU and shows 30% identity to that from *Pseudomonas* sp. strain CBS-3.

Halogenated aromatic compounds such as PCBs, pentachlorophenol, and various herbicides and pesticides have been introduced into the environment in enormous quantities over the past forty years. Many of these compounds are highly resistant to both biotic and abiotic degradation and therefore tend to accumulate in the environment. Resistance to biodegradation is largely due to the presence of halogen substituents. Although many microorganisms can degrade aromatic compounds, relatively few can degrade halogenated aromatic compounds.

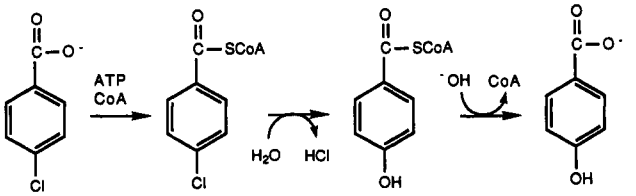
In recent years, a number of microorganisms that can degrade halogenated aromatic compounds have been isolated from contaminated soils. *Arthrobacter* sp. strain 4-CB1 (previously known as *Acinetobacter* sp. strain 4-CB1) was isolated from PCB-contaminated soil by selection for growth on 4-chlorobenzoate as a sole carbon source (Adriaens et al., 1989). (4-Chlorobenzoate is a breakdown product of some PCB congeners.) The pathway for degradation of 4-chlorobenzoate in this microorganism is shown in Scheme 1 (Copley & Crooks, 1992).

We are particularly interested in the mechanism and evolutionary origin of 4-chlorobenzoyl CoA dehalogenase, the enzyme that catalyzes the replacement of the chlorine substituent of 4-chlorobenzoyl CoA (4CBCoA) with a hydroxyl group. Here we report the purification and characterization of this enzyme from *Arthrobacter* sp. strain 4-CB1 and a comparison of this enzyme with corresponding enzymes from other microorganisms.

MATERIALS AND METHODS

General. Arthrobacter sp. strain 4-CB1 was a gift from P. Adriaens and D. Focht (Department of Soil and Environmental Sciences, University of California, Riverside). Coenzyme A (trilithium salt) was purchased from US Biochemicals.

Scheme 1



Substituted benzoyl chlorides, ω-aminodecyl-agarose, 6-aminocaproic acid, 4-chlorobenzoate, Chelex chelating resin, and Reactive Green 19 dye ligand column material were purchased from Sigma Chemicals. Bovine γ-globulin for protein concentration determination and molecular weight standards for gel filtration and SDS–polyacrylamide gel electrophoresis (PAGE) were purchased from Bio-Rad. Fractogel EMD TMAE 650 (S) anion-exchange resin was purchased from EM Separations. The Superdex 200 gel filtration column was purchased from Pharmacia. 4-(2-Aminoethyl)benzenesulfonyl fluoride–HCl (AEBSF) and leupeptin protease inhibitors were purchased from Calbiochem Corp. All chemicals were used as received without further purification.

4-Halobenzoyl CoA substrates were synthesized as described previously (Crooks & Copley, 1993). Synthesis of 6-[*p*-(trifluoromethyl)benzamido]caproic acid (the ligand used to elute the affinity column) was accomplished by combining 4-(trifluoromethyl)benzoyl chloride in acetonitrile with a stoichiometric amount of 6-aminocaproic acid in water. The pH was maintained at 8.0 by addition of triethylamine. After stirring overnight at room temperature, the solvents were removed under reduced pressure. The product was sometimes purified by reverse-phase HPLC using a Vydac C-18 column, although further purification proved to be unnecessary for use in eluting the affinity column. ^{1}H NMR (400 MHz; in D_2O) δ 1.2–1.3 (2H, m, CH_2), 1.4–1.5 (4H, m, CH_2), 2.0 (2H, t, J = 7.4 Hz, CH_2), 3.2 (2H, t, J = 6.9 Hz, CH_2), 7.65 and 7.69 (4H, apparent AB pattern, J_{ab} = 8.4 Hz, aromatic protons).

Growth Conditions. Arthrobacter sp. strain 4-CB1 was grown at 30 °C in minimal media containing 4-chlorobenzoate

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as the sole carbon source. Growth conditions have been described previously (Adriaens et al., 1989).

Purification of 4-CBCoA Dehalogenase. Cells from 6 L of growth medium were harvested by centrifugation at 6000*g* at 4 °C for 20 min. The supernatant was decanted and the pellet resuspended in disruption buffer [50 mM MOPS, pH 7.2, containing 5 mM dithiothreitol (DTT), 10 mM KCl, 0.5 mM AEBSF, 1 mM EDTA, 50 μM leupeptin, and 10 μg/mL DNase I]. Resuspended cells were disrupted by three passages through a French pressure cell at 20000 psi. Debris and unbroken cells were removed by centrifugation at 6000*g* for 20 min. The supernatant was decanted and saved. The pellet was resuspended in a small volume of disruption buffer and passed through the French pressure cell twice at 20000 psi. This solution was combined with the first supernatant and subjected to centrifugation at 35000*g* for 25 min. The supernatant was loaded onto a 10-mL Reactive Green 19 dye column pre-equilibrated with 50 mM MOPS, pH 6.9, containing 2 mM DTT. The column was washed with 50 mL of the equilibration buffer. 4-CBCoA dehalogenase flowed through the dye column, while 4-hydroxybenzoyl CoA thioesterase, which removes CoA from the product of the dehalogenation reaction, bound tightly. (Removal of the thioesterase is helpful for accurate measurement of dehalogenase activity.) Fractions containing dehalogenase activity were combined and loaded onto a 15-mL Fractogel EMD-TMAE 650 (S) strong anion-exchange column pre-equilibrated with 50 mM MOPS, pH 6.9, containing 2 mM DTT. The column was washed with 75 mL of the equilibration buffer. Proteins were eluted with a 200-mL linear gradient of 0–0.5 M KCl in the equilibration buffer, followed by an additional 50 mL of buffer containing 0.5 M KCl. Fractions containing dehalogenase activity were pooled and loaded onto an affinity column (described below) pre-equilibrated with 50 mM MOPS, pH 7.6, containing 15% glycerol and 2 mM DTT. The column was washed with the same buffer containing 250 mM KCl. 4-CBCoA dehalogenase was eluted with 25 mM 6-[*p*-(trifluoromethyl)benzamido]caproic acid in 50 mM Tris-HCl, pH 8.0, containing 15% glycerol, 250 mM KCl, and 2 mM DTT. Fractions containing dehalogenase activity were pooled and concentrated in an Amicon stirred cell equipped with a YM-10 membrane to a final volume of approximately 2 mL. The enzyme solution was cleared by ultracentrifugation and loaded onto a Superdex 200 gel filtration column equilibrated with 50 mM MOPS, pH 7.2, containing 2 mM DTT, 150 mM KCl, and 10% glycerol. Fractions containing dehalogenase activity were combined and concentrated to approximately 2 mL. Additional glycerol was added to give a final concentration of 30%, and the solution was stored at −20 °C in siliconized tubes.

Preparation of Affinity Column. 4-(Trifluoromethyl)-benzoyl chloride (200 μmol) in 50 mL of acetonitrile was slowly added to 10 mL of ω-aminodecyl-agarose resin (containing 10–20 μmol of functional groups/mL) in 50 mL of aqueous 0.1 M $KHCO_3$. The solution was gently shaken at room temperature and the amount of 4-(trifluoromethyl)-benzoyl chloride in solution was measured spectrophotometrically until no further uptake was observed. Using this procedure, 182 μmol of 4-(trifluoromethyl)benzoyl chloride bound to the resin (91% of the theoretical maximum).

Enzyme Assays. (1) Continuous UV Assay. Assays for dehalogenase activity were carried out in a 3-mL quartz cuvette equipped with a stir bar. Filtered phosphate buffer (20 mM final concentration, pH 7.2) and substrate were allowed to equilibrate at 30 °C. Reactions were initiated by addition of enzyme solution, and the change in absorbance at 300 nm was monitored over time. The $\Delta\epsilon_{300}$ for conversion of 4-CBCoA to 4-hydroxybenzoyl CoA at pH 7.2 is 8.5 $mM^{-1}cm^{-1}$.

(2) Stopped-Time Assay. A stopped-time assay was developed to allow accurate kinetic measurements in the presence of high concentrations of inhibitors or poor substrates and to provide a rapid method for screening column fractions for dehalogenase activity. The assay is based upon the absorbance of the phenolate form of 4-hydroxybenzoyl CoA at pH > 11 (λ_{max} = 330 nm, ϵ_{330} = 30.1 $mM^{-1}cm^{-1}$). (4-Halobenzoyl CoA substrates have no significant absorbance at 330 nm at pH > 11.) For determination of kinetic parameters, the assay was carried out in a final volume of 2.1 mL containing 20 mM phosphate buffer, pH 7.2 at 30 °C. After addition of enzyme solution, 500-μL aliquots were removed at intervals and added to tubes containing 900 μL of 20 mM NaOH. This concentration of hydroxide is sufficient to irreversibly inactivate the enzyme and convert the product to its highly stable phenolate form. An additional 400 μL of 10 mM NaOH were added to bring the final volume to 1.8 mL, and the absorbance at 330 nm was measured. The continuous and stopped-time assays agree to within 5%.

To screen column fractions for dehalogenase activity, the reaction was carried out in a final volume of 250 μL. After incubation for 10–20 min at 30 °C, reactions were quenched by addition of 250 μL of 20 mM NaOH to each tube, and the absorbance at 330 nm was measured.

Determination of Kinetic Parameters. Stopped-time assays were carried out as described above over a range of substrate concentrations from 4 to 400 μM. Kinetic parameters were obtained using the Enzfitter program (Sigma). Protein concentrations were determined using the Bradford assay (Bradford, 1976). Bovine γ-globulin was used as the standard.

Determination of pH Stability. Purified enzyme was incubated at 4 °C in buffers ranging in pH from 4 to 10. All buffers contained 2 mM DTT. After 4 h, phosphate buffer was added to neutralize each solution. The solutions were allowed to equilibrate at pH 7.2 for 15 min at 4 °C before assaying for activity using the continuous assay described above.

Determination of Optimum pH for Dehalogenase Reaction. Assays for dehalogenase activity were carried out in buffers ranging in pH from 6.61–9.60. The buffers used were 409 mM MOPS, pH 6.61; 178 mM MOPS, pH 7.20; 120 mM MOPS, pH 7.82; 319 mM Tricine, pH 7.70; 169 mM Tricine, pH 8.20; 117 mM Tricine, pH 8.82; 309 mM borate, pH 8.80; and 133 mM borate, pH 9.60. All of these buffers have an ionic strength of 0.1 M. The continuous assay for dehalogenase activity was used. Values for $\Delta\epsilon_{300}$ at each pH value used were as follows: pH 6.61, 8.8 mM^{-1} cm^{-1}; pH 7.20, 8.5 mM^{-1} cm^{-1}; pH 7.70, 7.9 mM^{-1} cm^{-1}, pH 7.82, 8.2 mM^{-1} cm^{-1}; pH 8.20, 7.6 mM^{-1} cm^{-1}; pH 8.82, 6.8 mM^{-1} cm^{-1}; pH 9.60, 6.3 mM^{-1} cm^{-1}.

Determination of Subunit and Holoenzyme Molecular Weights. The subunit molecular weight of the enzyme was determined by SDS–PAGE using a 5% stacking gel and a 10% separating gel. Protein standards used were phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa). The native molecular weight of the enzyme was calculated from the retention time of the peak of dehalogenase activity eluted from a Superdex 200 size exclusion column. Protein standards included thyroglobulin (670 kDa), γ-globulin (158 kDa),

Table 1: Purification of 4-Chlorobenzoyl CoA Dehalogenase from 11 g of Wet Cell Paste

step	protein (mg)	activity (units)	sp act. (units/mg)	yield (%)	purification (*x*-fold)
crude extract	418	58.9	0.14	100	1.0
Green Dye	220	61.9	0.28	105	2.0
anion exchange	134	53.7	0.40	91	2.9
affinity[a]		14.0		24	
gel filtration	12.8	12.9	1.01	22	7.2

[a] The protein concentration cannot be measured after the affinity chromatography step because the ligand used to elute the column interferes with the Bradford assay.

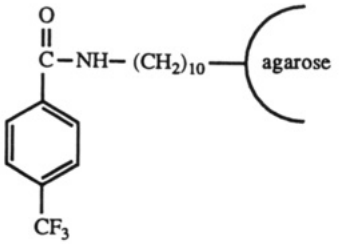

FIGURE 1: Schematic diagram of the affinity column material used to purify 4-CBCoA dehalogenase.

ovalbumin (44 kDa), myoglobin (17 kDa), and vitamin B_{12} (1.35 kDa).

Determination of N-Terminal Sequence. N-Terminal sequencing was conducted by the University of Wisconsin Biotechnology Center using an automated Model 475A gas phase sequencer with on-line Model 120A PTH analyzer and Model 610A data collection and analysis system (Applied Biosystems, Foster City, CA). The purified protein was subjected to 24 rounds of Edman degradation twice with identical results.

Analysis of Membrane Fatty Acids. The microorganism used for these studies was originally identified as *Acinetobacter* sp. 4-CB1 (Adriaens et al., 1989). *Acinetobacter* is a common Gram-negative soil microorganism. In order to confirm this identification, the microorganism was submitted to Microbial ID, Inc. (Newark, DE), for analysis of the membrane fatty acids. The analysis showed that 88% of the membrane fatty acids were branched and that no 3-OH fatty acids were present. The membrane fatty acid profile was consistent with *Arthrobacter* sp., a Gram-positive microorganism. The fatty acid composition of Gram-negative membranes is quite different. The fatty acids are almost exclusively straight chain, rather than branched, and always contain a hydroxyl group at the 3-position (Myron Sasser, MIDI, Newark, DE, personal communication). Thus, this microorganism has been reassigned as *Arthrobacter* sp. 4-CB1.

RESULTS AND DISCUSSION

Enzyme Purification. 4-CBCoA dehalogenase was purified from 11 grams of cell paste with an overall yield of 12.8 mg (see Table 1). The purification procedure involved initial chromatography on a Green Dye 19 column to remove 4-hydroxybenzoyl CoA thioesterase (which interferes with the accurate quantitation of dehalogenase activity), followed by anion exchange chromatography. The most important step in the purification was affinity chromatography using a column bearing an immobilized (trifluoromethyl)benzamide moiety (see Figure 1). This ligand was chosen because the trifluoromethyl group closely mimics bromine both in size and electron-withdrawing properties, but it cannot be displaced from the aromatic ring by the enzyme. An amide linkage was chosen rather than a thioester linkage because of its greater

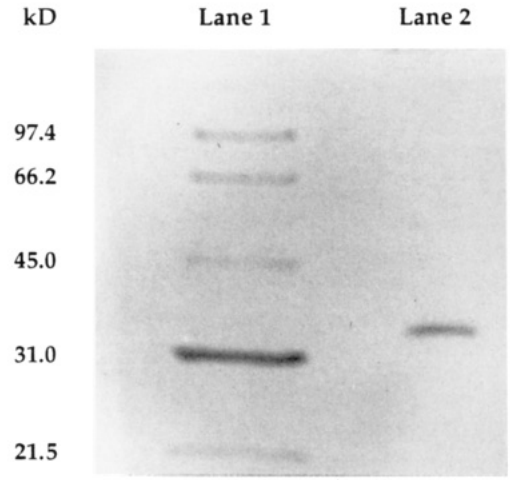

FIGURE 2: SDS–polyacrylamide gel of purified 4-CBCoA dehalogenase. Lane 1, protein mass markers. Lane 2, 4-CBCoA dehalogenase. Molecular masses of markers are indicated on the left.

stability in the presence of DTT and at slightly alkaline pH. 4-CBCoA dehalogenase bound to the affinity column and was eluted with 6-[*p*-(trifluoromethyl)benzamido]caproic acid. 6-[*p*-(Trifluoromethyl)benzamido]caproic acid and the remaining small amounts of protein contaminants were removed by size exclusion chromatography using a Superdex 200 column. The enzyme was homogeneous by SDS–PAGE (see Figure 2) and isoelectric focusing (data not shown).

Maintenance of enzymatic activity both during and after purification was critically dependent on the presence of DTT. In the absence of DTT, the enzyme quickly loses activity and Lineweaver–Burk plots of $1/v$ vs $1/[S]$ become nonlinear, suggesting formation of a mixed population containing some damaged enzyme.

Physical Characterization of 4-CBCoA Dehalogenase. Purified 4-CBCoA dehalogenase migrated as a single band on SDS–PAGE with an apparent subunit molecular mass of 33 kDa (see Figure 2). The native molecular mass was determined by gel filtration to be 116 kDa. Therefore, the native enzyme is apparently a homotetramer. For purposes of comparison, the *Pseudomonas* sp. strain CBS-3 dehalogenase is a homotetramer of 30-kDa subunits (Babbitt et al., 1992), and the subunit molecular mass of the *Arthrobacter* sp. strain SU enzyme is 30 kDa (based upon the size of the open reading frame assigned to the dehalogenase enzyme) (Schmitz et al., 1992).

The isoelectric point of the native enzyme was determined by isoelectric focusing to be 6.1.

In preparation for determination of the pH profile for the enzyme, we explored the stability of the enzyme at pH values between 6.0 and 10.0. The enzyme was incubated for 4 h at 4 °C in buffers ranging in pH from 6 to 10. The solutions were neutralized and allowed to equilibrate at pH 7.2 for 15 min at 4 °C before assaying for activity. The enzyme retained full activity after incubation in buffers ranging in pH from 6.5 to 10.0. However, below pH 6.5, the enzyme was irreversibly damaged. Enzyme treated at pH 6.0 had only 40% of its original activity even after reequilibration at pH 7.2 (data not shown).

Profiles for log $k_{cat,rel}$ and log $(k_{cat}/K_M)_{rel}$ as a function of pH are shown in Figure 3. The accessible pH range does not allow determination of the full pH profiles, due to the inactivation of the enzyme below pH 6.5 and to the rapid nonenzymatic hydrolysis of the substrate above pH 10. Log-$(k_{cat}/K_M)_{rel}$ increases only slightly with pH. The slope of the best-fit line to the data shown in Figure 3a is only 0.1. The

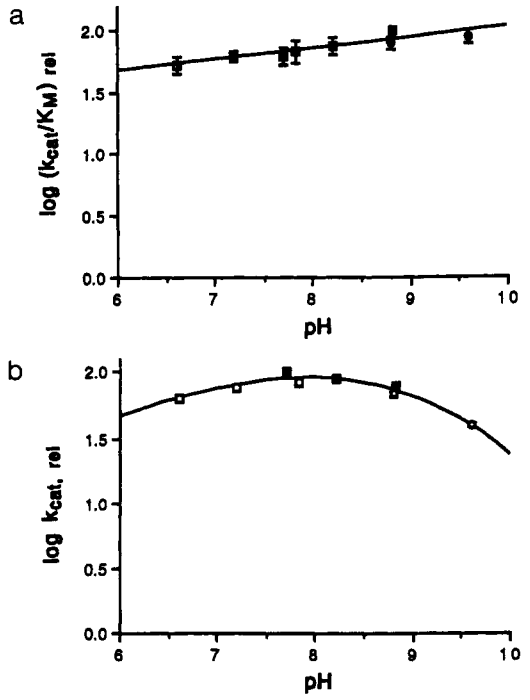

FIGURE 3: Profiles for log $k_{cat,rel}$ (a) and log $(k_{cat}/K_M)_{rel}$ (b) as a function of pH. The buffers used were (□) MOPS; (■) tricine; and (○) borate. Error bars for some points were smaller than the symbols used in the graph.

Table 2: Kinetic Parameters for Dehalogenation of 4-X-benzoyl CoA Substrates

X	K_M (μM)	k_{cat} (s^{-1})
I	14 ± 2	2.00 ± 0.09
Br	36 ± 3	2.40 ± 0.10
Cl	34 ± 3	1.30 ± 0.05
F	75 ± 5	0.0030 ± 0.0001

profile for log $k_{cat,rel}$ (see Figure 3b) shows some curvature, but the accessible pH range is too limited to conclude that the profile is bell-shaped. The pH optimum for k_{cat} occurs at pH 8.0.

Substrate Specificity. We have previously reported that 4-CBCoA dehalogenase from *Arthrobacter* sp. strain 4-CB1 catalyzes the dehalogenation of 4-fluoro- and 4-bromobenzoyl CoA in addition to that of 4-CBCoA (Crooks & Copley, 1993). Recently, we investigated the ability of the enzyme to dehalogenate 4-iodobenzoyl CoA and found the kinetic parameters to be very similar to those found for the 4-chloro and 4-bromo substrates. The kinetic parameters for the four substrates are summarized in Table 2.

There are two additional interesting aspects to the kinetic data presented in Table 2. First, the k_{cat} for dehalogenation of 4-CBCoA is only 1 s^{-1}. This value is quite low compared to that of most metabolic enzymes and is especially striking because all carbon for cell growth on 4-chlorobenzoate (the sole carbon source in the standard growth medium) must pass through this enzyme. Cells grown on 4-chlorobenzoate obviously compensate for the low k_{cat} by producing large amounts of the enzyme. In five different preparations, the dehalogenase accounted for 10.3 ± 2.5% of the soluble protein in the crude extract (data not shown).

A second striking observation is that, in the series of 4-halobenzoyl CoA substrates, the K_M values vary only slightly, while the size of the leaving group varies dramatically. If the values for K_M accurately reflect values for K_D for these substrates, then the size of the leaving group does not seem to influence binding.

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Pseudo.                 met-tyr-glu-ala-ile-gly-his-arg-val-glu-asp-gly-
SU          met-ser-ser-asn-ser-asp-his-his-ile-ser-val-glu-his-thr-asp-gly-
4-CB1           ala-val-asn-ser-asp-his-his-ile-ser-val-glu-his-thr-asp-gly-

Pseudo.     val-ala-glu-ile-thr-ile-lys-leu-pro
SU          val-ala-thr-ile-arg-phe-thr-arg-pro
4-CB1       val-ala-thr-ile-arg-phe-thr-arg-pro
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FIGURE 4: N-Terminal sequences of 4-CBCoA dehalogenases from *Arthrobacter* sp. strain 4-CB1 (*4-CB1*), *Pseudomonas* sp. CBS-3 (*Pseudo.*), and *Arthrobacter* sp. strain SU (*SU*). Residues in italics are uncertain assignments. The *Arthrobacter* sp. strain 4-CB1 sequence was determined by Edman degradation, and the *Pseudomonas* (Babbitt et al., 1992) and *Arthrobacter* sp. strain SU (Schmitz et al., 1992) sequences were inferred from the DNA sequences.

The specificity of the enzyme with respect to the position of nucleophilic attack was investigated by using 2-chlorobenzoyl CoA and 3-chlorobenzoyl CoA. Neither of these molecules is dehalogenated by the enzyme.

N-Terminal Sequence. The sequence of the 24 N-terminal amino acid residues of 4-CBCoA dehalogenase was determined by Edman degradation. Figure 4 shows the N-terminal sequence of 4-CBCoA dehalogenase from *Arthrobacter* sp. 4CB-1, along with the sequences of the 4-CBCoA dehalogenases from *Pseudomonas* sp. strain CBS-3 (Babbitt et al., 1992) and *Arthrobacter* sp. strain SU (Schmitz et al., 1992). The N-terminal sequences of the *Arthrobacter* strains are almost identical. The first two residues of the 4-CB1 sequence differ from the SU sequence, but the assignments of these residues after Edman degradation were uncertain. The *Arthrobacter* sequences show 30% identity to the *Pseudomonas* sp. strain CBS-3 sequence in this region.

Reassignment of Acinetobacter sp. 4CB-1 as Arthrobacter sp. 4CB-1. Arthrobacter sp. 4CB-1 was originally identified as *Acinetobacter* sp. 4CB-1 (Adriaens et al., 1989) using classical taxonomic techniques. Our finding that the N-terminal sequence of the dehalogenase protein was almost identical to that of the corresponding enzyme from *Arthrobacter* sp. strain SU prompted us to reassess the assignment of this microorganism. Indeed, analysis of the membrane fatty acids demonstrated that the microorganism is actually an *Arthrobacter* sp.

The reason for the difficulty in identifying this microorganism is the variability in the characteristics of *Arthrobacter* under different culture conditions. In young cultures, *Arthrobacter* is rod-shaped, while in older cultures, it becomes coccoid. Furthermore, Gram staining of *Arthrobacter* gives variable results (Skerman, 1959). The original cultures of sp. 4CB-1 that were examined were Gram-negative coccoid bacteria that were oxidase-negative, and the microorganism was consequently identified as an *Acinetobacter* sp. strain. We regard the membrane fatty acid composition as definitive, however, and this microorganism will henceforth be designated as *Arthrobacter* sp. strain 4CB-1.

Comparison of Arthrobacter sp. Strain 4-CB1 Dehalogenase with Corresponding Enzymes from Other Microorganisms. 4-CBCoA dehalogenase has been identified or at least implicated in the degradation of 4-chlorobenzoate in a number of microorganisms, including *Acinetobacter* sp. strain 4-CB1 (Copley & Crooks, 1992), *Pseudomonas* sp. strain CBS-3 (Scholten et al., 1991), and several strains of *Arthrobacter* (Schmitz et al., 1992; Tsoi et al., 1991; Marks et al., 1984). As described above, *Acinetobacter* sp. strain 4-CB1 has been reassigned as *Arthrobacter* sp. strain 4-CB1. The enzymes have been purified to homogeneity only from

Arthrobacter sp. strain 4-CB1 and *Pseudomonas* sp. strain CBS-3 (Chang et al., 1992), while the genes have been sequenced only from *Pseudomonas* sp. strain CBS-3 (Babbitt et al., 1992) and *Arthrobacter* sp. strain SU (Schmitz et al., 1992).

The *Arthrobacter* sp. strain 4-CB1 and *Pseudomonas* sp. strain CBS-3 dehalogenases are similar with respect to size and to k_{cat}. Furthermore, both enzymes are specific for dehalogenation at the 4-position. They differ, however, in their ability to dehalogenate 4-fluorobenzoyl CoA. The *Arthrobacter* sp. strain 4-CB1 enzyme dehalogenates 4-fluorobenzoyl CoA at a rate 430-fold lower than that for 4-CBCoA. Dehalogenation of 4-fluorobenzoyl CoA by the *Pseudomonas* enzyme is much slower: the k_{cat} is 10^5-fold lower than that for 4-CBCoA (Liang et al., 1993). We hope that insight into this intriguing difference will be provided by pre-steady-state kinetic studies of the dehalogenation reaction. Such studies have been recently carried out with the *Pseudomonas* enzyme (Liang et al., 1993). Pre-steady-state kinetic studies of the *Arthrobacter* sp. strain 4-CB1 enzyme are in progress.

ACKNOWLEDGMENT

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