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Steady-State and Pre-Steady-State Kinetic Analysis of Halopropane Conversion by a Rhodococcus Haloalkane Dehalogenase†

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ABSTRACT: Haloalkane dehalogenase from Rhodococcus rhodochrous NCIMB 13064 (DhaA) catalyzes the hydrolysis of carbon–halogen bonds in a wide range of haloalkanes. We examined the steady-state and pre-steady-state kinetics of halopropane conversion by DhaA to illuminate mechanistic details of the dehalogenation pathway. Steady-state kinetic analysis of DhaA with a range of halopropanes showed that bromopropanes had higher $k_{cat}$ and lower $K_M$ values than the chlorinated analogues. The kinetic mechanism of dehalogenation was further studied using rapid-quench-flow analysis of 1,3-dibromopropane conversion. This provided a direct measurement of the chemical steps in the reaction mechanism, i.e., cleavage of the carbon–halogen bond and hydrolysis of the covalent alkyl–enzyme intermediate. The results lead to a minimal mechanism consisting of four main steps. The occurrence of a pre-steady-state burst, both for bromide and 3-bromo-1-propanol, suggests that product release is rate-limiting under steady-state conditions. Combining pre-steady-state burst and single-turnover experiments indicated that the rate of carbon–bromine bond cleavage was indeed more than 100-fold higher than the steady-state $k_{cat}$. Product release occurred with a rate constant of 3.9 s$^{-1}$, a value close to the experimental $k_{cat}$ of 2.7 s$^{-1}$. Comparing the kinetic mechanism of DhaA with that of the corresponding enzyme from Xanthobacter autotrophicus GJ10 (DhlA) shows that the overall mechanisms are similar. However, whereas in DhlA the rate of halide release represents the slowest step in the catalytic cycle, our results suggest that in DhaA the release of 3-bromo-1-propanol is the slowest step during 1,3-dibromopropane conversion.

Haloalkane dehalogenases comprise a group of enzymes that hydrolyze carbon–halogen bonds in a wide range of haloalkanes, some of which are notorious environmental pollutants. The potential use of haloalkane dehalogenases in bioremediation applications has stimulated intensive investigation of these enzymes (1), and there is a growing interest in the application of these enzymes as industrial biocatalysts (2). Three different haloalkane dehalogenases have been purified and characterized in detail: the enzymes from Xanthobacter autotrophicus GJ10 (DhlA) (3), Spingomonas paucimobilis UT26 (LinB) (4), and Rhodococcus rhodochrous NCIMB13064 (DhaA) (5). In addition, a number of haloalkane dehalogenases were previously isolated from Gram-positive haloalkane-utilizing bacterial strains m15-3 (6), HA1 (7), GJ70 (8), and Y2 (9). However, DNA sequencing showed that the corresponding haloalkane dehalogenase genes of these strains were identical to the dhaA gene of R. rhodochrous NCIMB13064 (10). The X-ray crystal structures of the haloalkane dehalogenases DhlA (11), LinB (12), and DhaA (13) are now available, and reveal that the enzymes belong to the α/β-hydrolase fold superfamily (14). Haloalkane dehalogenases have a globular structure and are composed of two domains: a large central catalytic domain with an α/β-hydrolase fold structure and a second domain which lies like a cap on the main domain. The latter domain emerges as a large α-helical excursion between β-strands 6 and 7 of the catalytic core. The interface of the two domains forms the hydrophobic active site. The catalytic triad residues are a nucleophilic aspartate, a base catalyst histidine, and an aspartate or glutamate as the third member. These amino acids form the basis of the dehalogenation reaction and are located in the main domain. Whereas there is significant sequence similarity in the catalytic core, the sequence and structure of the cap domain diverge considerably between different haloalkane dehalogenases. The cap domain was proposed to play a prominent role in determining substrate specificity (15).

Considerable understanding of the catalytic mechanism of haloalkane dehalogenases has emerged from studies with the enzyme from X. autotrophicus GJ10 (DhlA) (16–20). This enzyme contains a catalytic triad formed by Asp124, His289, and Asp260. The overall catalytic mechanism for dehalogenation occurs in four kinetically distinguishable steps. After formation of the Michaelis complex, the dehalogenation reaction proceeds via a nucleophilic attack of Asp124 on the halo-substituted carbon atom of the substrate, thereby displacing the halogen ion and forming an alkyl–enzyme intermediate. This covalent intermediate is subsequently

† This work was supported by a grant from Ciba Specialty Chemicals Inc. (Basel, Switzerland).
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1 Abbreviations: DhaA, haloalkane dehalogenase from R. rhodochrous; DhlA, haloalkane dehalogenase from X. autotrophicus GJ10; LinB, haloalkane dehalogenase from S. paucimobilis UT2; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.
DhaA is most active on longer chain (C_2 to C_8) haloalkanes (5). The dhaA gene encodes a protein of 293 amino acids whose sequence is ~27% identical to that of DhIA. DhaA and DhIA have conserved amino acid residues involved in catalysis, suggesting that they have a similar reaction mechanism, yet they are adapted to different haloalkanes (21). Furthermore, DhaA has some structural features that might cause details of the dehalogenation mechanism to be different from those of DhIA. First, DhaA lacks a tryptophan residue equivalent to Trp175 of DhlA that is involved in halide release (26). The enzyme solution was concentrated spectrophotometrically at 280 nm. An absorbance of 1 corresponds to 0.54 mg/mL, as calculated with DNAStar (DNASTAR, Inc., Madison, WI). The purity of the dehalogenase was analyzed by SDS-PAGE.

**Materials and Methods**

**Materials.** All halogenated compounds that were used were obtained from commercial suppliers (Across, Merck, and Aldrich). \( ^3 \)H_2O (99.8%, v/v) was purchased from Isotec Inc. (Miamisburg, OH).

Protein Expression and Purification. The haloalkane dehalogenase gene dhaA was amplified by PCR using total DNA of *Rhodococcus* sp. strain m15-3 as a template. The isolated dhaA gene was cloned into the T7-based expression vector pGEM", and overexpressed in *Escherichia coli* BL21(DE3) (10). Because of the introduction of an Nco restriction site for translational fusion into the ATG start codon, the second amino acid was changed from Ser to Ala.

The haloalkane dehalogenase was expressed and purified by DEAE-cellulose and hydroxylapatite chromatography according to the method of Schanstra et al. (28). Typically, 1.5 g of purified DhaA was isolated from a 5 L culture of *E. coli* BL21(DE3). The enzyme solution was concentrated with an Amicon ultrafiltration cell using a PM10 filter and stored at ~20 or 4 °C in TEMAG [10 mM Tris-So, (pH 7.5), 1 mM EDTA, 1 mM \( \beta \)-mercaptoethanol, 3 mM sodium azide, and 10% (v/v) glycerol]. The concentration of the purified DhaA was measured spectrophotometrically at 280 nm. An absorbance of 1 corresponds to 0.54 mg/mL, as calculated with DNAStar (DNASTAR, Inc., Madison, WI). The purity of the dehalogenase was analyzed by SDS-PAGE.

**Steady-State Kinetics.** Dehalogenase assays were carried out at 30 °C in 50 mM NaHCO_3/NaOH buffer (pH 9.4) using colorimetric detection of halide release from halogenated substrates (3). Solvent kinetic isotope effects were determined by performing dehalogenase assays in buffer containing halogenated substrate, and increasing concentrations of \( ^2 \)H_2O. The K_m and V_max values were calculated from the initial rate of (halo)alcohol production, determined by gas chromatography, or from halide production rates, determined colorimetrically (26). The K_M value for 1,3-dibromopropane was determined by following substrate depletion by gas chromatography. A tube with a sufficient amount of enzyme and 50 \( \mu \)M 1,3-dibromopropane was incubated at 30 °C, and at different times within 20 min, samples of 1 mL were taken. The samples were extracted with 1.5 mL of diethyl ether containing 0.05 mM 1-bromohexane as the internal standard. The organic phase was analyzed by gas chromatography. Potassium bromide (80 mM), 3-bromo-1-propanol (20 mM), and 2-bromoethanol (20 mM) were tested as product inhibitors of 1,3-dibromopropane conversion by DhaA. Conversion of 1,3-dibromopropane was followed by gas chromatography.

**Rapid-Quench-Flow Measurements.** Single-turnover and pre-steady-state burst experiments on 1,3-dibromopropane conversion were carried out in 50 mM NaHCO_3/NaOH (pH 9.4) and 1 mM dithiothreitol at 30 °C using a Kintek RQF-3 rapid-quench-flow apparatus (KinTek Corp., State College, PA). The concentrations of enzyme and substrate cited in the text are those after mixing and during the enzymatic reaction. The reaction was initiated by rapidly mixing equal volumes (50 \( \mu \)L) of a concentrated enzyme solution and a freshly prepared 1,3-dibromopropane solution. At appropriate times, ranging from 2 ms to 0.5 s, the reaction was quenched with 120 \( \mu \)L of 0.8 M H_2SO_4. The quenched reaction mixture was directly extracted with 1.5 mL of ice-cold diethyl ether containing 0.05 mM 1-bromohexane as the internal standard, and then neutralized by the addition of NaHCO_3. The ether phase containing noncovally bound substrate and halo alcohol was analyzed by gas chromatography, and the amount of bromide in the water phase was measured by ion chromatography.
Kinetics of Haloalkane Dehalogenase

Data Analysis. Numerical integration was performed by using Dynafit (29) (Biokin, Ltd., Madison, WI). The entire data set was fit to a single mechanism and a single set of rate constants. The 95% confidence interval for the rate constants was determined by a systematic search in the parameter space using an algorithm implemented in the program. An iterative process, varying one rate constant at a time, was used to fit the data. Initial estimates of \( k_2, k_3, \) and \( k_4 \) were obtained from the fits of the pre-steady-state burst data to the biphasic burst equation (30):

\[
\text{product} = A \times \exp(-k_0 t) + k_3 t
\]

where \( A \) is the amplitude of the burst, \( k_0 \) is the first-order rate constant of the pre-steady-state exponential phase, and \( k_3 \) is the rate of the linear phase which corresponds to the steady-state rate. The data were fit to the biphasic burst equation by nonlinear regression analysis using SigmaPlot (Jandel Scientific).

Analytical Methods. Concentrations of organic halogenated compounds were quantitatively determined by gas chromatography on a Chrompack 4385 gas chromatograph equipped with a flame ionization detector and an HP wax column (length of 25 m, inner diameter of 0.2 mm, and film thickness of 0.2 \( \mu \text{m} \)) (Hewlett-Packard). Nitrogen was used as the carrier gas (50 kPa), and the temperature program was as follows: isothermal for 3 min at 45 °C followed by an increase to 220 °C at a rate of 10 °C/min.

Bromide concentrations in the water phase were quantitated by ion chromatography on a Dionex ion chromatograph with an AS12A column (length of 25 cm and diameter of 4 mm) and an AG12A precolumn (length of 4 cm and diameter of 4 mm). The carrier liquid was 270 mM Na2CO3 and 30 mM NaHCO3.

RESULTS AND DISCUSSION

Steady-State Kinetics of Halopropane Conversion. The dhaA gene was heterologously expressed under control of the T7 promoter in E. coli BL21(DE3), and the dehalogenase was purified to homogeneity. The purified enzyme was used to determine the steady-state kinetic constants on a range of haloalkanes, including the important environmental chemicals 1,2-dichloropropane and 1,2,3-trichloropropane. Both chloro- and bromopropanes are substrates for DhaA (Table 1). In agreement with previous results, bromopropanes are better substrates with turnover numbers \( (k_{cat}) \) that are higher than those of chlorinated analogues (25, 26). In terms of catalytic efficiency \( (k_{cat}/K_M) \), 1,3-dibromopropane was the best substrate. Furthermore, DhaA converted different brominated propanes with a similar \( k_{cat} \) value. The higher affinity of DhaA for brominated compounds might be a consequence of tight substrate binding in combination with a high rate of carbon–bromine bond cleavage, as was observed for 1,2-dibromoethane conversion by DhlA, the corresponding enzyme from X. autotrophicus GJ10 (20). DhaA dehalogenated mono- and dibrominated propanes with a catalytic efficiency similar to that of DhlA, whereas the catalytic efficiency of DhaA on 1,2,3-tribromopropane was ~30-fold higher (25, 26). This observation is consistent with the larger active site cavity of DhaA that can probably better accommodate large bulky substrates such as 1,2,3-tribromopropane (15). DhaA dehalogenated the different chlorinated propanes with a similar catalytic efficiency similar to that of DhlA, whereas the catalytic efficiency of DhaA on 1,2,3-tribromopropane was ~30-fold higher (25, 26). This observation is consistent with the larger active site cavity of DhaA that can probably better accommodate large bulky substrates such as 1,2,3-tribromopropane (15).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( k_{cat} ) (s−1)</th>
<th>( K_M ) (mM)</th>
<th>( k_{cat}/K_M ) (M−1 s−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-chloropropane</td>
<td>0.48</td>
<td>1.0</td>
<td>480</td>
</tr>
<tr>
<td>1,2-dichloropropane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,3-dichloropropane</td>
<td>1.3</td>
<td>0.63</td>
<td>2000</td>
</tr>
<tr>
<td>1,2,3-trichloropropane</td>
<td>0.08</td>
<td>2.2</td>
<td>36</td>
</tr>
<tr>
<td>1-bromopropane</td>
<td>2.7</td>
<td>0.6</td>
<td>4500</td>
</tr>
<tr>
<td>1,2-dibromopropane</td>
<td>2.3</td>
<td>1.0</td>
<td>2300</td>
</tr>
<tr>
<td>1,3-dibromopropane</td>
<td>2.7</td>
<td>0.005</td>
<td>540000</td>
</tr>
<tr>
<td>1,2,3-dibromopropane</td>
<td>3.6</td>
<td>0.3</td>
<td>12000</td>
</tr>
<tr>
<td>1,2-dibromoethane</td>
<td>14.3</td>
<td>4.0</td>
<td>3600</td>
</tr>
<tr>
<td>3-bromo-1-propanol</td>
<td>3.0</td>
<td>3.3</td>
<td>910</td>
</tr>
<tr>
<td>2-bromoethanol*</td>
<td>&gt; 3.0</td>
<td>&gt; 11.0</td>
<td>&gt; 272</td>
</tr>
</tbody>
</table>

* Not detectable, detection limit of <10 milliunits/mg of protein at 10 mM substrate. Due to the high background, only \( k_{cat}/K_M \) could be determined, setting lower limits for the \( k_{cat} \) and \( K_M \) values. The \( k_{cat} \) values had errors of less than 15%, and the \( K_M \) values had errors of less than 25%.

With considerable differences in maximum turnover as well as affinity. Within this series of substrates, DhaA has the highest efficiency toward 1,3-dichloropropane. Both 1,2-dichloropropane and 1,2,3-trichloropropane are poor substrates. DhaA slowly dehalogenated 1,2,3-trichloropropane with a low \( k_{cat} \) and high \( K_M \), whereas no significant activity was detected for 1,2-dichloropropane. The opposite applies for DhlA, which is not active on 1,2,3-trichloropropane but slowly dehalogenated 1,2-dichloropropane (20, 26).

Compared to DhaA, the Xanthobacter dehalogenase has a more restricted substrate range. The latter dehalogenase exhibits the highest activity on smaller compounds such as its natural substrate 1,2-dichloroethane and the brominated analogue and nematocide 1,2-dibromoethane (20). Interestingly, DhaA converted 1,2-dibromoethane at a steady-state rate that is 4–5-fold higher than that found with DhlA, even though 1,2-dichloroethane is not a substrate for DhaA (20, 25, 26).

DhaA was also active on polar compounds such as 3-bromo-1-propanol and 2-bromoethanol, which are the products of enzymatic dehalogenation of 1,3-dibromopropane and 1,2-dibromoethane, respectively. Maximum turnover rates for these halo alcohols were similar to those found for the brominated propanes, whereas the \( K_M \) values were higher. Both halohydrins were examined as product inhibitors of 1,3-dibromopropane conversion. DhaA was inhibited by 3-bromo-1-propanol, whereas 2-bromoethanol did not inhibit 1,3-dibromopropane conversion. Since 3-bromo-1-propanol is a substrate for DhaA with a reasonably high affinity, this substrate is most likely a competitive inhibitor.

Bromide also inhibited DhaA. In the presence of 80 mM bromide, the maximum turnover rate on 1,3-dibromopropane dropped 10-fold. Since the \( K_M \) is too low to measure accurately, we were not able to determine a clear effect on the \( K_M \) in the presence of bromide. However, it seems likely that halide binds to both the free enzyme and the enzyme–substrate complex, since X-ray crystallography data for different haloalkane dehalogenases indicate that halides can bind in the active site region of these enzymes (12, 13, 16, 31). This suggests that bromide exerts a noncompetitive type of inhibition, most likely mixed, on DhaA. Surprisingly, Schindler et al. (25), using an assay dependent on pH changes, did not find halide inhibition of DhaA at concentrations up to 80 mM.
Transient-State Kinetic Analysis of 1,3-Dibromopropane Conversion. The chemical reaction steps involved in dehalogenation of 1,3-dibromopropane by DhaA were examined using rapid-quench-flow kinetic methods. To identify the rate-limiting step in the overall reaction pathway, we conducted a pre-steady-state burst experiment and followed product formation. The reaction was initiated by mixing an excess of 1,3-dibromopropane (final concentration of 2.5 mM) with DhaA (final concentration of 540 \( \mu \)M). After various times, the reactions were quenched and the amounts of bromide and 3-bromo-1-propanol were quantitated. A pre-steady-state burst was found both for bromide and 3-bromo-1-propanol, with observed exponential burst rate constants of 250 and 10 s\(^{-1}\), respectively (Figure 2A). This fast initial rate was followed by a slower steady-state rate of product formation of 2.8 s\(^{-1}\) (\( k_{\text{on}} \) divided by the active enzyme concentration), which is in good agreement with the \( k_{\text{cat}} \) of 2.7 s\(^{-1}\) determined from halide production rates that were found after manual mixing. The active enzyme concentration was obtained from the amplitude of the bromide burst and corresponded apparently to 65% of the enzyme concentration as was determined spectrophotometrically.

The observation of a pre-steady-state burst for both products indicated that a step after chemical catalysis, presumably product release, is rate-limiting. Furthermore, it implies that there are at least three steps following substrate binding (Scheme 1): formation of the alkyl–enzyme intermediate (step 2), hydrolysis of the alkyl–enzyme intermedi-
The mechanism involves substrate binding, formation of an alkyl–enzyme intermediate and simultaneous cleavage of the carbon–bromine bond, hydrolysis of the alkyl–enzyme intermediate, and finally release of the products from the enzyme active site. On the basis of the observation of a product burst, the first three steps occur at a rate that is faster than the rate of steady-state turnover, whereas step 4, product release, is proposed to limit maximum turnover. The overall dehalogenase reaction was considered irreversible since no products are observed in the reverse reaction. To determine the rate constants for the individual steps, both pre-steady-state burst and single-turnover data were combined and fitted to the mechanism presented in Scheme 1 by numerical integration using Dynafit. The adjustable parameters were all five rate constants that appear in the reaction mechanism, and the resulting best fit values are summarized in Table 2.

Initially, the amplitude of the bromide burst was lower than expected. A decreased burst amplitude may be governed by several factors, such as characteristics of the reaction kinetics, an internal equilibrium, or that a fraction of the enzyme is not participating in the reaction (30). In a recent paper, Lewandowicz et al. suggested, on the basis of the measurement of a chlorine kinetic isotope effect, that the dehalogenation step during conversion of 1,2-dichloroethane by DhlA is irreversible and that the overall irreversibility is caused by the hydrolysis of the covalent intermediate (33). Therefore, we also performed burst simulations using a model that includes a reversible dehalogenation step. However, these simulations could not explain the data of both pre-steady-state burst and single-turnover experiments. Furthermore, the observation that during a single turnover essentially all substrate is transiently present as a covalent intermediate excludes reversibility of the first chemical step (see above).

The mechanism presented in Scheme 1 could describe the experimental data if the active enzyme concentration present during the burst experiment was approximately the same as the amount of 3-bromo-1-propanol produced, there was no significant accumulation of a covalent enzyme–substrate intermediate, which is in agreement with a slow step occurring after hydrolysis of the intermediate. The exponential burst phases of bromide and 3-bromo-1-propanol production largely define rate constants $k_3$ and $k_4$, respectively. The steady-state reaction rate $k_5$ defines the rate constant of product release $k_6$, which corresponds to a simplified step representing an unknown unimolecular rate-limiting event.

The kinetics of a single turnover of 1,3-dibromopropane conversion by DhaA are shown in Figure 2B. Excess DhaA (750 μM) was rapidly mixed with 1,3-dibromopropane (400 μM), and substrate depletion and product formation were monitored. Within the first 15 ms of the reaction, substrate disappeared and simultaneously bromide production reached its maximum, whereas hardly any 3-bromo-1-propanol was observed. Substrate depletion and 3-bromo-1-propanol production curves crossed at 70 μM, indicating that hydrolysis of the covalent intermediate occurred at a much lower rate than its formation. This resulted in a very rapid accumulation of the alkyl–enzyme intermediate. The initial dehalogenation step appears to be irreversible, since all halide was completely released during the initial phase of alcohol production.

**Proposed Kinetic Mechanism for Haloalkane Dehalogenase DhaA**. A minimal model consisting of four steps consistent with the pre-steady-state data (Figure 3 and Scheme 1) was constructed.

**Scheme 1**

\[
E + RX \xrightarrow{k_1} E \cdot RX \xrightarrow{k_2} E \cdot R \cdot X' \xrightarrow{k_3} E \cdot ROH \cdot X' \xrightarrow{k_4} E + ROH + X
\]

The rates clearly show that substrate binding and chemical catalysis are not the steps that limit maximum turnover. Using single-turnover conditions, the rate of substrate binding appeared to be faster than the rate of chemical catalysis. At a 1,3-dibromopropane concentration of 400 μM, the pseudo-first-order rate constant ($k_1[S]$) was 400 s$^{-1}$. The data did not allow extraction of a unique value for the rate of substrate dissociation $k_{-1}$, but a solution could be obtained using computer simulations to the mechanism in Scheme 1 together with the rate constants in Table 2.

**Table 2. Pre-Steady-State Kinetic Constants of DhaA for 1,3-Dibromopropane (Scheme 1) at pH 9.4 and 30 °C**

<table>
<thead>
<tr>
<th>reaction</th>
<th>kinetic parameter</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>substrate binding</td>
<td>$k_1$</td>
<td>1.0 μM$^{-1}$ s$^{-1}$ (0.8–2.5)</td>
</tr>
<tr>
<td>substrate dissociation</td>
<td>$k_{-1}$</td>
<td>60–300 s$^{-1}$</td>
</tr>
<tr>
<td>carbon–bromine bond cleavage</td>
<td>$k_2$</td>
<td>300 s$^{-1}$ (249–372)</td>
</tr>
<tr>
<td>hydrolysis of the alkyl–enzyme intermediate</td>
<td>$k_3$</td>
<td>14.8 s$^{-1}$ (13.5–16.3)</td>
</tr>
<tr>
<td>product release</td>
<td>$k_4$</td>
<td>3.9 s$^{-1}$ (3.4–4.6)</td>
</tr>
</tbody>
</table>

* Determined from steady-state parameters and computer simulation. Numbers in parentheses represent the 95% confidence intervals.
The rate-determining step for 1,2-dibromoethane conversion than the maximum turnover rate with 1,3-dibromopropane. The active site cavity may allow faster diffusion of the halide ion that is cleaved off (12, 21). Another structural feature that may facilitate halide release in DhaA is the different topological position of the carboxylic function of the triad. The triad acid group in DhaA follows β-strand 6 rather than β-strand 7 as in DhIA and most other αβ-hydrolase fold enzymes (13, 22). Mutagenesis of active site residues Trp175 and Asp260 of DhIA, and subsequent structural and kinetic analysis, corroborated the above observations. These experiments showed that halide release in these mutants was accelerated and not limiting maximum turnover as was found for wild-type DhIA (34, 35).

Summarizing, we defined the steps of a minimal dehalogenation mechanism that is sufficient to explain the steady-state kinetic data. Pre-steady-state kinetic analysis of 1,3-dibromopropane conversion by DhaA showed that substrate binding and chemical catalysis occur faster than the steady-state rate which corresponds to product release. The overall mechanism by which DhaA catalyzes dehalogenation is similar to that of DhIA, but the rate-determining step of DhaA may be different. In DhIA, the less efficient stabilization of the halogen, the different topological arrangement of the triad acid Glu130, and the much larger and less buried active site cavity may allow faster diffusion of the halide ion out of the active site. Together with the high activity on 1,2-dibromoethane, these observations support the idea that bromide release is not rate-determining during conversion of 1,3-dibromopropane. Release of the product alcohol from the DhaA active site is therefore proposed to be the overall rate-limiting step, which is largely determined by the size and affinity of the product alcohol, explaining the difference in $k_{cat}$ between 1,2-dibromoethane and 1,3-dibromopropane conversion.

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Kinetics of Haloalkane Dehalogenase


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