Comparison of formation of reactive conformers for the S_N2 displacements by CH_3CO_2^- in water and by Asp124-CO_2^- in a haloalkane dehalogenase

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Contributed by Thomas C. Bruice, November 26, 2002

The S_N2 displacement of Cl^- from 1,2-dichloroethane by acetate (CH_3CO_2^-) in water and by the carboxylate of the active site aspartate in the haloalkane dehalogenase of *Xanthobacter autotrophicus* have been compared by using molecular dynamics simulations. In aqueous solution, six families of contact-pair structures (I–VI) were identified, and their relative concentrations and dissociation rate constants were determined. The near attack conformers (NACs) required for the S_N2 displacement reaction are members of the IV ($\text{CH}_3\text{COO}^- \cdots \text{CH}_2(\text{Cl})\text{CH}_2\text{Cl}$) family and are formed in the sequence $\text{II} \rightarrow \text{III} \rightarrow \text{IV} \rightarrow \text{NAC}$. The NAC subclass is defined by the $-\text{COO}^- \cdots \text{C}-\text{Cl}$ contact distance of ≤ 3.41 Å and the $-\text{COO}^- \cdots \text{C}-\text{Cl}$ angle of 157° – 180° . The mole percentage of NACs is 0.16%, based on the 1 M standard state. This result may be compared with 13.4 mole percentage of NACs in the Michaelis complex in the enzyme. It follows that NAC formation in the enzyme is favored by 2.6 kcal/mol. Because reaction coordinates from S to TS, both in water and in the enzyme, pass via NAC (i.e., $\text{S} \rightarrow \text{NAC} \rightarrow \text{TS}$), the reduction in the $\text{S} \rightarrow \text{NAC}$ barrier by 2.6 kcal/mol accounts for $\approx 25\%$ of the reduction of total barrier in the $\text{S} \rightarrow \text{TS}$ (10.7 kcal/mol). The remaining 75% of the advantage of the enzymatic reaction revolves around the efficiency of $\text{NAC} \rightarrow \text{TS}$ step. This process, based on previous studies, is discussed briefly.

X*anthobacter autotrophicus* haloalkane dehalogenase (DhlA) catalyzes the S_N2 displacement of the halogen substituent from haloalkanes by Asp-CO_2^- . This reaction can be compared with the reaction in water with CH_3CO_2^- (AcO^-) as a nucleophile (Scheme 1). The activation barrier ($\Delta G^\ddagger$) for the displacement of Cl^- from dichloroethane (DCE) is 15.3 kcal/mol for the enzymatic reaction (1) and ≈ 26 kcal/mol for the nonenzymatic counterpart in water (2). The first goal of this study is to devise means of determining the time-dependent mechanism of forming contact pairs from two separate reactants in water that satisfy the structural restraints of a near attack conformation (NAC). The second objective is to evaluate the contribution of NAC formation to the kinetic advantage of the DhlA reaction over the model reaction in water. These procedures can be generally applied to a study of various enzymatic catalysis over a water reaction.

Methods

Molecular Dynamics (MD) Simulation of 1,2-DCE and Acetate in Water.

To compare ground state structures of reactants in water with those in the enzyme active site, an MD simulation was performed on the system consisting of 1,2-DCE and an acetate ion (AcO^-) in a box of TIP3P water (3). Standard CHARMM (version 27) force field was used for AcO^- although OPLS force field parameters were adopted for DCE. The torsional parameters for DCE were adjusted so that only one of the two *gauche* conformations was sampled. It is known that for the S_N2 displacement of Cl^- , DCE should be in a *gauche* conformation (4–6). After the reactants were placed in a water box of size $20 \times 20 \times 20$ Å³, any TIP3P water molecule whose oxygen was within 2.6 Å from any atom of the reactants was deleted, and the system was energy-minimized by using a combination of the steepest descent (SD)

Scheme 1.

and adopted basis Newton-Raphson (ABNR) methods (7). An MD simulation was performed on the energy-minimized system with the position of carboxyl carbon of the acetate fixed at the center of the box. A periodic boundary condition was used to simulate a continuous water pool. The SHAKE algorithm was used to constrain bonds containing hydrogens to their equilibrium lengths (8). The Verlet leapfrog algorithm was used to integrate the equations of motion (9). A time step of 1.5 fs was used, and the nonbonded list was updated every 20 time steps. The nonbonded interactions were cut off at 10 Å. The Coulombic term was cut off by using a force shifting function, and the Lennard-Jones term was cut off with a switching function. The system was initially coupled to a 200 K heat bath for 15 ps by using a coupling constant of 5 s^{-1} . The system was subsequently coupled to a heat bath at 300 K for the rest of the simulation (23 ns) by using a coupling constant of 5 s^{-1} . The pressure was constantly maintained by a Berendsen algorithm (10) using an isothermal compressibility of $4.63 \times 10^{-5} \text{ atm}^{-1}$ and a pressure coupling constant of 5.0 ps. Coordinates were saved every 100 time steps.

When analyzing the trajectory, a configuration of AcO^- and DCE is classified as a contact pair when the closest intermolecular distance is within the van der Waals distance between two interacting atoms. The van der Waals radii used are 1.92 Å for sp^3 carbon, 1.76 Å for sp^2 carbon in acetate, 1.49 Å for acetate oxygen, and 1.75 Å for chlorine (11).

Results

Interactions between AcO^- and DCE (0.214 M for each) were investigated in water by using 23 ns MD simulation. The following results are based on the analysis of structures at every 0.15 ps of this MD trajectory. Reported numerical values in this paper are based on the correction to a 1 M standard state of both reactants.

Structures of Contact Pairs (AcO^- -DCE) in Water. In a 1 M aqueous solution of AcO^- and 1,2-DCE, collision dimers are present 43% of the time. Once these dimers form, the partners remain together for ≈ 25 ps on average and in few cases up to ≈ 80 ps.

Abbreviations: DhlA, *Xanthobacter autotrophicus* haloalkane dehalogenase; DCE, dichloroethane; AcO^- , acetate; NAC, near attack conformer; MD, molecular dynamics.

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Fig. 1. Six contact pairs of $\text{ClCH}_2\text{CH}_2\text{Cl}$ and CH_3CO_2^- . NAC is subspecies of IV where the angle of approach $-\text{COO}^- \cdots \text{C}-\text{Cl}$ is within the range of $157-180^\circ$. Atoms are colored by using standard atom representation: O (red), C (gray), H (white), Cl (green).

During this ≈ 25 - to 80 -ps time, the type of contact changes every few picoseconds. Fig. 1 shows structures of the various dimers.

The formation of six contact pairs can be observed to occur at a frequency in the decreasing order of **I**, **II** $\gg$ **III**, **IV** $>$ **V**, **VI** (Table 1). In $\approx 85\%$ of the dimeric states (**I** and **II**), the acetate- COO^- points away from the partner DCE. In these cases, there is little preference in choosing the complexing moiety of DCE ($-\text{CH}_2$ or $-\text{Cl}$). The slight preference to DCE-Cl (**I**) over DCE- CH_2 (**II**) may be due to the larger surface area of the $-\text{Cl}$. When acetate- COO^- is involved in a contact, the preference for the contact is with DCE- CH_2 (**III**, **IV**) over DCE-Cl (**V**, **VI**) due to the negative electrostatic interaction of acetate- COO^- and DCE-Cl. The atomic charges for the DCE methylene C is -0.06 electronic unit (e.u.), and the charge for $-\text{Cl}$ is -0.20 e.u. For **IV** and **VI**, it should be noted that the mole fractions are obtained by counting contacts involving either O of the carboxylate ion. Thus, the probability to be involved in forming a dimer for each O is the half of the values listed in Table 1.

From MD trajectories, one can also study the kinetic stability of each contact pair by calculating the first order rate constants

Table 1. Mole fraction of contact pairs of DCE and CH_3CO_2^- and the first-order rate constants for disappearance of each contact pair

Contact pairs	Symbol	Mole fraction, %	$k_{\text{diss}} \times 10^{12}, \text{s}^{-1}$
$-\text{O}_2\text{CCH}_3 \cdots \text{ClCH}_2\text{CH}_2\text{Cl}$	I	43	3.6
$-\text{O}_2\text{CCH}_3 \cdots \text{CH}_2(\text{Cl})\text{CH}_2\text{Cl}$	II	41	4.3
$\text{H}_3\text{CCO}_2^- \cdots \text{CH}_2(\text{Cl})\text{CH}_2\text{Cl}$	III	6	6.4
$\text{H}_3\text{CCOO}^- \cdots \text{CH}_2(\text{Cl})\text{CH}_2\text{Cl}$	IV	6	23
$\text{H}_3\text{CCO}_2^- \cdots \text{ClCH}_2\text{CH}_2\text{Cl}$	V	2	11
$\text{H}_3\text{CCOO}^- \cdots \text{ClCH}_2\text{CH}_2\text{Cl}^*$	VI	1	20

The DCE is present as one of two equivalent *gauche* conformers.

*Mole fraction of conformer **IV** and **VI** are calculated by counting contacts involving either O of the carboxylate.

for its dissociation (k_{diss}). The k_{diss} for contact pair decomposition was obtained from the statistics of the length of time for each contact pair to remain before it dissociates or rearranges into another contact pair. The first order plots of $\log$ (concentration of the contact pair) vs. time are shown in Fig. 2, and the slopes of each plot ($k_{\text{diss}}, \times 10^{12} \text{s}^{-1}$) are summarized in Table 1. All of these k_{diss} are within the range of 10^{12}s^{-1} . The half-life ($\log(2)/k_{\text{diss}}$) of each contact pair is in the decreasing order of **I**, **II** $>$ **III**, **V** $>$ **IV**, **VI**. This finding is comparable to the trend observed in the relative concentrations of contact pairs at equilibrium (**I**, **II** $>$ **III**, **IV** $>$ **V**, **VI**). **I** and **II** are the most probable contact pairs and dissociate most slowly. The differences between the order of the half-life and the order of concentration at equilibrium are in **IV** and **V**. The somewhat

Fig. 2. First order kinetics of contact pairs **I** ($\blacklozenge$), **II** ($+$), **III** ($\square$), **IV** ($\times$), **V** ($\triangle$), and **VI** ($*$). The coefficients of correlation are > 0.95 for all of the contact pairs.

Scheme 2.

faster dissociation of **IV** reflects an energetic cost in desolvating the acetate-COO⁻ when forming a contact with DCE, which will be discussed further (see below). It was observed from our simulation that formation of dimers involving the acetate-COO⁻ (**III** and **V**) do not require desolvation. This finding may explain their relatively longer half-lives compared with **IV** and **VI**, even though **III** and **V** appear less frequently than **IV** and **VI**.

Mechanism of NAC Formation in Water. Among the various contact dimers in water (Fig. 1), only those in which acetate-COO⁻ contacts (**IV**) the back side of CH₂-Cl can undergo an S_N2 displacement of Cl⁻. Therefore, our definition of the NAC is the —COO⁻...C—Cl distance ≤ 3.41 Å and the angle of attack given by —COO⁻...C—Cl being within ±15° deviation from the angle in the TS. We first discuss the mechanism of **IV** formation, followed by discussion on the character of the approach angles in **IV** and the NAC formation.

Dimers involving the AcO⁻...CH₂(Cl)CH₂Cl (**IV**) contact are disfavored. A dimer **IV** is not formed frequently (6% of total dimers), and even once formed it rapidly dissociates or rearranges into another contact pair (dissociation rate constant is 23.4 × 10¹² s⁻¹; Table 1). The instability of **IV** reflects an energetic cost in desolvating the AcO⁻ when forming a contact with DCE. Based on the observation of the hydration shell structures, the following picture is proposed (Scheme 2). With AcO⁻ in water, there are three water molecules associated with each O of the carboxylate, and one water molecule is held between them. This bridging water molecule is bound more strongly to one of the AcO⁻ oxygens and then moves to the other oxygen. For acetate, when complexed with DCE, the complexing O is hydrated by three water molecules and the noncomplexing O hydrated by three or four water molecules. These pictorial descriptions are supported by the time-average number of water molecules whose H is within 2.4 Å distant from O of AcO⁻ in free and in complexed states. In a free state, there are 3.5 water molecules within the sphere (*r* = 2.4 Å) around each O of the carboxylate, whereas in a complex **IV**, there are 3.0 water molecules for the complexing O and 3.6 water molecules for the noncomplexing O. Thus, desolvation required in formation of **IV** is equivalent to breaking of ≈0.5 water-hydrogen bond (Scheme 2).

It was observed that the desolvation of the complexing O of AcO⁻ is achieved not by a direct collision between two molecules, but via a formation of series of other contacts, which do not require a disruption of hydrogen bonds between water and the solutes. Statistics of contact pairs forming before **IV** shows that **II** → **III** → **IV** is the most probable path leading to **IV**, with the same carbon center of DCE involved in **II**, **III**, and **IV** contacts (Scheme 3). This characteristic of having a specific path to form **IV** is a distinct feature of **IV** compared with other contact pairs such as **I** or **II**. The path in Scheme 3 is obtained in the following manner. Throughout the trajectory, whenever the contact-pair **IV** is formed, the types of contact pair occurring for the time of τ just before the formation of **IV** were sampled. These statistics were repeatedly obtained with varying τ (1.5, 3.3, 5.0, 10.0, and 15.0 ps) to trace the time-evolution pattern of the

Scheme 3.

contact-pair just before **IV** (Fig. 3). The same procedures were repeated for the **I** and **II** contact pairs for a comparison purpose. Among the structures (**Y**) preceding the **X** (**X** = **IV**, **I**, or **II**) contact, the contact pair **Y** is designated by **Y'** if the complexing —CH₂Cl of DCE is different from that in **X** (i.e., if transition of **Y** → **X** involves a 1,2-migration). In Fig. 3, the dimer statistics just before **X** = **IV**, **I**, and **II** approximately reflect the general feature of the probability of each dimer shown in Table 1; i.e., **I**, **I'**, **II** and **II'** occur more often than **III**–**VI**'. However, three following features are seen in the statistics of **X** = **IV**, which distinguish from **X** = **I** or **II**. First, the number of contacts for **X** = **IV** is greater than for **X** = **I** or **II** regardless of the type of **Y** during the same sampling period τ . This result implies that **IV** prefers to form via other dimeric state(s) rather than by a direct

Fig. 3. Statistics of contact pairs (**Y**) formed for τ (ps) just before formation of **X**. τ is varied from 1.6 ps (red bar), 3.3 (violet), 5.0 (light yellow), and 10.0 (light blue) to 15.0 ps (dark blue). Notice that the contact pair whose type is the same as **X** is excluded in the statistics of **Y**.

Fig. 4. Statistics of angle of approach $\text{—COO}^- \cdots \text{C—Cl}$ in water (gray bars) and in enzyme (black bars).

collision. Second, the number of contact $\text{Y} = \text{III}$ before $\text{X} = \text{IV}$ becomes even larger than $\text{Y} = \text{I}$, I' , or II' when decreasing the sampling period τ . This result suggests that **III** is most likely followed by $\text{X} = \text{IV}$. Third, among $\text{Y} = \text{I}$, I' , **II** and II' , there is a stronger preference for **II** rather than **I**, I' or II' . This feature also becomes pronounced when decreasing τ , implying **II** also occurs just before **IV** with high probability. Between **II** and **III**, formation of **II** might precede formation of **III** because, as τ gets smaller, the population of **II** decreases more rapidly than does **III**. All of these features are not seen in the statistics of $\text{X} = \text{I}$ or **II**. In fact, the dimer statistics just before $\text{X} = \text{I}$ (Fig. 3B) and $\text{X} = \text{II}$ (Fig. 3C) are similar to the relative population of each conformer when sampled throughout the whole trajectory (Table 1). This result implies that there is no correlation between X (**I** or **II**) and Y (conformers formed just before X). These observations establish the proposed scenario of **II** $\rightarrow$ **III** $\rightarrow$ **IV** (Scheme 3).

Five percent of **IV** satisfies the attack-angle criterion of NAC ($\text{—COO}^- \cdots \text{C—Cl} \leq 157\text{--}180^\circ$). Examination of angles of approach to form **IV** shows that the population is distributed as a normal sine curve along the angle of approach, with the most configurations populating at $90\text{--}120^\circ$ (gray bars in Fig. 4). Mathematically the number of possible configurations at a certain angle of approach is a sine function of the angle of approach. Thus, a perfect sine distribution establishes no preference to a configuration of a certain angle of approach. For the case under inspection, the curvature is not completely symmetric due to the electrostatic repulsion between AcO^- and DCE—Cl at small angles of approach.

Discussion

Kinetic/Thermodynamic Properties of Contact Pairs (AcO^- ·DCE) in Water. The present work establishes methods to study behaviors of two reactants in water and compare this with a behavior of substrate in the enzyme. A behavior of ground state in water can be studied by examining various contact pairs. Six contact pairs of AcO^- ·DCE (**I**, **II**, **III**, **IV**, **V**, and **VI** in Fig. 1) have different populations and half-lives, depending on the nature of solute–solute interactions and solute–water interactions. The trend in both the population and the half-life follow the order of **I**, **II** $\gg$ **III**, **IV** $>$ **V**, **VI** (Table 1). It was observed that formation of **IV** ($\text{CH}_3\text{COO}^- \cdots \text{—CH}_2(\text{Cl})\text{CH}_2\text{Cl}$) and **VI** ($\text{CH}_3\text{COO}^- \cdots \text{—ClCH}_2\text{CH}_2\text{Cl}$) requires desolvation of AcO^- .

The Mechanism of NAC Formation in Water. Due to the requirement of desolvation of the acetate- COO^- , formation of **IV** in water does not occur via random collision of AcO^- and DCE. There is a specific path that is favored in **IV** formation (Scheme 3). First, AcO^- and DCE form a complex, which most frequently involves contact between the acetate- CH_3 and either DCE—Cl (**I**) or DCE—CH_2 (**II**). The two contact structures **I** and **II** are formed

without breaking hydrogen bonds to the waters solvating the two carboxylate oxygens of AcO^- . **I** or **II** frequently rearrange by rotation and translation of the DCE and AcO^- components relative to the other while retaining the dimeric state for $\approx 25\text{--}80$ ps. From the statistics of contact pairs present just before **IV** (Fig. 3), we propose the series of **II** $\rightarrow$ **III** $\rightarrow$ **IV** as a time-dependent mechanism of **IV** formation (Scheme 3). In this series of dimer formation, the —CH_2 carbon center of DCE, which is involved in **IV**, is the same carbon that is in contact with AcO^- in the preceding **II** and **III** (Scheme 3). Desolvation

Fig. 5. Corey–Pauling–Koltun view of the hydrophobic pocket of DhIA with substrate DCE bound to it. Residues (gray) surrounding the substrate DCE form extensive van der Waals contacts with DCE. (A) View from the side of $\text{—CH}_2\text{Cl}$ (gray at C and green at Cl) that is being attacked by Asp-124-COO^- (red at O). Only one water molecule (blue) is hydrogen bonded to the attacking O of Asp-124. This water molecule has moved away in the TS. (B) The dissected view of the structure in A. The carbon center shown is the nonelectrophilic C (gray), and Cl (green) is the nonleaving Cl.

of water occurs in the last step of **III** $\rightarrow$ **IV**. The number of water hydrogen bonds formed at the contact O of AcO^- is 3.5(**II**) $\rightarrow$ 3.5(**III**) $\rightarrow$ 3.0(**IV**).

NACs are subspecies of **IV** in which the attack angle of $-\text{COO}^- \cdots \text{C}=\text{Cl}$ is within $\pm 15^\circ$ deviation from the bonding angle in the TS for the $\text{S}_{\text{N}}2$ displacement of Cl^- . The mole fraction of all dimeric species (**I**, **II**, **III**, **IV**, **V**, **VI**) present at any time that are NACs is calculated to be 0.16%. This result can be compared with 13.4% of the mole percentage of $\text{DhlA} \cdot \text{DCE}$ conformers present as NACs in the Michaelis complex (12).

Comparison of Reaction Coordinates in Water and in Enzyme: Energy Contribution of NAC Formation to Free Energy of Activation. By using the mole fraction of NAC found in the MD simulations, the free energy difference in forming a NAC in water and in the enzyme can be quantitatively compared. Both reaction coordinates start from the same standard 1 M concentration of the DCE, AcO^- and enzyme in aqueous solution (Scheme 4). From the literature, $1/K_{\text{m}}$ for the formation of $\text{DhlA} \cdot \text{DCE}$ complex is $2,000 \text{ M}^{-1}$ (ΔG° of substrate binding to $\text{DhlA} = -4.5 \text{ kcal/mol}$; ref. 13). We have shown that 13.4% of this $\text{DhlA} \cdot \text{DCE}$ complex is represented by $\text{DhlA} \cdot \text{NAC}$ with all of the enzyme-bound DCE in *gauche* forms. Therefore, on average $\text{DhlA} \cdot \text{NAC}$ is 1.2 kcal/mol above the average energy of $\text{DhlA} \cdot \text{DCE}$ Michaelis complex. Now the reaction coordinate for the reaction in water can be assembled. Computational study by Jorgensen *et al.* (14) has shown that either of the two degenerate *gauche* forms is 0.62 kcal/mol more stable than the *trans* form in water. This result gives *gauche*-DCE [$\text{DCE}(\text{g})$] being 0.1 kcal/mol above the total ground state. Formation of the $\text{AcO}^- \cdot \text{DCE}(\text{g})$ complex in which the $-\text{CH}_3$ moiety of AcO^- is in contact with the DCE carbon center (**I** or **II**) is 0.5 kcal/mol above the free energy of 1 M solution of $\text{DCE}(\text{g})$ and AcO^- . This complex is equivalent to $\text{DhlA} \cdot \text{DCE}$ complex in the enzymatic reaction. We show that the

aqueous NAC resides 3.2 kcal/mol above the $\text{AcO}^- \cdot \text{DCE}(\text{g})$ complex. In summary, the free energy difference between the NAC and the ground state of AcO^- and DCE at 1 M concentration is 3.8 kcal/mol, whereas the free energy difference between $\text{DhlA} \cdot \text{DCE}$ and $\text{DhlA} \cdot \text{NAC}$ is 1.2 kcal/mol. The activation energy is 26 kcal/mol in water (2) and 15.3 kcal/mol at the enzyme active site (1). Thus, the free energy difference in formation of NAC (2.6 kcal/mol) accounts for $\approx 25\%$ of the advantage of the enzymatic reaction ($\Delta \Delta G^\ddagger = 11 \text{ kcal/mol}$).

The remaining 75% of the kinetic advantage of the enzyme over water stems from the ability of the enzyme to facilitate the reaction from NAC to TS. We have previously examined the E-NAC and E-TS structures by MD simulations (12). The enzyme reaction has also been explored by AM1/MM in which AM1 Hamiltonian was recalibrated to reproduce the energetics and geometries along the $\text{S}_{\text{N}}2$ reaction path for nucleophilic attack of AcO^- at DCE (15). A good portion of this advantage can be explained by the half-polar and half-hydrophobic surrounding of S and TS (Fig. 5), which assures both proton dissociation from nucleophilic Asp-124-COO^- and the advantage of a low dielectric for such an $\text{S}_{\text{N}}2$ displacement (16). Additional factors in catalysis are the movement of water molecules away from the active site on formation of TS and the assistance to departure of Cl^- in the TS by Trp-125 and Trp-175 (12). Quantitation of the importance to the energetics of $\text{NAC} \rightarrow \text{TS}$ of the heterogeneous active site, desolvation of nucleophile, and assistance to departure of the leaving group is presently impossible.

We gratefully acknowledge computer time on the University of California, Santa Barbara, SGI Origin2000 and at the National Partnership for Advanced Computational Infrastructure (San Diego Supercomputer Center). This study was supported by a grant from the National Science Foundation (MCB-9727937) and provost funds, University of California, Santa Barbara.

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