Comparison of Reaction Energetics and Leaving Group Interactions during the Enzyme-Catalyzed and Uncatalyzed Displacement of Chloride from Haloalkanes

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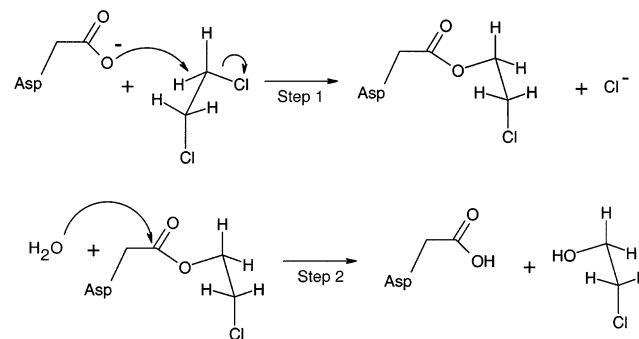*Received: November 12, 2002; In Final Form: February 24, 2003*

The S_N2 displacement of chloride from haloalkanes methyl chloride and 1,2-dichloroethane by a series of carboxylate nucleophiles is characterized using ab initio calculations. Comparison of reactivities of methyl chloride and 1,2-dichloroethane in the gas phase and aqueous solution reveals that relative reactivities of these two haloalkanes depend on the reaction environment. 1,2-Dichloroethane is more reactive in the gas phase than methyl chloride, but the order of reactivities is reversed in water. The origin of this effect is analyzed. The activation free energy barrier for the reaction between acetate and 1,2-dichloroethane is 25 kcal/mol (104.6 kJ/mol) in the aqueous environment. A similar reaction is catalyzed by the enzyme haloalkane dehalogenase with activation barrier of 15.3 kcal/mol (64.0 kJ/mol). The origin of this 10 kcal/mol (41.8 kJ/mol) catalytic effect is investigated by calculating how activation free energies depend on the dielectric constant of medium. Comparison of results obtained for the water environment and for the model of the active site environment indicates that a large percentage of this rate acceleration can be explained by the general hydrophobic nature of the active site. The enzyme also uses specific interactions with the chloride atom to facilitate the departure of this leaving group. We find that a single active site tryptophan residue interacts significantly more strongly with the leaving group in the transition state than in the ground state.

Introduction

The uncatalyzed aqueous hydrolysis of haloalkanes is a slow reaction that is accelerated by enzymes that belong to the haloalkane dehalogenase family. One of the best-studied members in this family is DhlA, the haloalkane dehalogenase from nitrogen-fixing bacterium *Xanthobacter autotrophicus* GJ10.[1,2] Structures for complexes of the enzyme with substrate 1,2-dichloroethane, covalently bound intermediate, and product halide ion have been determined at the atomic resolution.[2–4] These crystal structures reveal a mainly hydrophobic active site. Interestingly, dehalogenation of gaseous haloalkanes by solid haloalkane dehalogenase from *Rhodococcus rhodochrous* in the presence of water vapor has been reported recently.[5]

The enzymatic conversion of haloalkanes to corresponding alcohols involves two chemical reactions (Scheme 1). In the first step the active site carboxylate of Asp124 displaces halide ion in an S_N2 reaction yielding an alkyl enzyme intermediate.[6] In the second step, the alkyl enzyme intermediate is hydrolyzed by an activated water molecule, with His289 functioning as a general base catalyst.[7] The enzyme acts on several chlorinated and brominated substrates.[8] The kinetic mechanism of enzyme-catalyzed dehalogenation of 1,2-dichloroethane has been studied most.[8–12] The reaction appears to follow an ordered mechanism in which the displaced halide ion remains bound to the enzyme until the product of the second step, an alcohol or a halohydrin, dissociates.[9] The rate-limiting step in the overall conversion of 1,2-dichloroethane is thought to be an unimolecular isomerization of the enzyme preceding rapid halide dissociation.[9,13] The first chemical step, displacement of halide anion from haloalkane by the active site carboxylate, occurs with a rate constant of 50

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SCHEME 1

± 10 s^{-1}.[8] This corresponds to the activation free energy of 15.3 kcal/mol.[14] The uncatalyzed displacement of the halide atom from primary haloalkanes in water faces significantly higher barriers.[15] Rate constants for reactions of several haloalkanes with the acetate anion in water have been determined above the normal boiling point of water in sealed vials.[16–18] The reaction between hydroxide anion and methyl chloride in aqueous solution has been also studied experimentally, and an activation barrier of 24.6 kcal/mol has been determined.[19]

The origin of barrier lowering by haloalkane dehalogenases has been a subject of several previous studies.[3,10,20–27] Examination of crystal structures of the enzyme complexed with the substrate and the covalent intermediate reveals some factors that may contribute to the rate acceleration. First, the substrate and the nucleophilic carboxylate moiety are in a relatively nonpolar environment, and it is well-known that such S_N2 displacement reactions occur faster in nonpolar solvents than in water.[28] Second, two tryptophan residues are located in the active site in a manner such that favorable interactions with the leaving

TABLE 1: Basis Set and Electron Correlation Effects on the Strength of the C−Cl Bond[a]

	aug-cc-pVDZ	aug-cc-pVTZ	aug-cc-pVQZ	aug-cc-pV5Z	limit
MP2	6.77	8.12	8.60	8.84	8.95
MP3	6.39	7.69	8.14		8.38
MP4(SDQ)	6.16	7.47	7.97		8.25
CCSD	6.10	7.46	7.98		8.30
MP4(SDTQ)	5.69	6.90	7.37		7.67
CCSD-T	5.54	6.81	7.33		7.69

[a] Energy differences (in kcal/mol) between the minimum energy structure and the structure where the bond is stretched to 2.0 Å are shown. The values in the last column were obtained by single-exponential extrapolation of relative energies to the basis set limit.[37]

group may occur. Kinetic studies with mutant enzymes suggest that both Trp125 and Trp175 contribute somewhat to the rate acceleration.[10,20] Third, a favorable relative orientation of the substrate and the nucleophile in the active site is likely to contribute to the rate acceleration.[25] The relative significance of these factors is currently disputed.[14,29] We report now results from quantum mechanical calculations that allow the estimation of the relative importance of tryptophan residues as well as the nonpolar environment in the rate acceleration by haloalkane dehalogenase.

Computational prediction of reaction free energies and activation free energies of biochemical reactions in water or enzyme active sites with accuracy comparable to the experiment is a challenging task. Such accuracy, however, may be necessary to assess the relative importance of factors that contribute to the power of enzyme catalysis. We have now systematically evaluated the effects of computational details such as the level of electron correlation and size of the basis set on the energetics of S_N2 displacement reactions of chloride by a series of carboxylate nucleophiles.

Methods

It is known from numerous previous studies that electron correlation and basis set effects significantly affect barrier heights and reaction energies in S_N2 reactions.[23,30−33] To study the origin of this dependence and establish a reliable calculation methodology, two processes—adiabatic stretching of the C−Cl bond in methyl chloride and attachment of proton to formate anion—were examined. The C−Cl bond stretching was studied by optimizing methyl chloride at the MP2 level with aug-cc-pVDZ, aug-cc-pVTZ, and aug-cc-pVQZ basis sets[34] to the energy minimum and to a structure where the C−Cl bond is stretched to 2.0 Å. Single point calculations up to CCSD-T level were then performed with each basis. The MP2/aug-cc-p5Z single point calculation was performed using MP2/aug-cc-pQZ geometries. Theoretical proton affinity of formate was calculated similarly from quantum mechanical energies of formate and formic acid by adding thermal corrections obtained at MP2/aug-cc-pVDZ and MP2/aug-cc-pVTZ levels. Results of these preliminary studies (Tables 1 and 2) indicate that the C−Cl bond strength is underestimated with small basis sets, that the MP2 level significantly overestimates this bond strength, and that at least CCSD-T/aug-cc-pVTZ level is necessary in order to obtain a converged value for the proton affinity of formate. Previously, triples' contribution has been found to be significant in describing the barrier height in identity S_N2 reactions.[31] However, the MP4(SDQ)/aug-cc-pVTZ level appeared to be a good compromise between computational speed and accuracy as the lack of a triples effect nearly canceled out basis set effects at this level. The suitability of various electron correlation methods and basis sets in describing displacement reactions by carboxylates was further investigated by calculating the reaction energy and the activation energy for the reaction between the

TABLE 2: Basis Set and Electron Correlation Effects on the Proton Affinity of Formate[a]

	aug-cc-pVDZ	aug-cc-pVTZ	aug-cc-pVQZ
MP2	347.13	348.61	348.64
MP3	352.83	354.48	354.51
MP4(SDQ)	350.98	352.85	353.04
CCDS	351.79	353.55	
MP4(SDTQ)	348.39	349.74	349.76
CCSD-T	350.20	351.62	

[a] Values in the table are relative energies of formate anion compared to formic acid. The experimental gas phase proton affinity of formate is 345 ± 2 kcal/mol,[38] from which the "experimental" energy difference of 352.2 ± 2 kcal/mol can be obtained after zero point and thermal correction (7.25 kcal/mol at the MP2/aug-cc-pVTZ level).

formate anion and methyl chloride. Reactants and products were optimized at the MP2/aug-cc-pVTZ level of theory because this level is known to produce accurate geometries.[35] Reaction exothermicity was determined by single point calculations using three correlation consistent basis sets (aug-cc-pVDZ, aug-cc-pVTZ, and aug-cc-pVQZ), and the electron correlation effects were described via Møller−Plesset perturbation theory (MP2, MP3, and MP4) or by the coupled-clusters approach including triples via perturbative treatment (CCSD-T).[36] Thermal and entropic contributions to the free energy were calculated at the MP2/aug-cc-pVDZ level. Results from these studies (Table 2S in the Supporting Information and Table 3 in the Results section) also suggested that the MP4(SDQ)/aug-cc-pVTZ level is suitable for characterizing energetics of the displacement of chloride by carboxylates.

The transition state structure for the reaction between methyl chloride and formate was calculated at the MP2/6-31+G(d,p), MP2/aug-cc-pVDZ and MP2/aug-cc-pVTZ levels to test how much the structure depends on the choice of the basis set. Calculated transition state structures did not depend significantly on the choice of basis set. Frequency calculations at the MP2/aug-cc-pVDZ level confirmed that the optimized structure is the first-order saddle point. It has been demonstrated that the MP2 theory performs well in predicting the saddle point structure for an identity S_N2 displacement reaction with methyl chloride.[31] A partial reaction profile for the formate−methyl chloride reaction in the gas phase was obtained by varying the distance from the nucleophilic carboxylate oxygen to the electrophilic carbon and optimizing all other degrees of freedom at the MP2/aug-cc-pVDZ level. At distances larger than 2.8 Å it became necessary to fix the O−C−Cl angle to a value 179.9° to speed up the convergence. The ion−dipole complex along the reaction path was reoptimized at the MP2/aug-cc-pVTZ level. Single point calculations were performed at the MP4-(SDQ)/aug-cc-pVTZ level. Thermal and entropic contributions were calculated at the MP2/aug-cc-pVDZ level and added to the MP4(SDQ)/aug-cc-pVTZ energy differences to obtain the free energy profile in the gas phase. Charge distributions in molecules were analyzed using the CHelpG method with MP2

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electron densities.[39] Only valence electrons were correlated during the reaction energy profile evaluations.

Solvent effects on the reaction profile were estimated using a polarizable conductor (COSMO/CPCM) model.[40] Three environments with dielectric constants of 2.247 (as a model of a very hydrophobic active site), 10.36 (as a model of a weakly hydrophobic active site), and 78.39 (as a model for water) were considered. The calculations in the presence of a polarizable medium were performed at the MP2/aug-cc-pVTZ level. The free energy profile in solution was obtained by adding the zero point vibrational energy contribution and the solvation free energy contribution to the gas phase MP4(SDQ)/aug-cc-pVTZ energy difference. It should be noted that such an approach provides only an approximate value for the reaction free energy profile because (i) gas phase geometries are used, (ii) changes in vibrational partition functions upon going from the gas phase to solvent are neglected,[41] (iii) individual first-solvation shell effects are treated in an approximate way,[42] (iv) entropic and enthalpic contributions due to translational and rotational motions are assumed to cancel out,[43] and (v) the nonequilibrium solvation effects are ignored.[44,45] The use of gas phase geometries is likely to lead to overestimation of activation barriers, while neglecting the contribution from translational and rotational motions and the nonequilibrium solvation typically is expected to result in underestimation of activation free energies.[45,46]

To evaluate the effect of the nucleophile structure, reactions with acetate and propionate were also studied. The acetate reaction was characterized as described above, but for the propionate reaction only the gas phase reaction energy was evaluated. The reaction energies and activation free energies in the gas phase and in water were also calculated with 1,2-dichloroethane as the electrophile. The reaction between formate and 1,2-dichloroethane and between acetate and 1,2-dichloroethane were studied at the MP4(SDQ)/aug-cc-pVDZ//MP2/aug-cc-pVDZ level because optimization at the MP2/aug-cc-pVTZ level was prohibitively expensive for these systems. The solvent effects were evaluated at the MP2/aug-cc-pVDZ level, and the zero point energies for the acetate plus 1,2-dichloroethane reaction were evaluated at the MP2/6-31+G(d,p) level. Reactant complexes were not studied for these reactions because their free energy is expected to be above the isolated reactants in water.[47]

Interaction strengths of the ground state, the transition state, and the product (chloride ion) with a tryptophan residue in the active site of haloalkane dehalogenase were determined via quantum mechanical calculations. The reaction between the 1,2-dichloroethane and carboxylate of Asp124 was modeled as a nucleophilic substitution reaction between the formate anion and methyl chloride. The use of a smaller formate−methyl chloride system instead of the more realistic acetate−dichloroethane system is justified because the two systems had very similar transition state structures (e.g., R_{C-Cl} was 2.255 Å vs 2.260 Å at the MP2/aug-cc-pVDZ level) and gas phase reaction energy profiles. Furthermore, the extent of charge development on the leaving chloride atom was very similar in both systems along the reaction coordinate. The interaction energies of the ground state, the transition state, and the product state with the active site tryptophan residue were estimated by varying the distance between 3-methylindole and the chlorine atom and calculating the energy of the system at the MP2/aug-cc-pVDZ level. The mutual orientation of 3-methylindole and the transition state was such that the breaking carbon−chlorine bond was collinear with the indole N−H bond. Throughout, all electrons were included
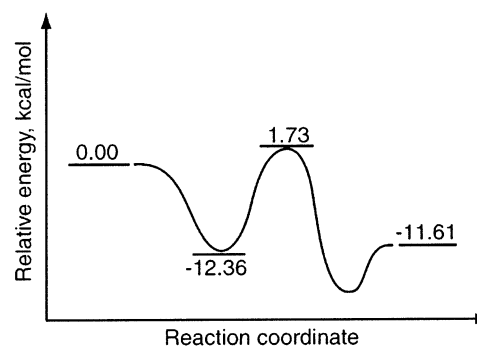

Figure 1. Reaction path in the gas phase for displacement of chloride from methyl chloride by formate.

in the correlation treatment. Basis set superposition error (BSSE) on interaction energies was corrected via a counterpoise scheme.[48,49] Similar calculations were performed with a single water molecule interacting with the methyl chloride, the transition state, and the chloride anion. To estimate how the environment modulates these interaction strengths, some of these calculations were repeated in the presence of the implicit solvent using a polarizable conductor (COSMO/CPCM) model.[40] The enzyme active site was described by a dielectric constant of 2.247 (benzene model), and the aqueous environment was described by a dielectric constant of 78.39 (water model). The interaction energies in the polarizable medium were performed at the MP2/aug-cc-pVDZ level and corrected for the basis set superposition error by assuming that the percentage of error remains the same in the gas phase and in dielectric environment. All quantum mechanical calculations were performed with the program Gaussian98.[50]

Results

Zero point energies, solvation energies, and symmetry groups for molecules discussed in this paper are given in Table 1S of the Supporting Information. This Results section is organized such that the net reaction energies are given first for each of the reactions considered, then activation energy data are presented, and the interaction energies with an enzymatic residue are given last.

Reaction Energies. The reaction between formate and methyl chloride follows a classical double-well potential energy path in the gas phase (Figure 1). Reaction energies with different basis sets and electron correlation treatments for this reaction are given in Table 2S in the Supporting Information. Results from MP4(SDQ)/aug-cc-pVTZ calculations are discussed below unless otherwise noted. The energy of isolated products, (Z)-methyl formate and chloride anion, is 11.6 kcal/mol lower than the energy of isolated reactants at this level. The reaction is exothermic (−9.0 kcal/mol) and exergonic (−6.4 kcal/mol) in the gas phase at 25 °C. Reasonable agreement is observed with experimental gas phase reaction enthalpy of −10.1 ± 2.3 kcal/mol.[51] Treatment of solvent effects via the polarizable conductor model predicts that the reaction free energies are −12.1, −13.8, and −15.5 kcal/mol for dielectrics of 2.247, 10.36, and 78.39, respectively. The reaction free energy in water can be estimated also using experimental free energies of hydration for chloride, formate, methyl chloride, and methyl formate.[38,52,53] Such a calculation leads to an aqueous reaction free energy of −13.9 kcal/mol as the products are solvated preferentially over reactants by 2.3 kcal/mol. However, the latter value must be treated with due caution because its uncertainty may be as high as 4−5 kcal/mol due to large uncertainties in the values of hydration free energies of formate and chloride anions.
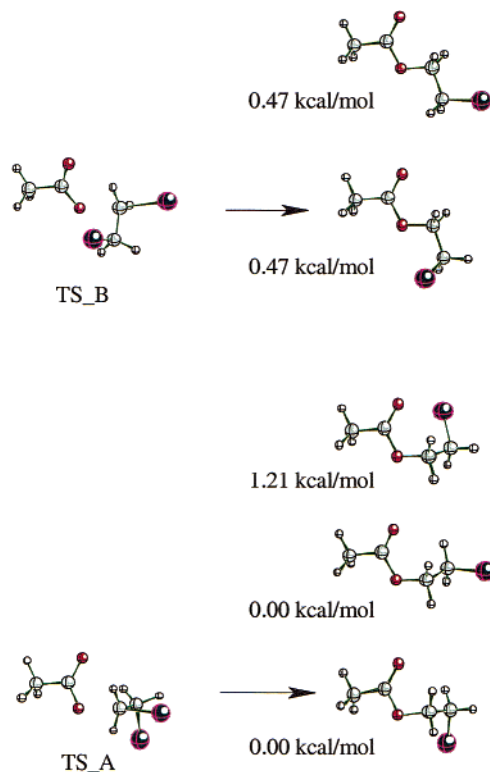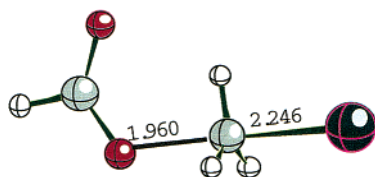
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CHART 1

0.47 kcal/mol

0.47 kcal/mol

TS_B

1.21 kcal/mol

0.00 kcal/mol

0.00 kcal/mol

TS_A

CHART 2

1.960   2.246

The reaction between acetate and methyl chloride is characterized by a reaction energy of −15.1 kcal/mol in the gas phase at the MP4(SDQ)/aug-cc-pVTZ level. Reaction energies at other levels are given in Table 3S in the Supporting Information. Thermal and entropic corrections yield a reaction free energy of −9.1 kcal/mol in the gas phase at 25 °C. The polarizable conductor model predicts that the reaction free energy in aqueous solution is −18.5 kcal/mol. Experimental free energies of solvation suggest that products methyl acetate and chloride are preferentially solvated by 6.8 kcal/mol,[38,52,53] leading to an aqueous free energy of −21.9 kcal/mol. The reaction energy for the propionate plus methyl chloride reaction was −15.3 kcal/mol in the gas phase. Very similar reactivity of acetate and propionate toward methyl chloride suggests that the acetate anion is a good model for the active site aspartate.

The reaction between formate and 1,2-dichloroethane leads to 2-chloroethyl formate. This species has five distinct low-energy conformers with the ester bond in the cis orientation. The lowest energy structure in the gas phase can be characterized as gauche−anti because the CO−CC dihedral is 81.2° and the OC−CCl dihedral is 175.2°. Another conformer, classified as gauche−gauche (the two dihedral angles are 77.5° and 62.0°, respectively) appears to be slightly more stable in aqueous solution. Conformers where the ester bond is in the trans orientation are expected to be higher in energy. The reaction energy at the MP4(SDQ)/aug-cc-pVDZ level is −15.2 kcal/mol when gauche 1,2-dichloroethane gives the lowest energy conformer of the product in the gas phase. On the basis of data

TABLE 3: Energy of the Transition State Relative to Isolated Reactants (in kcal/mol) with Different Basis Sets and Treatments of Electron Correlation[a]

	formate		acetate	
	aug-cc-pVDZ	aug-cc-pVTZ	aug-cc-pVDZ	aug-cc-pVTZ
MP2	−1.09	1.41	−2.01	0.52
MP3	0.02	2.35	−0.77	1.58
MP4(SDQ)	−0.88	1.73	−1.71	0.92
CCSD	−0.81	1.81	−1.62	
MP4(SDTQ)	−2.96	−0.65	−3.84	
CCSD-T	−2.88		−3.70	

[a] Results for reactions of methyl chloride with formate and acetate nucleophiles in the gas phase are shown.

TABLE 4: Reaction Profiles for the Reaction between Formate and Methyl Chloride in the Gas Phase and Three Dielectric Environments (All Values for 1 M Standard State)[a]

	reactant complex	transition state	isolated products
ΔE(gas)	−12.36	+1.73	−11.61
ΔG(gas)	−6.4	+9.8	−6.4
$\Delta G(\epsilon=2.247)$	−3.7	+13.0	−12.1
$\Delta G(\epsilon=10.36)$	+1.0	+19.6	−13.8
$\Delta G(\epsilon=78.39)$	+4.0	+23.3	−15.3

[a] Energies relative to isolated reactants are shown in kcal/mol.

in Tables 2S and 3S, we estimate that the MP4(SDQ)/aug-cc-pVTZ value would be −12.9 kcal/mol. The estimated reaction free energy in water is −15.2 kcal/mol.

The reaction between acetate and 1,2-dichloroethane in the gas phase leads to 2-chloroethyl acetate. The product can adopt five conformers where the ester bond is in the cis conformation (Chart 1). The form found in the enzyme active site (crystal structure Protein Data Bank code 2DHD) resembles one of the lowest energy conformers. Docking of the other lowest energy conformer to the active site does not reveal any steric conflicts, and it is possible that the observed structure of the enzyme-bound chloroethyl group represents a time average of two or more interconverting conformers. This hypothesis could be tested by molecular dynamics simulations of the enzyme-bound intermediate. The net energy for the acetate plus gauche 1,2-dichloroethane reaction in the gas phase was −19.3 kcal/mol at the MP4(SDQ)/aug-cc-pVDZ level. On the basis of the trends shown in Tables 2S and 3S (Supporting Information), we estimate that the MP4(SDQ)/aug-cc-pVTZ value would be −17.0 kcal/mol. Thermodynamic corrections yield an estimated reaction free energy of −10.5 kcal/mol in the gas phase and −19.0 kcal/mol in water. From a thermodynamic point of view, methyl chloride and 1,2-dichloroethane behave quite similarly while formate appears slightly less nucleophilic than acetate or propionate toward haloalkanes both in the gas phase and in water.

Activation Energies. The energy separation between the transition state structure (Chart 2) and isolated reactants depends on the choice of basis set and electron correlation treatment as shown in Table 3. As above, we use MP4(SDQ)/aug-cc-pVTZ//MP2/aug-cc-pVTZ results for further discussion. At this level, the transition state for the formate plus methyl chloride reaction lies 1.73 kcal/mol above the energy of isolated reactants. However, the reactants pass through a strong ion−dipole complex before forming the transition state in the gas phase (Table 4). The minimum energy structure occurs when the nucleophilic oxygen is 2.89 Å from the carbon atom on the BSSE-uncorrected MP2 energy surface. The energy separation between the ion−dipole complex and the transition state is 14.1

TABLE 5: Reaction Energies, Reaction Free Energies, and Activation Free Energies for Halide Displacement Reactions in the Gas Phase (g) and in Aqueous Solution (w)[a]

reactants	ΔE(g)	ΔG(g)	ΔG(w)	$\Delta E^{\ddagger}$(g)	$\Delta G^{\ddagger}$(g)	$\Delta G^{\ddagger}$(w)
formate + methyl chloride	−11.61	−6.4	−15.3	+1.73	+9.8	+23.3
acetate + methyl chloride	−15.09	−9.1	−18.5	+0.92	+9.3	+23.0
propionate + methyl chloride	−15.33	−9.3	−19.3	N/A	N/A	N/A
formate + 1,2-dichloroethane	(−12.9)	(−7.3)	(−15.2)	(−1.5)	(+7.7)	(+25.8)
acetate + 1,2-dichloroethane	(−17.0)	(−10.5)	(−19.0)	(−2.8)	(+6.1)	(+24.8)

[a] All values reported are relative to the isolated reactants as evaluated at the MP4(SDQ)/aug-cc-pVTZ level. Values in parentheses were obtained by adding a correction to the corresponding MP4(SDQ)/aug-cc-pVDZ energy difference. The correction was 2.30 kcal/mol for reaction energies and 2.60 kcal/mol for activation energies.

kcal/mol. The activation enthalpy at room temperature is 2.62 kcal/mol relative to isolated reactants and 14.4 kcal/mol relative to the ion−dipole complex. The small magnitude of the activation enthalpy relative to reactants suggests that the experimental observation of the reaction between formate and methyl chloride in the gas phase should be feasible.[54] The free energy profile for S_N2 reactions frequently differs from the energy profile due to thermal and entropic effects. Forming a reactant complex from isolated reactants carries a substantial entropic penalty in the gas phase. Thermal and entropic corrections for the reaction between formate and methyl chloride yield a transition state that is 16.2 kcal/mol above the reactant complex. Solvent effects have a pronounced influence on reaction profiles of S_N2 reactions.[28] In the current case, the transition state is less solvated by 10.2 kcal/mol in the medium with a dielectric constant of 2.247 (such as benzene) and by 20.4 kcal/mol in the medium with a dielectric constant of 78.39 (such as water) when compared to the isolated reactants. The ion−dipole complex is also less solvated than isolated reactants. In water, the reactant complex is not stable but lies 4.0 kcal/mol above the free energy of isolated reactants. The activation free energy values for the reaction in dielectric environments with dielectric constants 2.247, 10.26, and 78.39 were 16.8, 19.6, and 23.3 kcal/mol, respectively.

The structure of the transition state with acetate as the nucleophile is virtually identical to that of the transition state when formate is the nucleophile (R_{C-Cl} is 2.235 Å versus 2.246 Å at the MP2/aug-cc-pVTZ level). The acetate−methyl formate transition state lies 0.92 kcal/mol above the energy of isolated reactants (Table 3), suggesting that acetate is a slightly better nucleophile than formate in the gas phase. The free energy of the transition state is 9.3 kcal/mol above the free energy of isolated reactants in the gas phase at 1 M standard state; the corresponding free energy value in the aqueous solution at room temperature is 23.0 kcal/mol.

Energy profiles for formate−1,2-dichloroethane and acetate−1,2-dichloroethane reactions are similar to the formate−methyl chloride reaction profile in the gas phase except that the substrate, the transition state, and the product can adopt several conformations. 1,2-Dichloroethane exists in two conformers, of which the trans conformer is the global minimum in the gas phase.[55,56] The gauche conformer, which has a dipole moment of about 3 D, is stabilized by polar solvents relative to the trans form,[57] and the gauche form is predominant in the aqueous solution.[58,59] The gauche form is more reactive toward the nucleophilic displacement because the trans conformer gives rise to a transition state structure where the incoming nucleophile is eclipsing with the halogen at the adjacent carbon. Molecular dynamics simulations of the enzyme-bound 1,2-dichloroethane show that the gauche form of the substrate resides in the active site.[60]

The energy of the transition state for the formate plus 1,2-dichloroethane reaction was 4.1 kcal/mol below the energy of the isolated reactants in the gas phase at the MP4(SDQ)/aug-cc-pVDZ level. Data from Table 3 suggest that the MP4(SDQ)/aug-cc-pVTZ energy difference would be more positive by 2.6 kcal/mol. We thus estimate that the transition state is 1.5 kcal/mol below the isolated reactants at the MP4(SDQ)/aug-cc-pVTZ level. 1,2-Dichloroethane thus appears to be a slightly better electrophile than methyl chloride in the gas phase. However, the transition state with 1,2-dichloroethane is not well solvated compared to the transition state with methyl chloride. As a result, the aqueous phase free energy of activation for the formate plus 1,2-dichloroethane reaction (25.8 kcal/mol) is slightly higher than the free energy of activation with methyl chloride (23.3 kcal/mol).

Two transition state conformers that lead to the formation of *cis*-(Z)-chloroethyl acetate were identified (Chart 1). Using data from Table 3, we estimate that the gas phase energy of this transition state at the MP4(SDQ)/aug-cc-pVTZ level would be 2.8 kcal/mol below the isolated reactants. The second transition state, TS_B in Chart 1, was 1.6 kcal/mol above TS_A in the gas phase. Assuming that the reaction starts with gauche dichloroethane and proceeds via TS_A in the aqueous solution, we calculate the activation free energy barrier of 24.8 kcal/mol for the nucleophilic displacement of chlorine from 1,2-dichloroethane by acetate. The energetics of five halide displacement reactions with carboxylate nucleophiles is summarized in Table 5.

Assistance to Leaving Group Departure by Indole. The interaction energies of methyl chloride, methyl chloride−formate transition state, and chloride with 3-methylindole in the gas phase are shown in Figure 2. Figure 3 presents interaction energies of the transition state and chloride with 3-methylindole in two dielectric environments. Qualitatively similar results were obtained in calculations (using MP2/6-31+G(d,p) level) where the 1,2-dichloroethane−acetate transition state interacted with 3-methylindole. Energies for interaction with a single water molecule are substantially smaller as shown in Figure 4.

Discussion

The haloalkane dehalogenase reaction has been the subject of several previous theoretical studies. The reaction profiles in the gas phase and in a polarizable dielectric ($\epsilon = 78.3$) have been characterized at the MP2/6-31+G(d)//HF/6-31+G(d) level,[23] and the reaction path including some active site residues has been studied by pure semiempirical[21,22] or by hybrid QM/MM methods.[25,27] The noncatalyzed reaction in water has been modeled using empirical valence bond methodology,[14] and possible ground state configurations have been examined by classical molecular dynamics simulations.[47] In addition, molecular dynamics simulations of haloalkane dehalogenase, either free[61] or complexed with substrate and the transition state,[60] have been reported. However, several important questions such as the magnitude and the origin of rate acceleration by this enzyme remain disputed.[14,23,25,29,47]

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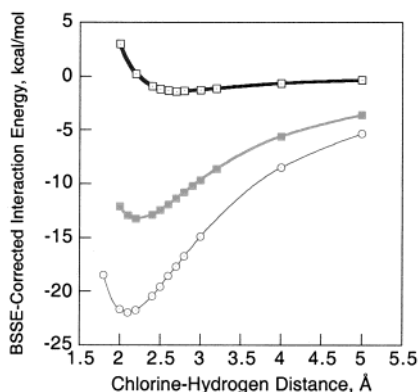
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Figure 2. Interaction energies of 3-methylindole with methyl chloride (open squares), the transition state (closed squares), and the chloride ion (open circles) in the gas phase.
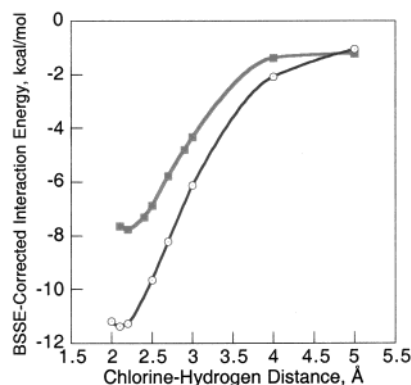

Figure 3. Interaction energies of 3-methylindole with the transition state (closed squares) and the chloride ion (open circles) in the dielectric mimicking benzene.
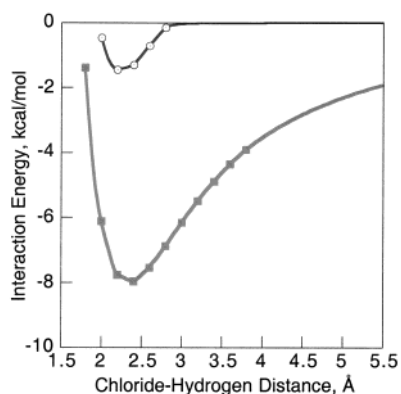

Figure 4. Interaction energies of a water molecule and the transition state in the gas phase (closed squares) and in the dielectric mimicking water (open circles). For comparison, the interaction energy between a water molecule and methyl chloride ground state in the gas phase is −0.6 kcal/mol.

Noncatalyzed Reaction. The first goal of current work was to obtain a reliable estimate of the magnitude of rate enhancement by haloalkane dehalogenase. The rate acceleration is defined here as a difference in standard free energies of activation for water-catalyzed and enzyme-catalyzed reaction between 1,2-dichloroethane and carboxylate of aspartate side chain. The experimental rate constant for the catalyzed displacement step can be obtained by analyzing rapid-kinetics data for dehalogenation of haloalkanes. The value of $50 \pm 10 \ s^{-1}$ has been determined for the dehalogenation of 1,2-dichloroethane in the active site of DHlA. Such analysis is needed due to the fact that the chemical displacement step is one of the fastest

steps in the overall reaction sequence.[8] Experimental rate constants for the uncatalyzed S_N2 displacement of chloride by carboxylates have been determined at elevated temperatures.[16−18] The rate constants at room temperature can be obtained by extrapolation of known rate constants,[27] estimated from similar reactions,[14] or calculated quantum mechanically.

The reaction between methyl chloride and formate has been studied in detail in the gas phase and in three dielectric environments (Table 4). The reaction free energy and the activation free energy for this reaction in water are −15.3 and 23.3 kcal/mol, respectively. The experimental rate constant for this reaction at 120 °C in 50% aqueous acetone has been measured as $16.0 \times 10^{-4} \ M^{-1} \ s^{-1}$.[16] From the transition state theory with unit transmission coefficient, activation free energy of 28.2 kcal/mol is obtained at 120 °C. No experimental activation enthalpy and activation entropy data are available for this reaction, and we used ab initio gas phase partition functions to estimate that $\Delta G^{\ddagger}$ is lowered by 3.4 kcal/mol when temperature is decreased from 120 to 25 °C. Because the translational and rotational entropy contribution to transition state formation is smaller in the water than in the gas phase,[43,62,63] this 3.4 kcal/mol estimate is actually a upper bound and the experimental activation free energy is between 24.8 and 28.2 kcal/mol. It appears that our calculation methodology slightly underestimates activation free energies for S_N2 reactions in water. We find that the reactant complex is thermodynamically unstable in aqueous solution by about 4 kcal/mol. A very similar value was reported recently for the acetate−dichloroethane complex using classical molecular dynamics simulations of this bimolecular collision process.[47] Current results show that acetate is a slightly better nucleophile than formate toward haloalkanes, consistent with the higher aqueous pK_a value of acetic acid as compared to formic acid. However, the activation free energies in water are rather insensitive to the structure of the carboxylate anion.

The purely theoretical estimate for the activation free energy for the noncatalyzed reaction between gauche 1,2-dichloroethane and acetate is 24.8 kcal/mol in water. To compare this value with experimental data, a correction due to the presence of nonreactive trans conformer in water must be made. On the basis of recent reanalysis[59] of experimental data, we calculate that the mole fraction of gauche form is between 0.58 and 0.71 at room temperature. The corresponding statistical correction to free energy of activation is 0.2−0.3 kcal/mol, and $\Delta G^{\ddagger}$ for the reaction between 1,2-dichloroethane and acetate is estimated to be about 25 kcal/mol. This estimate is substantially lower than the previously published value (32−38 kcal/mol) from our group[23] but agrees well with a value (25 kcal/mol) derived recently by extrapolating from related reactions.[14] The experimental $\Delta G^{\ddagger}$ for this reaction in water at 120 °C is 29.6 kcal/mol.[17] The dependence of rate constant on temperature over a narrow (20 degrees) temperature range gives an activation enthalpy $\Delta H^{\ddagger} = 19.6$ kcal/mol and an activation entropy $\Delta S^{\ddagger} = -26.5$ cal $M^{-1} \ K^{-1}$, from which $\Delta G^{\ddagger}$ of 27.5 kcal/mol can be obtained at 25 °C. As in the case of the formate plus methyl chloride reaction, the calculated $\Delta G^{\ddagger}$ value is slightly below the extrapolated experimental value. It is likely that the major part of the difference arises from the neglect of contributions from translational and rotational motions in reactants and the transition state. This hypothesis is supported by the magnitude of experimental activation entropy. The experimental $-T\Delta S^{\ddagger}$ term is 7.9 kcal/mol, which is expected to be largely due to the loss of translational and rotational degrees of freedom upon transition state formation. The contribution of translational and rotational motions to the enthalpy of activation is no larger than

−6RT (−3.5 kcal/mol).[43] The expected correction to activation free energy due to rotational and translational degrees of freedom is thus less than 4.4 kcal/mol. Using our theoretical value and the experimental free energy of activation for the enzymatic reaction, the net rate acceleration by the DHlA enzyme is about 10 kcal/mol while a purely experimental estimate gives a rate acceleration of about 12 kcal/mol.

Effect of β-Chloro Substituent in the Gas Phase and in Water. 1,2-Dichloroethane is less reactive than methyl chloride in water, consistent with a general trend that displacement of halogens from ethyl halides is slower than a similar displacement from methyl halides.[64] For example, the activation barriers with ethyl halides are frequently 1.3−1.9 kcal/mol higher than barriers with methyl halides in solution.[30,64] Steric effects are more dominant in the gas phase where the barrier with ethyl halides is 3.3−3.4 kcal/mol larger than the barrier with methyl halides.[30] However, comparison of transition state energies suggests that 1,2-dichloroethane is more reactive than methyl chloride in the gas phase. The apparent anomaly can be explained by the effect of the chlorine atom on an adjacent carbon because the ethyl chloride has been found to be significantly less reactive than 1,2-dichloroethane in the gas phase.[23] Experimental evidence for the rate accelerating effect of adjacent halogens in gas phase S_N2 reactions has been reported very recently.[65] Such rate acceleration by simple electronegative substituents has been explained as a field effect arising from a favorable charge−dipole interaction between the anionic reaction center and the dipole of the adjacent carbon−halogen bond.[65−67] The activating effect of the β-halogen is strongly masked in the solution,[68] and two explanations for this reversal of reactivity have been discussed.[65] The first explanation involves desolvation of polar atoms by the electron-withdrawing substituent; the second proposes that solvation alters the electronic structure of the S_N2 transition state such that the electrophilic carbon would gain a substantial positive charge in the solvated transition state.[65] We have tested the second proposal by reoptimizing the transition state for the formate plus methyl chloride system in the presence of two water molecules (Figure 1S in the Supporting Information) and find that the charge distribution of such a microsolvated transition state does not differ significantly from the charge distribution of the gas phase transition state. Also, the presence of the reaction field as in the COSMO model does not lead to a noticeable change in the Mulliken charge distribution. Our results support the model where the polar atoms of the attacking carboxylate and the leaving group are less solvated in the presence of a bulkier chloroethyl group. The observation[68] that fluorine, chlorine, and bromine substituents at the β-position lead to similar S_N2 reaction rates in methanol does not contradict the steric model because these halogens also affect gas phase reaction profiles. For example, bromine is expected to hinder the aqueous reaction more than chlorine due to its larger size, but it also lowers the transition state energy in the gas phase.[65] Furthermore, the transition state can adopt several conformers (Chart 1) and the population of each of the transition state conformers is likely to depend on the structure of the substituent at the β-carbon.

Nonspecific Solvation Effect. The role of nonspecific solvation in this rate acceleration can be estimated by comparing the activation free energies of a theoretical spontaneous reaction in hydrophobic environment with the enzyme-catalyzed reaction. The active site of haloalkane dehalogenase is partially hydrophobic, and it might be modeled with a dielectric constant in the range 2−10.[69] The activation barrier in dielectric of 2.247 thus provides the lower limit for the activation barrier in a

hypothetical enzyme that would catalyze the reaction only by virtue of a nonpolar environment. Similarly, the activation barrier in dielectric of 10.36 might provide an upper limit for this value. If the activation free energy is taken as the free energy difference between the transition state and the ion−dipole complex (shown in Table 4), then the activation free energy is 16.8 kcal/mol at $\epsilon = 2.247$ and 18.6 kcal/mol at $\epsilon = 10.36$. We note that we are using here data obtained with methyl chloride because data in Table 5 suggest that reaction energies with methyl chloride and 1,2-dichloroethane are very similar in nonpolar environment. Comparison of calculated activation free energy (e.g., 18.6 kcal/mol) with the value derived from the experimentally measured rate constant (15.3 kcal/mol) shows that a substantial part of the rate acceleration can be explained by nonpolar milieu in the active site. However, the haloalkane dehalogenase must use some specific interactions to lower the energy of the transition state relative to the ground state because the activation barrier even at $\epsilon = 2.247$ (16.8 kcal/mol) is still higher than the observed activation barrier. On the other hand, if the true dielectric of the active site is as low as 2.247, a large majority of the rate acceleration can be attributed simply to the change of polarity of the environment.

Role of Specific Interactions. Two tryptophan residues, Trp125 and Trp175, are located in the active site near the leaving halogen atom.[3] These residues interact with the leaving group in the transition state and are responsible for binding of the halide ion after the completion of the first chemical step. Experimental work shows that either Trp175 or Trp125 can be replaced with another aromatic amino acid with only moderate loss of catalytic activity.[10,20] For example, Trp175Tyr mutant performs the halide displacement step with about half the speed of the wild-type enzyme when 1,2-dibromoethane is used as a substrate.[10] The reaction with 1,2-dichloroethane appears to be slowed more, such that k_{cat}/K_m (which in this case correlates with the rate of halide displacement step) is decreased by 60-fold and the halide displacement step becomes rate limiting.[10] The mutation of Trp125 to phenylalanine leads to 20-fold reduction in k_{cat}/K_m for 1,2-dichloroethane.[20] Early semiempirical calculations of the reaction path in the enzyme were in accord with a proposal[3] that interaction of these two tryptophan residues with the transition state is important for catalysis. A decrease of activation energy by almost 10 kcal/mol was attributed to interaction with these two tryptophans.[21] More recent hybrid QM/MM calculations using system-specific parameterization suggested that the hydrogen bonding of chloride in transition state to tryptophans accounts for only 2−4 kcal/mol.[25] We have now calculated interaction strengths of the substrate, the transition state, and the product with a 3-methylindole molecule, which mimics one of the active site tryptophans (Figures 2 and 3). Comparison of interaction energies of the reacting system in the gas phase shows that the ground state, where the chloride atom carries a partial negative charge of −0.17, interacts very weakly (−1.4 kcal/mol) with the tryptophan residue. The interaction is significantly stronger (−13.3 kcal/mol) in the transition state (partial charge on the leaving chloride −0.72) and even stronger with the chloride anion (−22.0 kcal/mol). These interactions are attenuated by the dielectric environment. By modeling the active site as a homogeneous dielectric with $\epsilon = 2.247$, the transition state interaction is attenuated to −7.6 kcal/mol while the chloride ion interaction energy is −12.4 kcal/mol when the interaction distances are optimal.

It is interesting to compare current minimum-energy interaction distances to available experimental or molecular dynamics-derived data. The interaction energy between 3-methylindole

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and the ground state is most negative when the separation between the polar hydrogen of the indole moiety and the leaving chloride is 2.7 Å. In the molecular dynamics (MD) simulation of the E·S complex this distance fluctuated quite significantly and only one of two tryptophans was close enough to interact with the substrate. When such an interaction occurred, the corresponding distance was in the range of 2.4−3.2 Å.[60] The same distance in the transition state reaches an energy minimum near 2.2 Å according to current calculations. For comparison, the average distances in MD simulation of the E·TS complex were 2.24 ± 0.19 Å for Trp175 and 2.43 ± 0.29 Å for Trp125. This suggests that the active site near the leaving group is not unusually compressed or stretched in the ground and transition states. The minimum energy distance with the chloride anion is only 2.1 Å in the gas phase. Tryptophan−chloride distances in the crystal structure of the enzyme−chloride complex[3] are slightly longer, possibly suggesting that the product is not optimally bound. Such nonoptimal binding of the product facilitates the overall catalytic conversion because chloride release or the associated conformational change is the rate-limiting step during the turnover of 1,2-dichloroethane.

The interaction strengths shown above are only approximations to true interaction energies between the reacting system and enzyme because of at least four factors. First, the transition state that was used for interaction energy calculations was optimized in the gas phase. The transition states for S_N2 reactions with a negative leaving group and a negative nucleophile are relatively implastic, meaning that their structure does not depend significantly on the environment.[28] When the transition state structure was reoptimized in the presence of 3-methylindole (with distance between the leaving chloride and the polar hydrogen of 2.2 Å), a slightly earlier transition state ($r_{C−O} = 2.07$ and $r_{C−Cl} = 2.19$) was obtained. An early transition state with a similar structure was also observed in a previous QM/MM optimization of the transition state for a reaction between acetate and 1,2-dichloroethane.[25] Second, the enzyme active site contains two tryptophan residues (Trp125 and Trp175), both of which are likely to interact with the transition state and the chloride anion.[10,25,60] However, current data and studies with mutants where one of the tryptophans has been replaced[10] suggest that one tryptophan residue is sufficient for effective catalysis. Third, the orientation of the tryptophan residue relative to the transition state is different in the enzyme and in our calculations. In our calculations the two molecules are collinear; this choice was made to increase the speed of computations. A limited number of control calculations where the orientation was taken from a recent MD study of haloalkane dehalogenase[60] indicate that the interaction strengths may be 1 or 2 kcal/mol weaker in the active site orientation. Last, the interaction energies are strongly dependent on the dielectric constant of the environment. Dielectric constants of heterogeneous environments, such as enzyme active sites, are not uniquely defined and depend on both the site considered and residues included explicitly.[69] At one extreme, when all residues and solvent molecules are explicitly treated quantum mechanically, $\epsilon = 1$ is appropriate.[69,70] In calculating the interaction energies, the reacting system and one aromatic moiety were treated explicitly and the effect of the rest of the leaving group pocket, which is rather hydrophobic, was modeled by dielectric constant of 2.247. If this value underestimates the effective dielectric, the interaction of the tryptophan residue with the transition state becomes weaker. However, the general trend of

the transition state interacting stronger with the tryptophan residue than the reactant state remains independent of the value of ϵ.

In interpreting current data, it should be kept in mind that we have evaluated the interaction of the reacting system with only one active site residue. Based on structural data of the active site, it is likely that the second tryptophan does not interact with the ground state significantly and interacts with the transition state as strongly as the first tryptophan. Thus, the interaction with tryptophan residues clearly has a catalytic effect. Our data suggest that interaction with a single tryptophan residue is sufficient to explain the catalytic effect of the enzyme. One might ask if there are residues that interact more strongly with the ground state than with the transition state. Visual examination of crystal structures and previously generated MD trajectories of the enzyme−substrate complex reveals hydrogen bonding between the nonattacking carboxylate oxygen and two backbone amide groups. These interactions are likely to be slightly stronger in the ground state because the partial charge of the nonnucleophilic oxygen is more negative in the ground state (carboxylate oxygen) than in the transition state (partially carbonyl oxygen).

Interactions with a Water Molecule. One of the main findings of this work is that the active site tryptophan residue interacts significantly more strongly with the leaving group in the transition state than with the same group in the reactant state. Inspection of results in Figure 4 shows that the interaction of a single water molecule is also stronger with the transition state compared to the ground state. Figures 2 and 4 reveal that the gas phase interaction of the transition state with one tryptophan residue is stronger than the interaction of the transition state with one water molecule. This difference is further amplified when one considers the effect of the environment: the water−chloride interaction in the transition state is only 1.4 kcal/mol when the dielectric constant is 78.3 but the 3-methylindole−chloride interaction is 7.9 kcal/mol when the dielectric constant is 2.247. The number of water molecules solvating the leaving group in the transition state during the aqueous hydrolysis is between one and two for most of the time according to a recent MD simulation of the noncatalyzed reaction.[47] Thus, tryptophan residue stabilizes the transition state in the enzymatic reaction more than a water molecule does in water environment, and the tryptophan residue at the enzyme active site contributes significantly to the catalytic advantage of the dehalogenase enzyme when compared to water.

Conclusions

Displacement of chloride from methyl chloride or 1,2-dichloroethane by carboxylate anions is an exothermic process. High-level ab initio calculations suggest that the free energy of the transition state for the reaction between formate and methyl chloride in the gas phase is 9.8 kcal/mol above the free energy level of isolated reactants and 16.2 kcal/mol above the free energy of the ion−molecule complex. The activation free energies with methyl chloride are predicted to be 16.8, 19.6, and 23.3 kcal/mol in homogeneous environments with dielectric constants of 2.247, 10.36, and 78.39, respectively. 1,2-Dichloroethane is less reactive than methyl chloride in the aqueous solution, probably because the bulkier electrophile hinders solvation of polar atoms in the transition state. We estimate that the activation free energy for the reaction between 1,2-dichlororoethane and acetate is 25 kcal/mol in water, and thus the enzyme haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10 lowers the activation barrier by about 10 kcal/mol.

Part of this rate acceleration can be explained by low dielectric constant of the active site. Approximating the enzyme active site by homogeneous dielectric of 10.36, we estimate that about 4 kcal/mol of the total catalytic effect is due to the general nonpolar nature of the active site. It should be kept in mind that the real active site of haloalkane dehalogenase is not homogeneous dielectric and the real nonspecific contribution of the environment may be different than 4 kcal/mol. The reaction is further catalyzed by an interaction between the active site tryptophan residue and the leaving group. Comparison of interaction strengths of a single active site tryptophan residue with the ground state, the transition state, and the product reveals that the tryptophan residue interacts significantly more strongly with the product and the transition state than with the ground state. A similar, but less pronounced, trend was observed when interactions of a water molecule with the ground state, transition state, and the product were compared. The precise estimate of this contribution is challenging because the interaction strength is strongly dependent on the dielectric environment and the number of water molecules interacting with the leaving group in water. We estimate that the specific hydrogen bonding interactions with the active site tryptophan can contribute no more than 5 kcal/mol to the rate acceleration when compared to the aqueous reaction. A previous study has demonstrated that about 2.6 kcal/mol of rate acceleration can be explained by the ability of the enzyme to position the substrate in the active site in a favorable orientation for an S_N2 displacement by the active site carboxylate.[47] The sum of these three contributions (11.6 kcal/mol) falls between our theoretical estimate and the extrapolated experimental rate acceleration.

Supporting Information Available: Zero point energies and free energies of solvation for all the compounds, gas phase reaction energies of reactions of methyl chloride with formate and acetate using different basis sets, and data on microsolvated transition state. This material is available free of charge via the Internet at http://pubs.acs.org.

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