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COMMUNICATION

The Structures of the Enzyme–Substrate Complex and Transition State Formed in the S_N2 Displacement of Cl^- from 1,2-Dichloroethane at the Active Site of *Xanthobacter autotrophicus* Haloalkane Dehalogenase

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The mechanism of an enzymatic reaction is better understood when the structures of ground and transition states are known. We have set out to determine enzyme–substrate (ES) and transition state (TS) for the S_N2 displacement of Cl^- from 1,2-dichlorethane (DCE) by Asp 124–CO_2^- at the active site of *Xanthobacter autotrophicus* haloalkane dehalogenase. This is the initial step (Eq. [1]) of the enzymatic reaction (*1*).

$$(E\text{–Asp124–}CO_2^- + ClCH_2CH_2Cl + H_2O) \rightarrow (E\text{–Asp124–}CO_2\text{–}CH_2CH_2Cl + H_2O + Cl^-) \quad [1]$$

$$(E\text{–Asp124–}CO_2\text{–}CH_2CH_2Cl + H_2O + Cl^-) \rightarrow (E\text{–Asp124–}CO_2H + Cl^-) + HOCH_2CH_2Cl \quad [2]$$

$$(E\text{–Asp124–}CO_2H + Cl^-) \rightarrow E\text{–Asp124–}CO_2^- + Cl^- + H^+ \quad [3]$$

Our choice of subject is dependent upon knowing that: (i) TS structures for S_N2 displacements of anionic leaving groups by anionic nucleophiles are comparable in the gas and liquid phase (*2*, *3*), (ii) S_N2 displacements are associated with only one TS (no intermediates) such that use of quantum chemical calculations is simplified, and (iii) a crystallographic structure of *X. autotrophicus* haloalkane dehydrogenase is available at 1.9 Å and the structure of this enzyme with the DCE substrate is at the active site at 2.4 Å (Brookhaven Protein Database: lede and 2dhc, respectively) (*1*, *4*). In passing, the dehalogenase enzymes, as a whole, are of great interest in the bioremediation of haloalkane pollution by, among other agents, solvents, herbicides, pesticides, hydraulic fluids, plasticizers, and chemical intermediates (*5*).

We initiated the problem with the *ab initio* calculation of the TS structure and energies for the S_N2 displacement of Cl^- from DCE by $CH_3CO_2^-$ (AcO^-) in the gas phase [Hartree–Fock, HF/6-31 + G(d) level of theory] and in solution (self-consistent isodensity polarized continuum model) (*6*). Repulsive Cl···O interac-

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tions disfavor carboxylate attack upon the *trans* conformation of DCE such that the reaction involves nucleophilic attack at C(1) of the *gauche* conformation. The calculated TS structures were found, as anticipated, to be very similar when formed in the gas phase and in solution. In order to carry the quantum mechanical calculation to the active site of the enzyme, a semiempirical approach is required because of the large number of atoms involved. The *ab initio* and semiempirical PM3 structures for the TS of the S_N2 displacement of Cl^- from DCE by AcO^-, as judged by the extent of bond formation (O···C) and breaking (C···Cl), were quite comparable. Thus, for HF-SCRF/6-31 + G(d) and PM3, r(C···Cl)/(C–Cl) equals 1.30 and 1.24, respectively, and r(C–O)/(C···O) equals 0.66 and 0.74, respectively.

The next step was to use PM3 in order to optimize the TS for the reaction of Asp124–CO_2^- with DCE when including the side chains of the amino acids at the active site (Glu56, Trp125, Phe128, Phe172, Trp175, Leu179, Val219, Phe222, Pro223, Val226, Leu262, Leu263, and His289) (*7*). To retain the overall structure of the enzyme during the calculations, the peptide backbone atoms were held fixed to their crystallographic coordinates (2dhc) (*4*). The TS in the model active site is slightly looser than the TS in the gas phase (*6*). The C···O and the C···Cl distance in the model active site compared to the gas phase are 1.965 Å vs 1.942 Å and 2.228 Å vs 2.196 Å, respectively.

We now report molecular dynamics (MD) simulations of the ES complex and enzyme TS using the entire enzyme structure. The starting coordinates for the ES complex were those of the crystal structure of the enzyme with DCE in the active site (2dhc) (*4*). The DCE parameters used for the ES complex were from Jorgensen *et al.* (*8*). The parameters used for the enzyme were from Cornell *et al.* (*9*), and the water molecules were treated as TIP3P residues (*10*). The starting structure for the enzyme with a S_N2 TS was created as follows. In the PM3 optimization of the TS and surrounding cavity, the peptide backbone atoms were held fixed to their original coordinates (*loc cit*). Thus, it was a simple procedure to overlay the peptide backbones of the PM3-optimized TS and surrounding amino acids onto the peptide backbones of the crystal structure of the ES complex, remove the overlapped structure, and join in place the PM3 optimized structure carrying the TS. In order to maintain TS integrity throughout the MD calculations, we assigned large force constants to the TS bonds being made and broken and the associated O···C···Cl angle.

Minimization for 5000 steps of both the ES complex and the enzyme TS species was followed by MD simulations to 540 ps by using AMBER 4.1 programs (*11*). The partial charges used for the TS structure were derived from *ab initio* calculations at the 6-31G(d) level of theory, using the electrostatic potential at points selected by the GHelpG scheme (*12*). Heating and equilibrations were complete at about 150 ps. In the discussion of results which follow, the proton of the neutral imidazole species of His289 was present on the imidazole δN.[2]

[2] It is not clear from the crystallographic data (Ref. *4*) as to whether the N-protonated position of the imidazole of His289 is δN or εN. Though εN–H is most usual, we find that the His289 imidazole must be protonated on the δN. We will describe in a complete account of results how we carried out MD simulations for both the ES species and the TS species assuming both δN and εN protonation (four simulations in all). The results reported here are with the proton on δN.

After heating to ambient temperature (300 K) and equilibration, the ES structure has the following characteristics. DCE has converted from the *trans* conformation of the ES crystal structure to a *gauche* conformation (that favored in *ab initio* calculations for the reaction of AcO^- with DCE in the gas phase). The nonnucleophilic oxygen (OD1) of Asp124–CO_2^- is hydrogen bonded to Water396 and to two backbone amide hydrogens of Glu56 and Trp125. The nucleophilic oxygen (OD2) of Asp124–CO_2^- is hydrogen bonded to Water323. These three hydrogen bonds provide the electrostatic stabilization of the carboxylate anion. At the same time, the ligation of Water323 to OD2 blocks nucleophilic attack on C(1) of DCE. However, near attack conformations (NACs) (*13*, *14*) are consistently formed between 510 ps and termination of MD (540 ps) because Water323 periodically drifts away from the carboxylate group. At such times, the C(1) of DCE approaches the OD2 of Asp124–CO_2^- to distances ~3.00 Å—a distance defining a NAC(*13*, *14*). Throughout the MD simulation, the indole NH of Trp125 is hydrogen bonded to either Cl(1) or Cl(2) of DCE. The formation of NACs is associated with Trp125 hydrogen bonding to the Cl(1) of DCE. Thus, the reactive conformations (NACs) have Water323 dissociated from Asp–CO_2^-, hydrogen bonding of Cl(1) to Trp125, and OD2 in line to the C(1)–Cl(1) bond and at a distance of ~3 Å from C(1). The weak hydrogen bond between the Cl(1) substituent and Trp125 serves to guide the substrate into position for reaction in the completely hydrophobic active site. At no time during the MD does the indole N–H of Trp175 become associated in hydrogen bonding to the substrate. Involved in the structuring of the enzyme active site is a completely persistent hydrogen bond between OD2 of Asp260–CO_2^- and H–δN of His289.[2] A presentation of a NAC is shown in Fig. 1.

In the MD simulation of the TS, both Trp125 and Trp175 are persistently hydrogen bonded to the departing Cl(1). Thus, on proceeding from ground to transition state, the Trp125 hydrogen bond (2.43 ± 0.29 Å) is maintained and joined by hydrogen bonding (2.24 ± 0.19 Å) to the departing incipient Cl^- by Trp175 (Fig. 2). Given the standard deviation for the hydrogen bond distances in the molecular distances, the chloride is equidistant from the two tryptophans. A catalytically significant change in the positions of Water323, Water392, and Water396 occurs in comparing the simulated MD structure of ES to the MD simulated structure of enzyme TS. In the ES complex, Water323 and Water396 are positioned to stabilize the negative charge on Asp124. Now, in the TS, a tight hydrogen bonding matrix is formed by Water323 and Water396, Gly55, Gln123, His289, and a third water (Water392), shown in Fig. 3. Water323 and Water396 are held in such a manner that they exactly position the third water (Water392) as a nucleophile to attack the ester carbonyl formed after Cl^- departs from TS to provide Asp124–CO_2–CH_2CH_2–Cl. The imidazole of His289 is in position to be a general-base catalyst for the forthcoming attack of Water392 on the ester intermediate. His289 is positioned by an essential hydrogen bond between δN–H and OD2 of Asp260.[2]

CONCLUSION

The bimolecular rate constants for S_N2 displacement of a negatively charged leaving group by a negatively charged nucleophile can be 10^{16} greater in the gas phase than in water (*15*). Desolvation of nucleophile and solvent rearrangement