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A QM/MM study of a nucleophilic aromatic substitution reaction catalyzed by 4-chlorobenzoyl-CoA dehalogenase

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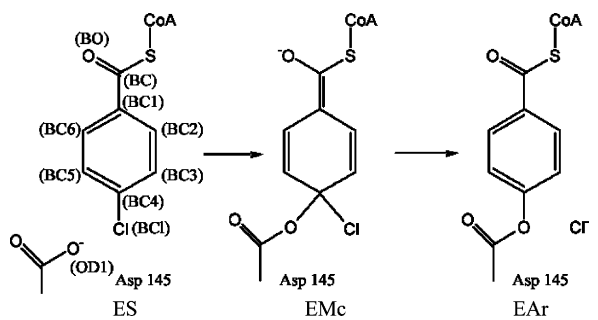
Calculated using a QM/MM method, the free energy profile for the conversion of 4-chlorobenzoate to 4-hydroxybenzoate catalyzed by 4-chlorobenzoyl-CoA dehalogenase indicates the existence of a stable Meisenheimer complex.

4-Chlorobenzoyl-CoA dehalogenase found in some soil bacteria catalyzes the conversion of 4-chlorobenzoate (4-CBA) to 4-hydroxybenzoate (4-HBA). In addition to its potential in bioremediation,[1] this enzyme presents a unique opportunity to study the catalytic mechanism of an aromatic nucleophilic substitution reaction. Experimental evidence[2–4] suggests that the first step of the catalyzed reaction proceeds *via* the nucleophilic aromatic substitution (S_NAr) mechanism (Scheme 1),[5] in which a carboxylate oxygen of Asp145 attacks the benzoyl C4 to displace Cl^-, forming an arylated complex (EAr). Subsequently, hydrolysis of the EAr produces the enzyme–product (EP) complex. The kinetic data have suggested an enzyme–Meisenheimer complex (EMc) as an intermediate in the substitution step, although it has not been directly detected because of its transient nature. Here, we report the free energy profile of the substitution reaction obtained using a combined quantum mechanical and molecular mechanical (QM/MM) method,[6] which indicates a stable EMc intermediate in the enzymatic reaction.

The Meisenheimer complex is typically unstable without electron withdrawing groups on the benzene ring.[5] In 4-CBA-CoA dehalogenase, the complex is thought to be stabilized by H-bonds between the benzoyl carbonyl and backbone amides of Gly114 and Phe64, with potential enhancment from the helical dipole terminated at Gly114.[7] Furthermore, the polarization of the benzoyl π electrons is facilitated by the surrounding aromatic side chains.[7] The effects of these electrostatic interactions on substrate binding and catalysis have been established by spectroscopic[8–12] and mutagenetic[9,11–13] studies.

Theoretically, the S_NAr reaction has been modeled in the gas phase and in solution by Hartree–Fock, density functional theory (DFT), and semiempirical PM3 methods.[14] Using a continuum solvation model, it was found that polar solvent stabilizes the reactant much more than the transition state.[14] Interestingly, a very shallow (< 0.1 kcal mol^{-1}) potential well, corresponding to the Meisenheimer complex, was found in the PM3 calculation, but it disappears in solution. Molecular dynamics (MD) simulations indicated that OD1 of Asp145 is oriented to form a "near-attack configuration"[15] (see Scheme 1 for atom definition). However, these studies did not address the role of the enzyme during the bond breaking and forming processes.



Scheme 1

The current QM/MM study on the S_NAr step was performed using CHARMM.[16] The starting geometry was modified from the EP structure (PDB code 1NZY)[7] by replacing the hydroxyl O in 4-HBA with Cl. Two subunits (A and B) were needed as several B residues are essential in stabilizing the phosphoryl groups of the substrate in unit A and in preventing water from entering the active site. The protein was solvated in a $r = 25$ Å sphere of TIP3P water[17] centered at Cl, augmented with stochastic boundary conditions.[18] A Poisson–Boltzmann charge scaling scheme[19] was introduced to correct for solvent shielding.

The system was divided into MM and QM regions. The CHARMM all atom force field[20] was used in the MM region. The quantum part, consisting of the Asp145 side chain and the benzoyl group of 4-CBA, was treated with the PM3 method.[21] This is because an *ab initio* or DFT treatment is not currently feasible for MD studies of this large system. The PM3 method has been extensively tested against *ab initio* and DFT methods, and found to yield good results for this[14] and other dechlorination processes.[22] The two QM–MM boundary atoms, namely C_α of Asp145 and C_β of the mercaptoethylamine part of the CoA, were treated with the generalized hybrid orbital method.[6]

We first simulated the active site dynamics at 300 K using the QM/MM MD method. The results confirmed that the benzoyl carbonyl maintains H-bonding with backbone amides of Gly114 and Phe64 throughout the 1 ns simulation, presumably *via* a cooperative scheme.[23] Consistent with mutagenesis data,[13] the H-bond with Gly114 ($\bar{r}$(O···H) = 2.1 ± 0.2 Å) is much stronger than that with Phe64 ($\bar{r}$(O···H) = 2.8 ± 0.6 Å). The MD results also indicated that OD1 is oriented to attack BC4 from the back side of the leaving group Cl^-, partially due to H-bonding with the Trp137 side chain. This is in agreement with an earlier MD study using an MM force field.[15]

The potential of mean force (PMF) for the catalyzed S_NAr reaction was determined by umbrella sampling[24] along the reaction coordinate defined by $R_\phi = R_{(BC4\text{-}BCl)} - R_{(OD1\text{-}BC4)}$. MD simulations were performed using 27 separate windows, in each of which a harmonic bias potential with a force constant between 50 to 80 kcal mol^{-1} Å^{-2} was used. The initial configuration for each run was determined by the adiabatic reaction path along R_ϕ. In the MD simulation, the system was first equilibrated for 50 ps, followed by data collection for an additional 50 ps. The time step was 1 fs and the SHAKE algorithm[25] was used to maintain the covalent bonding involving H atoms.

Fig. 1 shows the PMF for the catalyzed S_NAr reaction. The most striking feature and key finding of the present study is the middle well at $R_\phi = 0.6$ Å, corresponding to the EMc complex. The fact that it is much deeper than that in the gaseous reaction underlines the significant stabilization of the Meisenheimer complex by the enzyme environment. Our results also offer strong evidence in support of the hypothesis that the anionic σ-complex is stabilized by redistributing the additional charge brought by the attacking nucleophile. This is illustrated in Fig. 2, where the Mulliken charges of several representative atoms plotted along the reaction path clearly demonstrate the transient migration of the π electrons in the benzoyl moiety during the formation of the EMc complex.

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Displayed in the same figure are changes of some bond lengths during the reaction. We note for instance the shortening of the BC1–BC bond and the quinone-like structure of the benzene ring. Such changes have been inferred from UV and Raman spectra.[8,10,12]

To better understand the catalytic role of the enzyme, the PMF is compared with the PM3 free energies for a model gas phase S_NAr reaction[14] in Fig. 1. The first transition state in the PMF is about 17 kcal mol^{-1}, 1.6 kcal mol^{-1} lower than the gas phase counterpart. The stabilization of the transition state is likely due to H-bonding of the carbonyl group, which has been estimated from kinetic data to be 1.7 kcal mol^{-1}.[26] It is worth noting that this dominant barrier is 20 kcal mol^{-1} less than that of the solution reaction (37 kcal mol^{-1}),[14] underscoring the catalytic strategy of the enzyme to desolvate the benzene ring. On the other hand, the EMc complex converts to EAr over a relatively low (3.8 kcal mol^{-1}) barrier, which seems unaffected by the enzyme. The EAr complex is destabilized by the enzyme by ~12 kcal mol^{-1} relative to the gas phase model, which can be rationalized by the tight active site and the lack of polar residues to bind the bulky Cl^-. However, the dynamics of the chloride ion probably warrants further studies.

The existing kinetic model for this reaction assumes that the conversion of EMc to EAr is rate-limiting.[27] Due to difficulties in detecting the EMc complex, this assumption was based on the fitting of the observed EAr accumulation and thus contains large uncertainty. The kinetic model was also heavily influenced by data for the reaction with fluoro-substituted 4-CBA, where an EMc complex was successfully trapped thanks to the poor F^- leaving group.[28] Based on the free energy profile, we propose a refined kinetic scheme in which the rate of the substitution step is determined by the formation of EMc, rather than that of EAr. Preliminary analysis show that our model does not contradict the EAr kinetic data.[26]

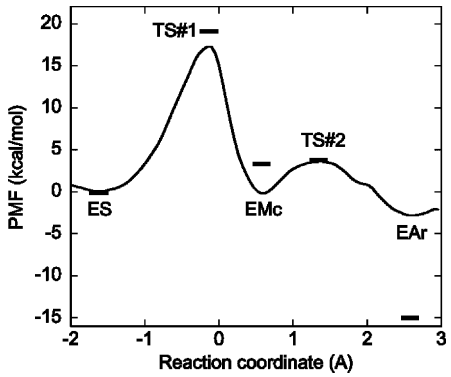

Fig. 1 Computed potential of mean force of the catalyzed S_NAr reaction (curve) and free energies for the model reaction between 4-Cl–Ph–CO–S–Me and acetate ion in the gas phase (bars).

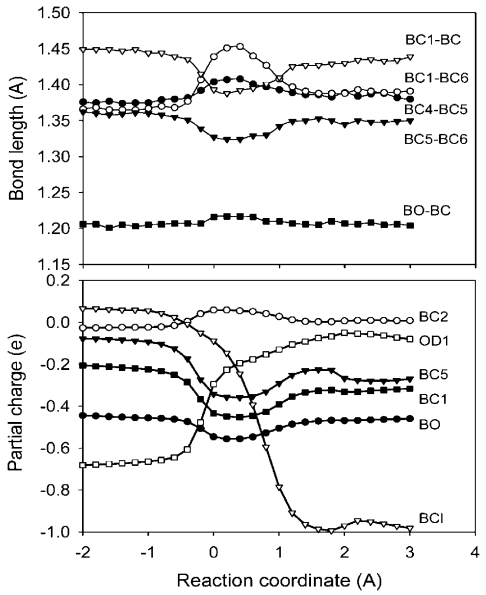

Fig. 2 Bond lengths and Mulliken charges of selected benzoyl atoms along the reaction path. The change of bond lengths in the benzene ring is largely symmetric so some are not shown.

To summarize, the present QM/MM free energy simulations demonstrate that the EMc complex is significantly stabilized by the enzyme, resulting in a discrete intermediate along the reaction pathway of the nucleophilic aromatic substitution in 4-CBA-CoA dehalogenase. The free energy profile sheds further light on the reaction mechanism and allows the refinement of the kinetic model.

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