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Semiempirical QM/MM potential with simple valence bond (SVB) for enzyme reactions. Application to the nucleophilic addition reaction in haloalkane dehalogenase

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Abstract. We present a method for the correction of errors in combined QM/MM calculations using a semiempirical Hamiltonian for enzyme reactions. Since semiempirical models can provide a reasonable representation of the general shape of the potential energy surface for chemical reactions, we introduce a simple valence bond-like (SVB) term to correct the energies at critical points on the potential energy surface. The present SVB term is not a stand-alone potential energy function, but it is used purely for introducing small energy corrections to the semiempirical Hamiltonian to achieve the accuracy needed for modeling enzymatic reactions. We show that the present coupled QM-SVB/MM approach can be parameterized to reproduce experimental and ab initio results for model reactions, and have applied the PM3-SVB/MM potential to the nucleophilic addition reaction in haloalkane dehalogenase. In a preliminary energy minimization study, the PM3-SVB/MM results are reasonable, suggesting that it may be used in free energy simulations to assess enzymatic reaction mechanism.

Keywords: Simple valence bond – Combined QM/MM – Haloalkane dehalogenase

Introduction

Haloalkane dehalogenases (DHase) constitute a class of enzymes that offer the possibility of bioremediation of environmental contaminants such as haloalkanes. DHase acts on a wide range of substrates [1, 2, 3, 4]. The enzyme DHase from the bacterium *Xanthobacter autotrophicus GJ10*, is a 35 kDa protein with 310 amino acid residues [5]. It is a globular α/β protein, capable of removing halogen atoms from halogenated hydrocarbons to the corresponding alcohols and halide ions. The enzymatic process involves an initial nucleophilic substitution reaction by Asp124 to yield an ester intermediate [6, 7, 8, 9, 10], which is subsequently hydrolyzed to form an alcohol [11]. In the latter step, a water and two charged residues (His289 and Asp260) in the active site are directly responsible for the nucleophilic attack in the enzyme mechanism [12]. These three residues constitute the so-called 'catalytic triad residues' in haloalkane dehalogenases, similar to hydrolases and proteases, such as serine protease.

Crystal structures of haloalkane dehalogenase have been determined in the presence of the substrate 1,2-dichloroethane, the ester intermediate, and the product, providing one of the rare cases where the enzyme structure was solved in the presence of its native substrate and reaction intermediate [6]. Using high resolution X-ray crystallography at varying pH and temperature conditions, a two-step reaction mechanism of the hydrolysis of 1,2-dichloroethane (DCE) to 2-chloroethanol was observed [6]. The first step in the proposed mechanism involves the nucleophilic attack by the carboxylate oxygen of Asp124 on one of the chlorine-bearing carbon (C1) of DCE, displacing the chloride ion and forming an ester intermediate via an S_N2 displacement reaction (Scheme 1). The second step involves the hydrolysis of the ester intermediate to form a tetrahedral intermediate (TI). TI, decomposes to form the product alcohol in the third step, the alcohol leaves the active site followed by the release of the chloride ion formed during the S_N2 displacement step. Kinetic studies reveal that the release of the chloride ion to be the rate determining step [13, 14].

The free energy of activation of the S_N2 displacement reaction has been studied previously by several groups [8, 9, 10, 15], including our own effort [16]. The present investigation focuses on the second step, namely the

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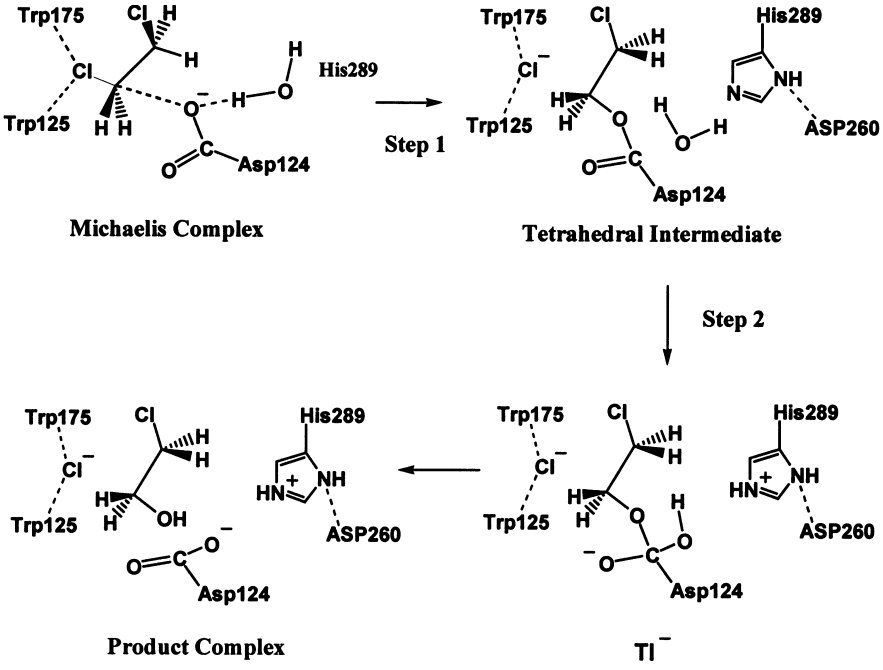


Scheme 1. Schematic representation of the steps involved in the catalytic mechanism of haloalkane dehalogenase

hydrolysis of the ester intermediate to form a tetrahedral intermediate on the C_γ atom of Asp124 residue (Scheme 1). Our goal is to eventually determine the free energy reaction profile for the formation of the tetrahedral intermediate and its decomposition, which requires time-consuming free energy simulations. Because of the computational cost, a semiempirical method must be used in combined quantum mechanical and molecular mechanical (QM/MM) simulations. However, the standard semiempirical AM1 [17] or PM3 [18] models do not yield correct proton affinities for the relevant acids and bases involved in the dehalogenation reaction. To overcome this problem, we have used a simple valence bond-like empirical potential to correct the intrinsic errors in the semiempirical model. This approach implicitly assumes that the general shape and features of the potential surface for the dehalogenation reaction are adequately represented by the AM1 and PM3 model [19]. The only quantity that needs to be corrected is the reaction energy. The present paper summarizes the approach used to generate this simple valence bond (SVB) term and the results of a preliminary study of the potential energy surface for the general base catalyzed addition reaction using this QM-SVB/MM potential.

Method

Potential energy function

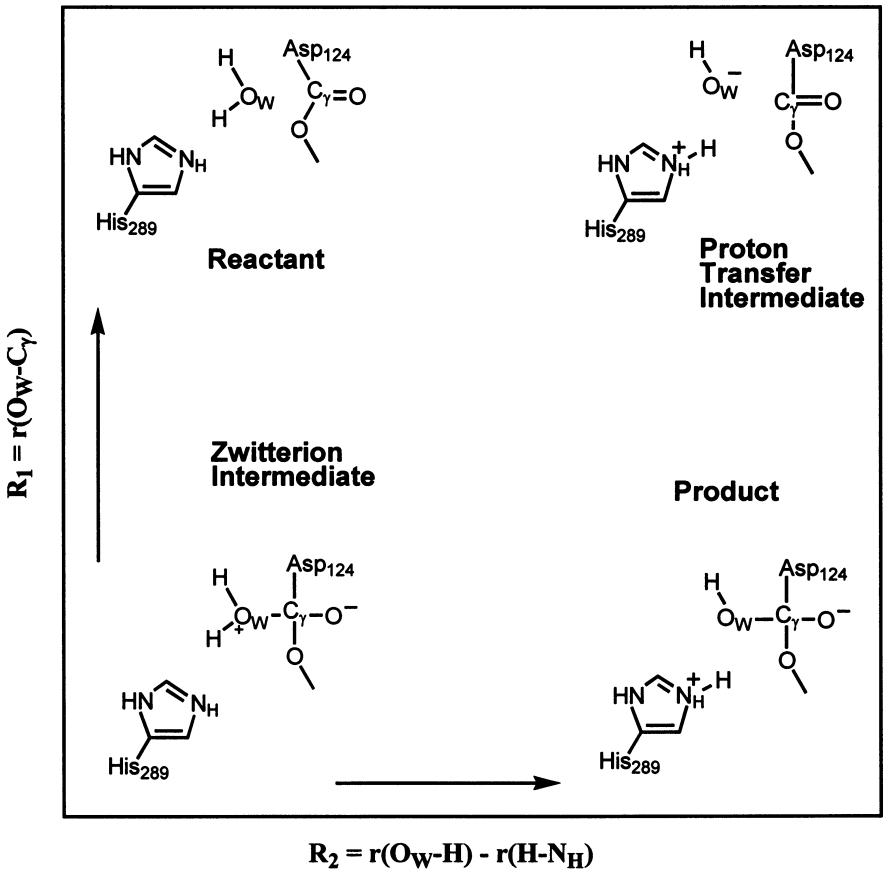
We use a combined quantum mechanical and molecular mechanical (QM/MM) potential for the study of the dehalogenation reaction in haloalkane dehalogenase. Combined QM/MM methods have been reviewed previously [20, 21, 22, 23]. Therefore, we only outline some key aspects of the method. The main step of this approach is to partition the enzyme system into a quantum mechanical region, consisting of the substrate and residues directly involved in the chemical step, and a molecular mechanical region, which includes the rest of the protein-solvent system. In the present study, the QM region consists of the Asp124-ester intermediate, resulting from the initial nucleophilic substitution step, a water molecule, and His289. The QM system is treated by the semiempirical PM3 model [18] and the MM region is approximated by the CHARMM22 force field [24]. The connection between the QM and MM region is represented by the generalized hybrid orbital (GHO) method [25, 26], and the C_α atoms of Asp124 and His289 are treated as the boundary atoms. With this representation, the total potential energy of the system is given as follows:

$$E_{tot} = E_{qm} + E_{qm/mm} + E_{mm} + E_{SVB}(R_1, R_2) \quad (1)$$

where E_{qm} is the quantum mechanical energy for the QM region, $E_{qm/mm}$ is the QM/MM interaction energy, which include both electrostatic and van der Waals terms, E_{mm} is the interaction energy of the MM system, and the last E_{SVB} term represents a correction to the semiempirical E_{qm} energy. The variables, R_1 and R_2, in Eq. 1 are the reaction coordinates that characterize the nucleophilic attack at the carbonyl group and the simultaneous proton transfer reaction between water and His289 (see below).

The reaction coordinate

The initial step in the hydrolysis reaction of the ester-enzyme intermediate in DHase is the formation of a tetrahedral intermediate [6]. The reaction (Scheme 1) involves a nucleophilic addition of a water molecule to the carbonyl group, which is catalyzed by the general base His289 and is accompanied by a proton transfer from the nucleophilic water. Thus, an important question in elucidating the reaction mechanism is to determine whether the nucleophilic addition and the proton transfer processes are concerted or stepwise reaction. An effective and general approach to this problem is to construct the More O'Ferrall-Jencks diagram by treating the two processes independently [27]. As illustrated in Scheme 2, we define the reaction coordinate R_1 for the nucleophilic addition process as the distance between the oxygen of water (O_W) and the carbonyl carbon (C_γ) of the ester-intermediate from Asp124 (Eq. 2). For the proton transfer reaction, we define the reaction coordinate R_2 as the difference in distance between the transferring hydrogen atom with the donor (O_W) and acceptor (N_H) atoms (Eq. 3).



Scheme 2. Schematic representation of the two-dimensional surface depicting the possible mechanisms for the nucleophilic addition reaction

$$R_1 = r_{OC} \tag{2}$$

$$R_2 = r_{OH} - r_{HN} \tag{3}$$

The concerted reaction mechanism that involves simultaneous formation of the tetrahedral intermediate and the proton transfer from water to His289 corresponds to a path diagonally in going from the reactant state at the upper left-hand side corner to the product state at the lower right-hand corner. There are two step-wise reaction paths: the first is the nucleophilic attack to yield a zwitterionic intermediate, followed by proton transfer (lower left-hand side corner), and the second path involves an initial proton transfer and subsequent nucleophilic addition by a hydroxide ion (upper right-hand side corner). Of course, there is a spectrum of possible paths connecting these possibilities, and it is typically not possible to distinguish these processes on the basis of kinetic data. On the other hand, free energy calculations of such a two-dimensional surface can be used to analyze the most favorable paths for these complex processes involving general base catalyzed hydrolysis reactions.

A simple valence bond (SVB) term for semiempirical QM/MM potential

It would be ideal to use high-level ab initio molecular orbital or density functional theory (DFT) to represent the reactive part of the system such that accurate results can be obtained directly from QM calculations. However, these calculations are still too time-consuming to be practical at the present time even for simple model reactions in solution [28, 29, 30]. Consequently, approximate quantum chemical models have to be used in free energy calculations using combined QM/MM potentials. Although semiempirical methods are computationally efficient, allowing adequate sampling of conformational space in statistical mechanical Monte Carlo or molecular dynamics simulations of proteins and solutions, it is often difficult to achieve the desired chemical accuracy with standard models such as the AM1 or PM3 method. Nevertheless, these methods can provide a reasonable description of the general shape and features of the potential energy surface (PES) for chemical transformations. It is the relative energies at the critical points, including the reactant, the product, and the transition state, that require special corrections in order to improve the accuracy of the semiempirical method. Here, we describe an approach to empirically correct the energies for enzymatic reactions using semiempirical QM models.

The performance of semiempirical QM models can be improved by developing new semiempirical parameters for specific reactions [31, 32]; however, in practice, the parameterization process is far from straightforward because the Gaussian terms for core-core interactions in the AM1 or PM3 formalism often have large variations and make significant contributions in the new parameter set. If these empirical Gaussian terms still need to be optimized, it might be simply to include an empirical term to improve the performance of the semiempirical model. Thus, a simple analytical function may be included in the combined QM/MM potential energy function to obtain accurate activation barrier and energy of reaction. To achieve this goal, we have chosen to use a simple valence bond-like (SVB) potential in our study.

Let $E_{ai}(R_1, R_2)$ be the target PES, which may be obtained from high-level ab initio and density functional calculations, or from experiment. The SVB correction term to the semiempirical energy, specifically, the correction to the semiempirical PM3 energy $E_{qm}^{PM3}(R_1; R_2)$ in the present study, is defined by Eq 4.

$$E_{SVB}(R_1, R_2) = E_{ai}(R_1, R_2) - E_{qm}^{PM3}(R_1, R_2) \tag{4}$$

In principle, $E_{SVB}(R_1, R_2)$ is a function of all variables. However, in practice this is not necessary because the correction term is relatively small in comparison to the variation in the PES. Furthermore, in most applications, only energies at the stationary points, such as the energy of reaction and the free energy of activation, are available from experiments. Thus, it might be sufficient to use the energies at

these stationary points to adjust parameters of the SVB potential and this is the procedure we use to parameterize the SVB correction term. It is interesting to note that other methods including the widely used empirical valence bond [33] method also make use of only energies of key stationary points in the parameterization process. In this case, the entirely potential energy surface is assumed to be well-defined, even when there are just three data points used in the parameterization.

The analytical expression of the SVB potential for the hydrolysis reaction in dehalogenase is a sum of two empirical potential function terms, describing the nucleophilic addition process and the proton transfer reaction (Scheme 2), respectively:

$$E_{SVB}(R_1, R_2) = \Delta V_{NA}(R_1) + \Delta V_{PT}(R_2) \tag{5}$$

Thus, the goal is to adjust parameters of the analytical functions in Eq. 5 to reproduce the energies at critical points on the potential surface defined by Eq. 4.

The correction term for the proton transfer (PT) reaction between water and His289 is modeled by a two-state VB expression that includes the effective diabatic states, M_1 and M_2, corresponding to the ionizations of water and an imidazolium ion, respectively.

$$\begin{aligned}\Delta V_{PT} = &\frac{1}{2}(M_1[r_{\mathrm{OH}}] + M_2[r_{\mathrm{HN}}]) \\ &- \frac{1}{2}\left[(M_1[r_{\mathrm{OH}}] - M_2[r_{\mathrm{HN}}])^2 + 4V_{12}^2(r_{ON})\right]^{1/2} + \Delta D_{OH}\end{aligned} \tag{6}$$

where ΔD_{OH} is defined in Eq. 7, r_{OH} and r_{HN} are distances as explained in Eqs. 2 and 3, and r_{ON} is the distance between the donor (O_W) and acceptor (N_H) heavy atoms, and they are implicit variables of the reaction coordinate R_2. V_{12} is a coupling function between the two effective diabatic states. The Morse potential M_1 and M_2 in Eq. 6 is written as follows:

$$M(r_{\mathrm{AB}}) = \Delta D_{\mathrm{AB}}\left[e^{-2\alpha_{\mathrm{AB}}\left(r_{\mathrm{AB}} - r^o_{\mathrm{AB}}\right)} - 2e^{-\alpha_{\mathrm{AB}}\left(r_{\mathrm{AB}} - r^o_{\mathrm{AB}}\right)}\right] \tag{7}$$

In Eq 7, ΔD_{AB} represents the difference in dissociation energy between the target D^o_{AB} and PM3 $D^{\mathrm{PM3}}_{\mathrm{AB}}$ values and is a parameter explained in detail in the parameterization section, r^o_{AB} is the equilibrium bond distance between atoms A and B, and α_{AB} is related to the force constant k_{AB} and the experimental bond dissociation energy D^o_{AB} by $\alpha_{AB} = \left(k_{\mathrm{AB}}/2D^{\mathrm{o}}_{\mathrm{AB}}\right)^{1/2}$ The coupling term in Eq 6 is typically approximated by an exponential function:

$$V_{12}(r_{ON}) = D_{12}e^{-\alpha_{12}\left(r_{\mathrm{ON}} - r^{\mathrm{o}}_{\mathrm{ON}}\right)} \tag{8}$$

where the parameters D_{12} and α_{12} are adjusted to obtain the desired barrier height.

In the present study, the correction term for the nucleophilic addition coordinate is modeled by a single Morse term. Thus,

$$\Delta V_{NA}(R_1) = M(R_1) \tag{9}$$

We note that the present SVB term should be distinguished from the empirical valence bond (EVB) method used by Warshel and others [33]. The EVB potential is used to describe the entire reaction potential energy surface using analytical expressions for the valence bond-like states, which is fitted to two experimental parameters, the free energy of activation and the free energy of reaction for model reactions. The SVB term used here is not a stand-alone potential energy function itself, but it is an effective correction term to increase the accuracy of semiempirical QM methods.

Parameterization

The empirical parameters for the SVB correction in Eqs. 5–9 can be obtained by considering the energies of reaction for the following three reactions:

$$H_2O \rightarrow OH^- + H^+ \tag{10}$$

$$ImH^+ \rightarrow Im + H^+ \tag{11}$$

$$Im + H_2O + CH_3CO_2CH_3 \rightarrow ImH^+ + TI^- \tag{12}$$

where Im and ImH^+ are imidazole and imidazolium ion, and TI^- represent the tetrahedral intermediate of the ester hydrolysis reaction (Scheme 1). Eqs. 10 and 11 are related to the proton affinities of hydroxide ion and imidazole, and we use the experimental data to correct the errors in PM3 calculations [34]. Thus, the parameter D_{AB} in Eq. 6 is the difference between experimental [34] and PM3 proton affinities, and we used equilibrium bond distances from ab initio HF/aug-cc-pVDZ calculations to approximate r^o_{AB}. The exponent parameters are computed using the normal mode bond-stretching frequencies from experiment. We have used a constant value of 0.01 kcal/mol for the coupling parameter V_{12}, i.e., $D_{12} = 0.01$ kcal/mol and $\alpha_{12} = 0.0$, since we have not optimized the barrier height for the proton transfer reaction.

There is no experimental data for reaction 12. Consequently, we decided to use ab initio results at the MP2/aug-cc-pVDZ level in the parameterization process. Density functional calculations (B3LYP/aug-cc-pVDZ) yield similar results. In this case, we determined the energy of reaction for reaction 12 at the MP2 level (129.7 kcal/mol), which is subtracted from the experimental result for the proton transfer reaction from water to imidizole (166.9 kcal/mol) [34] to yield an energy of reaction (−37.2 kcal/mol) for the addition of OH^- to methyl acetate. The reason that we used this energy subtraction scheme, rather than the MP2 results directly, is to incorporate the contribution from experimental proton affinities that have been used in the parameterization of reactions 10 and 11 into the overall reaction. The PM3 value for the addition reaction is −50.4 kcal/mol, and thus, a net correction of +13.2 kcal/mol is needed for Eq. 9, which is reflected in the direction along the R_1 coordinate in Fig. 1. The final parameter set is listed in Table 1, and the energy contour from the SVB correction term for semiempirical QM/MM potential for the hydrolysis step in haloalkane dehalogenase is shown in Fig. 1. For reaction 10, the PM3 results underestimate the proton affinity of a hydroxide ion by 0.8 kcal/mol in comparison with experiment, while the discrepancy is −13.7 kcal/mol for imidazole. Therefore, the overall reaction for the proton transfer process from water to imidazole is overestimated by 12.9 kcal/mol, and a correction of 12.9 kcal/mol is reflected along the reaction coordinate R_2 in Fig. 1. Overall, a small, net correction of 0.4 kcal/mol is introduced by the SVB term for the reaction of Eq. 12 (energy difference along the diagonal direction in Fig. 1); however, this is due to fortuitous error cancellations in the PM3 proton affinity and reaction energy for the nucleophilic addition.

Results and discussion

We have applied the SVB correction term along with the combined QM/MM potential using the PM3 model [18] and the CHARMM-22 force field [24] to determine the potential energy surface of the nucleophilic-proton transfer reaction, leading to the tetrahedral intermediate in the ester hydrolysis reaction in haloalkane dehydrogenase. Eventually, we will use this potential function to determine the two-dimensional free energy surface to assess the reaction mechanism in terms of step-wise and concerted processes as depicted in Scheme 2.

In the present calculation, the crystal structure of the ester intermediate from the first reaction step was used as the starting coordinates. The combined PM3-SVB/CHARMM-TIP3P potential was used in all calculations. The QM region included the crystallographic water that is recognized as the nucleophile in the hy-

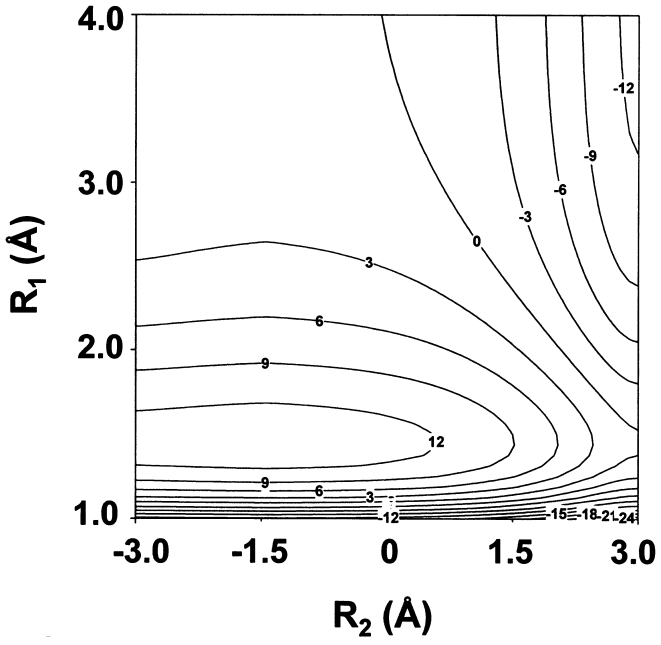


Fig. 1. SVB correction term for the nucleophilic addition reaction

Table 1. Parameters used in the 'E_{SVB}' correction term

	D_{AB}	α_{AB}	r^{o}_{AB}	V_{12}
O–H	0.8	2.223	0.95	0.01
H–N	13.7	2.137	1.00	
C–O	13.2	1.952	1.45	

drolysis step, the side chain of His289 which acts as a general base catalyst, and the side chain of Asp124 with the 2-chloroethyl moiety attached to it. To incorporate solvent effects, a 24 Å sphere of pre-equilibrated TIP3P water [35] was placed around the enzyme with the center of mass position for all QM atoms as the origin. The rest of the enzyme was treated classically. The QM region was treated by the PM3 model, whereas MM atoms were represented by the CHARMM-22 force field. There are two boundary atoms to connect the QM and MM region, which were treated using the generalized hybrid orbital method (GHO) [25, 26]. To equilibrate the system, we first carried out ca. 100 ps molecular dynamics simulation at 300 K. Then, we performed simulated-annealing molecular dynamics to a final temperature of 30 K in 20 ps. This structure was further minimized to a final gradient of 0.0001 kcal/mol-Å and an energy change less than 0.001 kcal/mol.

The reference structure obtained was used as the starting structure to map out a two-dimensional potential energy surface (Fig. 2). The two reaction coordinates, corresponding to the nucleophilic addition and proton transfer processes, have been defined in Eqs. 2 and 3. We note that the potential energy surface obtained by geometry optimization at fixed reaction coordinate values only provides a rough estimate of the reaction process. It is necessary to obtain the free energy surface through free energy simulations to assess the nature of the reaction mechanism. Thus, our discussion is only limited to the key features of the potential energy surface. Note that the potential energy contour depicted in Fig. 2 will be used as the initial guess for a biasing potential in future umbrella sampling simulations.

From the minimum energy path in Fig. 2, it appears that the protonation of His289 and the formation of the tetrahedral intermediate take place in an asynchronous concerted manner. There is a tendency of pronounced bond formation in the nucleophilic attack as the proton transfer from the nucleophilic water to His289 occurs later. However, we do not detect a stable species corresponding to the step-wise reaction intermediate in either corners in the More O'Ferrall-Jencks diagram. The zwitterionic tetrahedral intermediate (not a stable species) located at the lower left corner in Fig. 2 is about 20 kcal/mol higher in energy than the enzyme-ester intermediate of the dehalogenase reaction.

To provide some insights on the role of amino acid residues in the active site, we performed an energy decomposition analysis. The calculations were carried out by annihilating atomic charges of each residue, beginning from the nearest ones to the QM region. Energy change from the proceeding structure gives a reasonable indication of the electrostatic contribution to the interaction energy between the QM reactive species with each individual residue. This type of analysis has been previously used by Karplus, Bash, Brooks and coworkers [36, 37, 38, 39]. Two such calculations were performed as shown in Fig. 3. The series shown in solid line corresponds to the relative stabilization energy of

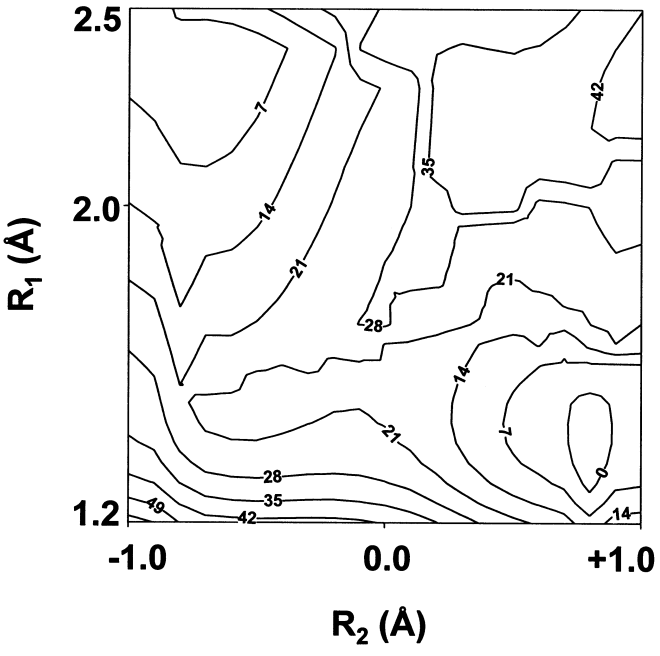


Fig. 2. Corrected two-dimensional potential energy surface for the nucleophilic addition reaction. The coordinate R_1 is for the addition step, and R_2 is for the proton transfer from water to imidazole

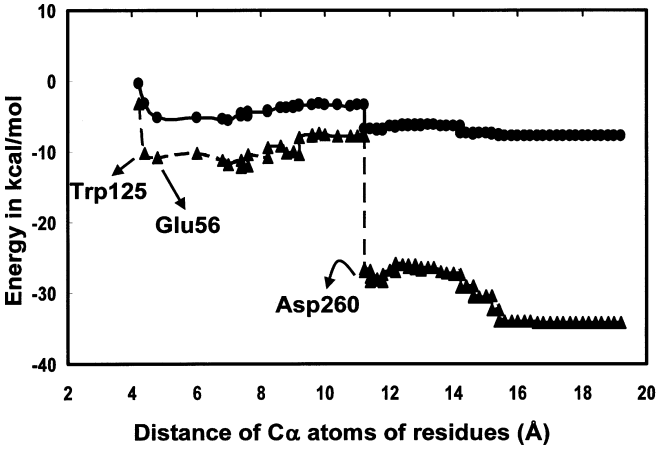


Fig. 3. Relative electrostatic stabilization rendered by residues near the active site

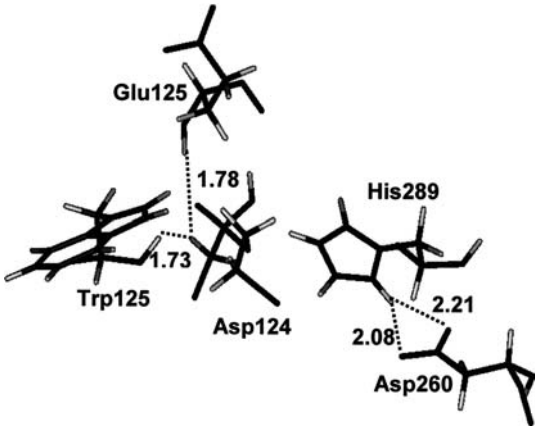


Fig. 4. Schematic representation showing the Glu56 and Trp125 residues stabilizing the negative charge developed on Asp124, called the oxy-anion hole and the positive charge on the imidazolium ring of His289 stabilized by Asp260. *Dashed lines* represent the hydrogen bonding interactions and their corresponding distances in Å

residues in the reactant structure as compared to the tetrahedral intermediate (product), and the series shown in dashed curves corresponds to that of reactant compared to the transition structure. In Fig. 3, residues are ordered according to the distance of the C_α atoms of the MM residues from the QM region centered at the C_γ atom of the ester intermediate.

We immediately notice that the positive charge developed on the imidazole ring of His289 is stabilized by Asp260, and more importantly, the stabilizing effect is greater at the transition state that the product state. A second important feature in the ester hydrolysis reaction, which is common to many protease and hydrolyase catalysis, is the development of a negative charge the carbonyl oxygen of ester bond from the side chain of Asp124. The so-called oxyanion hole is stabilized by hydrogen bonding interactions with backbone amides of Trp125 and Glu56 (Fig. 4) [6]. Figure 3 also indicates that all other residues make relatively small contributions individually, but they generate significant contributions collectively. It will be interesting to examine if similar observations can be found from molecular dynamics simulations.

Conclusions

In this paper, we present a method for the correction of errors introduced in combined QM/MM calculations using semiempirical Hamiltonians. Noticing the fact that semiempirical models provide a reasonable representation of the general shape of the potential energy surface for chemical reactions, we introduce a simple valence bond (SVB) term to correct the energies at critical points on the potential energy surface. The present SVB term is not a stand-alone potential energy function such as the widely-used empirical valence bond method or the London-Eyring-Polanyi-Sato form used in gas-phase dynamics calculations. The SVB term is used purely for introducing small energy corrections to the semiempirical Hamiltonian to achieve the accuracy needed for modeling enzymatic reactions. Such a correction term is useful because ab initio calculations that include electron correlation with large basis functions are too expensive for molecular dynamics calculations of enzyme systems. We have shown that the present coupled QM-SVB/MM approach can be parameterized to reproduce experimental and ab initio results for model reactions. The resulting potential has been applied to the nucleophilic addition reaction in haloalkane dehydrogenase. In a preliminary energy minimization study, the PM3-SVB/MM results seem to be reasonable and can be used in molecular dynamics free energy simulations to assess enzymatic reaction mechanism. Our computational results show that the nucleophilic addition and proton transfer process take place in an asynchronous concerted fashion.

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