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Concerted Hydrogen Atom and Electron Transfer Mechanism for Catalysis by Lysine-Specific Demethylase

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Abstract

We calculate the free energy profile for the postulated hydride transfer reaction mechanism for the catalysis of lysine demethylation by lysine-specific demethylase LSD1. The potential energy surface is obtained by using combined electrostatically embedded multi-configuration molecular mechanics (EE-MCMM) and single-configuration molecular mechanics (MM). We employ a constant valence bond coupling term to obtain analytical energies and gradients of the EE-MCMM subsystem, which contains 45 QM atoms and which is parametrized with a density functional calculations employing specific reaction parameters obtained by matching high-level wave function calculations. In the MM region, we employ the Amber ff03 and TIP3P force fields. The free energy of activation at 300 K is calculated by molecular dynamics (MD) umbrella sampling on a system with 102090 atoms as the maximum of the free energy profile along the reaction coordinate as obtained by the weighted histogram analysis method with 17 umbrella sampling windows. This yields a free energy of activation of only 10 kcal/mol, showing that the previously postulated direct hydride transfer reaction mechanism is plausible, although we find that it is better interpreted as a concerted transfer of a hydrogen atom and an electron.

Keywords

enzyme catalysis; free energy of activation profile; hydride transfer; electrostatically embedded multiconfiguration molecular mechanics; quantum mechanics; specific reaction parameters

1. INTRODUCTION

Combined quantum-mechanics/molecular-mechanics (QM/MM) methods are a powerful tool for modeling reactions evolving electronic structure changes in enzyme-catalyzed reactions and other biomolecular and catalytic processes.^{1–17} In QM/MM calculations, an accurate energy calculation is carried out for a QM subsystem by using either wave function

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theory or density functional theory. The surrounding environment of the QM subsystem is treated by molecular mechanics. Standard molecular mechanical force fields are based on empirical potentials describing small-amplitude vibrations, noncovalent van der Waals interactions, and electrostatic interactions, and when parametrized in the usual way they do not describe bond breaking and so cannot be applied to the whole system when a chemical reaction is involved. It is an open challenge to implement QM/MM calculations employing reliable QM methods for molecular dynamics simulations because the size of the QM region required to treat the active site reliably is large, and extensive molecular dynamics (MD) conformational sampling is required to calculate reliable free energies, and yet, to obtain accurate energies and gradients for the MD simulation, it is desirable to employ a high level of electronic structure theory (density functional theory or wave function calculations with dynamical electron correlation) for the QM part. For large QM subsystems and typical MD simulation time scales (thousands of picoseconds), reliable electronic structure methods are expensive.

One approach to overcoming the size and time scale challenges is to facilitate the energy and gradient calculations needed for QM/MM MD simulations by the method proposed by Higashi and one of the present authors, in particular the electrostatically embedded multi-configuration molecular mechanics (EE-MCMM) method.^{18–20} Employing EE-MCMM as the QM method provides a very efficient means to carry out quantum mechanical/molecular mechanical (QM/MM) calculations in molecular dynamics simulations. Instead of directly calculating the energy and gradient of the QM part at each MD step, the EE-MCMM method employs an analytic potential fitted in a particularly convenient fashion to the QM calculations, and this fitting requires a limited number of QM electronic structure calculations for an electronically embedded QM region with the electronic embedding based on QM partial charge–MM partial charge interactions. As originally applied, the electronic structure calculations were used to generate a four-dimensional Hessian expansion at each of several interpolation nodes, and these four-dimensional Hessians were joined together by Shepard interpolation of a valence bond coupling matrix element, thereby yielding an analytical function for the potential energy surface that includes both the electronic energy and nuclear repulsion of the QM subsystem and an environment-dependent self-consistent treatment of the QM–MM electrostatic interaction energy. By adding in the nonelectrostatic interaction terms between the QM and MM regions and the energy in the MM region, the total QM/MM energy and gradient can be obtained analytically, which facilitates extensive sampling of the conformational space along the reaction coordinate by MD. This method has been successfully applied to study the S_N2 reaction of between Cl^- and CH_3Cl^- in aqueous solution^{18,19} and to study $RCH_2C(O)O^- + CH_2ClCH_2Cl \rightarrow RCH_2C(O)OCH_2CH_2Cl + Cl^-$, where R is haloalkane dehalogenase, and the carboxylate nucleophile participating in the reaction is the side chain of Asp124.²⁰ It has also used for the direct simulation of excited-state intramolecular proton transfer and vibrational coherence of 10-hydroxybenzo[h]quinoline in solution.²¹ Here we show that it can be used with a convenient simplification (explained below) that reduces the cost even further.

In the current study, we employ the simplified EE-MCMM/MM/MD method to calculate the free energy profile for the hydride transfer reaction catalyzed by lysine-specific demethylase (LSD1). LSD1 is an amine oxidase that plays important roles in the epigenetic regulation of

gene transcription in eukaryotic cells by catalyzing the demethylation of mono-*N*-methylated and di-*N*-methylated lysyl residues in the *N*-terminal tails of histones.^{22–26} LSD1 requires flavin adenine dinucleotide (FAD) as a coenzyme, and it oxidizes the carbon-nitrogen bond between the methyl group and the ϵ -amine of *N*-methylated lysyl residues. Several mechanisms have been proposed for the mechanism of the amine oxidation, including both carbanion mechanisms and direct hydride transfer from the substrate (the methyl group and the ϵ -amine of the *N*-methylated lysyl residues) to the flavin group of the cofactor,²⁷ with the latter mechanism shown for a model reaction in Scheme 1, where the FAD and the side chain of the amino acid are both truncated with methyl groups in the model. In the current work we simulate the free energy profile of this direct reaction path in an untruncated model of the whole solvated protein-substrate complex. The energy and gradient needed in the MD sampling steps are obtained by EE-MCMM/MM calculations based on electronic structure Hessian calculations at three geometries (reactant, product, and transition state). The EE-MCMM parametrization of the calculations is based on the MPW1K density functional²⁸ with specific reaction parameters for a 45-atom subsystem.

2. CALCULATIONS

2.1. Model system and choice of electronic structure method

First we performed calculations on the model system of Scheme 1 in order to select a suitable electronic structure model. For this 39-atom model, we optimized the geometry of the reactant van der Waals (vdW) complex, the transition structure (a stationary point with one imaginary frequency corresponding to the reaction coordinate), and the product vdW complex by several density functional^{28–30} methods; all density functional calculations in this article are carried out with the 6-31+G(d,p)³² or the 6-31G(d,p)³¹ basis set. The choice of density functionals examined is not intended to be a systematic exploration of density functional theory; rather four approximate density functionals are selected because previous experience^{28–30,32} shows that they provide reasonably accurate barrier heights for a variety of reaction types, and therefore they are good candidates for starting points of a treatment employing specific reaction parameters. Standard density functional calculations were carried out with the MPW1K,²⁸ M06-2X,²⁹ M08-HX,³⁰ and M08-SO³⁰ density functionals using an integration grid with 99 radial shells and 974 angular points per shell. The M06-2X and M08-HX density functional calculations were performed by using the *Gaussian 09* program,³³ and the M08-SO density functional calculations were carried out using a locally modified version of *Gaussian 09* program, namely *MN-GFM5.0*.³⁴ The optimized structures of the stationary points as obtained with the M06-2X density functional are shown in Figure 1.

In addition to the density functional calculations two multi-coefficient wave function correlation methods, namely BMC-CCSD³⁵ and G3SX,³⁶ were used to calculate single-point energies at the geometries optimized with the density functionals. The BMC-CCSD and G3SX calculations were carried out using *MLGAUSS2.0*.³⁷ Note that in these calculations, we are calculating just the potential energy without the vibrational contributions.

The reaction energies and forward and reverse barrier heights are tabulated in Table 1. The BMC-CCSD calculations in Table 1 show that the results are not sensitive to the method used to optimize the geometries of the stationary points. We chose the results obtained by G3SX//M06-2X/6-31+G(d,p) as our best estimates. Table 1 shows that, if we accept the G3SX result as the most accurate one, the density functional calculations using MPW1K, M06-2X, M08-HX, and M08-SO overestimate the barrier heights for both forward and reverse reactions. By contrast, the BMC-CCSD calculations underestimate the barrier heights. We therefore employ a specific-reaction-parameter (SRP) density functional to obtain a barrier for the reaction that agrees with the G3SX value; to obtain this functional we modified the MPW1K functional by changing the percentage of Hartree-Fock (HF) exchange and by scaling the gradient correction to the correlation energy with the 6-31G(d,p) basis set. The new functional, called MPW-SRP, has 38.6% Hartree-Fock exchange (as compared to 42.8% in MPW1K) and the gradient correction in the correlation is scaled by a factor of 1.74 (as compared to 1.00 in MPW1K). The MPW-SRP results agree with G3SX//M06-2X/6-31+G(d,p) results very well, and MPW-SRP/6-31G(d,p) is selected to carry out the QM part of all QM/MM calculations in the solution phase in the next step.

2.2. Enzyme setup and EE-QM/MM calculations

LSD1 has three structural domains: a SWIRM (Swi3p, Rsc8p, and Moira) domain, an amine-oxidase-like domain, and a tower domain. The tower domain binds CoREST, which is a SANT-domain-containing transcriptional corepressor that mediates the demethylation. From the Protein Database (PDB code 2V1D³⁸), we obtained the structure of an LSD1•CoREST complex co-crystallized with a peptide substrate in the catalytic cavity. The substrate is a 16-residue peptide of the *N*-terminal H3 histone tail in carrying the K4M mutation, which in our setup was mutated back to dimethylated lysine. The CoREST was removed, and the tower domain was truncated at residues 422 and 519, which were then connected by a Gly-Gly-Gly loop.³⁹ Hydrogen atoms were added with the *CHARMM*⁴⁰ HBUILD module, the system was solvated in a 100 Å cubic box of TIP3P⁴¹ water molecules, and chloride ions were added to enforce electroneutrality; this yields a system with 102090 atoms, which were treated by the EE-MCMM combined quantum mechanical and molecular mechanical method, as discussed next.

Since we are studying the hydride transfer between C and N, there is the possibility of considerable short-range nuclear and electronic reorganization during the reaction. Note that the hydride-accepting nitrogen atom is located in an aromatic highly-conjugated ring-structured scaffold environment; thus it is important to include the atoms in the rings as part of the QM subsystem in order to adequately model the variable electron de-localizing effects of conjugated orbitals in the ring segment of FAD. Given that the ribitol group of FAD is not orbitally conjugated to the ring segment and is highly spatially separated from the reaction center, the ribitol group can remain in the MM subsystem, and it was placed there. The hydride-donating carbon atom is connected with the NCH₂CH₂CH₂CH₂ group of the demethylated-lysine side chain, and the electrons and orbitals in the closest nitrogen atom and the next carbon atom are included in the QM subsystem because of their proximity to the hydride transfer. The more distant carbons and hydrogens need not be included due to larger spatial separation from the reactive site; thus these atoms were left in the MM part of

the calculation. This yields a 45-atom QM subsystem that includes the *N*-dimethyl- ϵ -amine group of the Lys4 residue and part of protonated flavin cofactor; see Figure 2. The remaining 102045 atoms were treated by molecular mechanics.

The quantum mechanical electronic structure calculations used to parametrize the EE-MCMM calculations were carried out using MPW-SRP/6-31G(d,p); the MM force field was TIP3P⁴¹ for water and AMBER ff03⁴² for the MM part of the substrate and protein. To obtain the electrostatic interaction between QM and MM subsystem, partial charges of the QM region were obtained by Charge Model 4⁴³ (CM4), and partial charges of the MM region were obtained by restrained electrostatic potential^{42,44} (RESP). The interaction between the QM and MM regions was treated using link hydrogen atoms with the balanced redistributed charge (BRC) scheme in the MPW-SRP/6-31G(d,p)-AMBER ff03 interface.^{45,46} The QM-MM electrostatic interaction in the EE-QM calculations is smoothed to zero at 15 Å by using a tapering method.⁴⁷ (Note that the EE-QM internal energy of the QM subsystem depends on not only the coordinates of the QM subsystem, but also on the electrostatic potential Φ_a at each atom a , with $a = 1, 2, \dots, 45$. The charge response kernels^{18–20} $\chi_{a\beta}$ and $\kappa_{a\beta}$ are used to obtain the QM-MM electrostatic interaction energy in the optimization step discussed below.) The EE-QM/MM calculations were carried with GAMESSPLUS.^{47,48}

After short restrained minimization and gradual heating, the system was equilibrated at constant pressure and temperature (298.15 K, 1 atm) for 2 ns with the program *NAMD* v2.6.⁴⁹ Electrostatic interactions were treated with particle mesh Ewald⁵⁰ and switching cutoffs between 9 and 11 Å were used for Lennard-Jones interactions. After global equilibration, the system was prepared for QM/MM calculations: proton-transfer from the H3 dimethyl-Lys4 to Lys661 was mimicked by setting their protonation state to neutral and protonated, respectively. The entire flavin and the dimethyl-Lys4 side-chain moieties were treated quantum-mechanically with the semiempirical Austin Model 1, and the system was further equilibrated for 350 ps with CHARMM. Then the reactant, product, and transition structure geometries of the enzyme system are obtained by EE-QM/MM optimization. During the optimizations, the 459 atoms within a 10-Å radius around the central nitrogen atom, labeled N1 in Figure 2(a), were allowed to move, and all other atoms were constrained to fixed positions.

2.3. EE-MCMM calculations

The QM subsystem contribution to the potential energy in the EE-MCMM method is the lowest eigenvalue of a 2×2 diabatic Hamiltonian matrix,^{18–20}

$$V^{\text{EE-MCMM}} = \begin{pmatrix} V_{11} & V_{12} \\ V_{12} & V_{22} \end{pmatrix} \quad (1)$$

where V_{12} is the valence bond coupling term, where the diagonal elements of the matrix are the valence bond diabatic energies given by

$$V_{ii}(q, \Phi) = V_{ii}^{\text{MM}}(q) + V_{ii}^{\text{CRK}}(q, \Phi) + \delta_{i2} \Delta E \quad (2)$$

where $i = 1$ (for the reactant) or 2 (for the product), where E is the QM subsystem internal energy of the products relative to the reactants (excluding its electrostatic interaction V_{ii}^{CRK} with the MM subsystem), where V_{ii}^{MM} is a potential energy function describing the reactant and product, for which we employed the AMBER GAFF⁵¹ force field with modifications of the bond stretching and vdW terms,²¹ and where

$$V_{ii}^{\text{CRK}}(q, \Phi) = Q^{(i)\text{T}} \Delta \Phi^{(i)} + \frac{1}{2} \Delta \Phi^{(i)\text{T}} \chi^{(i)} \Delta \Phi^{(i)} + \Phi^{(i)\text{T}} \kappa^{(i)} \Delta q^{(i)} \quad (3)$$

where q and Φ represent the nuclear coordinates of the QM subsystem and the electrostatic potential from the MM region, and where Q , χ , and κ are the partial charge and the charge response kernels in the QM subsystem.

We set E equal to -8.54 kcal/mol based on optimized reactant and product QM segment geometries in the solution phase, as obtained in Section 2.2 and shown in Figure 2.

In previous work,^{18–20,52} the coupling term V_{12} was obtained by Shepard interpolation. In the current study, we set V_{12} equal to a constant value, which we parametrized to be 47.9 kcal/mol. This value was obtained by requiring the EE-QM/MM energy and EE-MCMM energy to be the same at the transition structure of Figure 2(b). This requires

$$V_{12} = \sqrt{(V_{11}^{\text{TS}} - V^{\text{EE-QM/MM,TS}})(V_{22}^{\text{TS}} - V^{\text{EE-QM/MM,TS}})} \quad (4)$$

where $V^{\text{EE-QM/MM,TS}}$ is the EE-QM/MM potential energy barrier for the transition state with respect to the reactant. This term includes both the electronic structure barrier in the gas phase and the polarization caused by the QM-MM electrostatic interactions. In the current study, $V^{\text{EE-QM/MM,TS}}$ equals 13.1 kcal/mol, which is obtained by calculating the EE-QM/MM energy of reaction and transition structure energy using the EE-QM/MM method.

The diabatic potential energies at the transition state geometry are V_{11}^{TS} , and V_{22}^{TS} , are calculated using eqs 2 and 3.

The EE-MCMM potential energy is used for the following MD simulation.

2.4. MD simulation and potential of mean force calculations

The reaction coordinate s defined by

$$s = r_1 - r_2 \quad (1)$$

where r_1 is the breaking C-H bond distance, and r_2 is the forming N-H bond distance. Umbrella sampling was carried out at 300 K to calculate the potential of mean force (PMF) profile along the defined reaction coordinate. The equations of motion were integrated by the velocity Verlet method with a time step of 0.5 fs. From reactant to product, the whole

reaction path is divided into 19 evenly spaced windows centered at values s from -1.97 to 1.63 Å. The restrained force constant in each window is set as $100 \text{ kcal}/\text{\AA}^2$. For each umbrella-sampling window, we began with a 50 ps MD trajectory calculation for equilibration, followed by 100 ps calculation for statistical sampling. The windows were combined by the weighted histogram analysis method (WHAM). The dynamics calculations were carried out by a locally modified version of *AMBER*,⁵³ namely *AMBERPLUS*.⁵⁴ The WHAM calculations were carried out with a program written by Grossfield.⁵⁵

3. RESULTS

The diabatic representation used here provides a global analytic potential energy surface that we found to be robust enough to model the whole free energy profile without spurious artifacts. In comparison to the value of 47.9 kcal/mol used here for the valence bond coupling matrix element, we note that a value of 34.6 kcal/mol was previously added to the off-diagonal matrix element in an effective Hamiltonian mixed molecular orbital and valence bond (EH-MOVB) approach to obtain a total matrix element of 40 kcal/mol .⁵⁶

The key geometrical parameters of the three solution-phase stationary points, as defined in Figure 2 and as optimized in the solution phase in the enzyme active site by EE-QM/MM employing MPW-SRP/6-31G(d,p) for the QM part, as described in Section 2.2, are given in Table 2. The geometrical results are similar to those obtained from B3LYP/6-31+G(d) and M06-2X/6-31+G(d) optimizations and from the EH-MOVB method.⁵⁶

The partial atomic charges on the three atoms (C, H, and N) in the reaction center are listed in Table 3. Table 3 shows that the transferring hydride ion has a positive partial charge that becomes progressively more positive as it transfers. For this reason the hydride transfer might actually be better described as a concerted transfer of a hydrogen atom and an electron, with the electron transferring 21% to the acceptor nitrogen and the remainder to the rest of the FAD rather than being localized on the transferring hydrogen atom.

A snapshot from the umbrella sampling MD simulations is shown in Figure 3, and the potential of mean force (PMF) obtained by the WHAM method is displayed in Figure 4. The free energy of activation is the maximum of this free energy profile, and the figure shows that this occurs at $s = 0.02$ Å, which is consistent with the EE-QM/MM transition structure in the solution phase (indicating negligible variational effect⁵⁷ on the location of the dynamical bottleneck) and is 10 kcal/mol . This value is reasonably consistent with, although smaller than, the phenomenological experimental value, which is obtained using experimental rate data,²⁸ and which is 18 kcal/mol . In a review⁵⁸ of six previous studies of quantum effects on enzyme kinetics, the inclusion of nuclear motion quantum effects (quantized vibrations and tunneling) increased the rate constant on average by a factor of 220, corresponding to a decrease in the phenomenological free energy of activation by 3 kcal/mol . Including these effects would make the disagreement with experiment larger. We note that if, as in earlier work,⁵⁶ we had calibrated our potential using the DFT barriers rather than the wave function barriers, which are higher (see Table 1 and the discussion in Section 2.1), our results would be in better agreement with the experimental value, but calculating the precise value of the barrier or rate constant was not our objective (and

therefore we did not include tunneling or zero point effects). The most significant finding is that the free energy of activation is calculated to be low enough that the postulated direct transfer mechanism is feasible, although it is better described as a concerted hydrogen atom and electron transfer.

4. CONCLUDING REMARKS

We employed the electrostatically embedded multi-configuration molecular mechanics with input from density functional calculations with specific reaction parameters to simulate the direct hydride transfer reaction catalyzed by lysine -specific demethylase LSD1. By using a constant valence bond coupling, a stable analytical EE-MCMM potential energy surface of the QM subsystem was obtained, which was further used in the molecular dynamics simulations to calculate the free energy profile for the reaction. This procedure requires a minimal number of high-level calculations for the QM segment in the simulation process; therefore it facilitates the sampling and simulation enormously.

We found a reasonably low barrier for the catalytic step. This confirms that the postulated reaction mechanism is feasible, although we find that in the density functional calculations the partial atomic charge on hydrogen is positive so that the simulated process is better described as a concerted transfer of a hydrogen atom and an electron.

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Figure 1.
The structures of the (a) reactant, (b) transition structure, and (c) product of the 39-atom model compounds used for gas-phase calculations that mimic the QM segment of the enzyme reaction.

Figure 2.
The EE-QM/MM structures of the (a) reactant, (b) transition structure, and (c) product of the 45-atom QM segment of the enzyme reaction in the solution phase.

Figure 3. Snapshot of the active site of LSD1 for the hydride transfer from substrate to protonated flavin adenine dinucleotide.

Figure 4.
Free energy profile as a function of reaction coordinate s .

Scheme 1.

Table 1

Calculated forward and reverse barrier heights and energies of reaction^a for the model compounds in gas phase (in kcal/mol)

	V_f	V_r	E
M06-2X/6-31+G(d,p)	15.20	23.32	-8.13
M08-HX/6-31+G(d,p)	15.84	24.28	-8.44
M08-SO/6-31+G(d,p)	16.75	26.64	-9.89
MPW1K/6-31+G(d,p)	19.95	29.54	-9.59
G3SX//M06-2X/6-31+G(d,p)	13.62	20.85	-7.24
BMC-CCSD//M06-2X/6-31+G(d,p)	11.77	19.84	-8.06
BMC-CCSD//M08-HX/6-31+G(d,p)	11.70	19.83	-8.13
BMC-CCSD//M08-SO/6-31+G(d,p)	11.85	19.85	-8.00
MPW-SRP/6-31G(d,p) ^b	13.59	20.82	7.23

^a All barrier heights and reaction energies in this table are zero-point-exclusive; i.e., they are potential energies.

^b Contains 38.6% Hartree-Fock exchange and 174% non-local correction

Table 2

Coordinates ($\text{\AA}$) of EE-QM/MM solution-phase geometries for the QM subsystem optimized by MPW-SRP/6-31G(d,p)

	r_1	r_2	s
Reactant vdW complex	1.10	2.69	-1.59
Transition structure	1.30	1.32	-0.02
Product vdW complex	2.75	1.01	1.74

Table 3

Partial atomic charges on the active C, H, and N atoms of the reaction center of the QM subsystem in the reactant and product complexes and the transition state in the solution phase.^a

	C	H	N
Reactant vdW complex	-0.05	0.04	-0.26
Transition structure	0.00	0.20	-0.34
Product vdW complex	0.22	0.31	-0.47

^aThe charges were obtained by the CM4 charge model with the MPW-SRP/6-31G(d,p) method used for the QM subsystem in EE-QM/MM calculations.