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Reaction Pathway for Cocaine Hydrolase-Catalyzed Hydrolysis of (+)-Cocaine

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Abstract

A recently designed and discovered cocaine hydrolase (CocH), engineered from human butyrylcholinesterase (BChE), has been proven promising as a novel enzyme therapy for treatment of cocaine overdose and addiction because it is highly efficient in catalyzing hydrolysis of naturally occurring (–)-cocaine. It has been known that the CocH also has a high catalytic efficiency against (+)-cocaine, a synthetic enantiomer of cocaine. Reaction pathway and the corresponding free energy profile for the CocH-catalyzed hydrolysis of (+)-cocaine have been determined, in the present study, by performing first-principles pseudobond quantum mechanical/molecular mechanical (QM/MM)-free energy (FE) calculations. According to the QM/MM-FE results, the catalytic hydrolysis process is initiated by the nucleophilic attack on carbonyl carbon of (–)-cocaine benzoyl ester *via* hydroxyl oxygen of S198 side chain, and the second reaction step (*i.e.* dissociation of benzoyl ester) is rate-determining. This finding for CocH-catalyzed hydrolysis of (+)-cocaine is remarkably different from that for the (+)-cocaine hydrolysis catalyzed by bacterial cocaine esterase in which the first reaction step of the deacylation is associated with the highest free energy barrier (~17.9 kcal/mol). The overall free energy barrier (~16.0 kcal/mol) calculated for the acylation stage of CocH-catalyzed hydrolysis of (+)-cocaine is in good agreement with the experimental free energy barrier of ~14.5 kcal/mol derived from the experimental kinetic data.

Introduction

Cocaine addiction and overdose have been a major public health problem affecting over 40 million Americans since 1980.¹ Patients with cocaine addiction and overdose suffer heart attacks or strokes which are life-threatening. The disastrous medical and social

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consequences of cocaine addiction, such as violent crimes, loss in individual productivity, illness, and death, have made the development of an effective pharmacological treatment a high priority.^{2, 3} It is highly desired for development of an anti-cocaine medication to design an efficient cocaine-metabolizing enzyme which can be used an exogenous enzyme to rapidly detoxify cocaine and convert cocaine to biologically inactive metabolites.⁴

Butyrylcholinesterase (BChE) is the principal cocaine-metabolizing enzyme which hydrolyzes cocaine to biologically inactive metabolites in human plasma⁵ and, thus, cocaine hydrolysis catalyzed by BChE is the cocaine-metabolizing pathway most suitable for amplification in therapeutic development for treatment cocaine abuse. However, the wild-type BChE has a rather low catalytic efficiency ($k_{\text{cat}} = 4.1 \text{ min}^{-1}$, $K_{\text{M}} = 4.5 \text{ }\mu\text{M}$) against naturally occurring (–)-cocaine.⁶ Our computational studies^{7–12} on BChE-catalyzed hydrolysis of (–)-cocaine and subsequent structure-and-mechanism-based computational design have led to the discovery of various BChE mutants with a considerably improved catalytic efficiency against (–)-cocaine.^{13–24} Among these BChE mutants and cocaine-metabolizing enzymes reported so far, the A199S/F227A/S287G/A328W/Y332G mutant of human BChE has been studied most extensively in animal models of cocaine overdose or addiction due to its extremely high catalytic efficiency ($k_{\text{cat}}K_{\text{M}} = \sim 1.84 \times 10^9 \text{ M min}^{-1}$) and the high *in vivo* activity.²⁵ For example, pretreatment with this cocaine hydrolase (CocH) using a dose of 0.01 mg per mouse (i.v.) fully protected mice from the acute toxicity of a lethal dose of cocaine (180 mg/kg, i.p.), which shows that the CocH is efficient for cocaine detoxification.²⁵ This CocH has been recognized as a *true* CocH in literature.²⁶ It has also been demonstrated by independent animal studies^{27–30} that the gene transfer using a viral vector encoding this CocH can yield plasma CocH levels that effectively detoxify cocaine, block cocaine-induced physiological effects, and diminish long-term cocaine intake for lengthy periods without immune reactions or cholinergic dysfunction.

Further, it has been demonstrated that the CocH can also efficiently hydrolyze (+)-cocaine.^{31, 32} However, the reaction pathway for CocH-catalyzed hydrolysis of (+)-cocaine remains to be uncovered. In the present study, we have employed the first-principles pseudobond quantum mechanical/molecular mechanical-free energy (QM/MM-FE)^{33–36} calculations, which has been demonstrated to be a powerful tool in simulating a variety of enzymes,^{25, 37–57} to reveal the reaction pathway for CocH-catalyzed hydrolysis of (+)-cocaine.

Based on the detailed mechanistic insights obtained from our recent computational studies on carboxylic esters (*e.g.* acetylcholine, butyrylcholine, and (–)-cocaine) for their hydrolyses catalyzed by human BChE (or mutant) or bacterial cocaine esterase (CocE),^{10, 15, 25, 43, 58–63} the ester hydrolysis always consists of two stages: acylation and deacylation as shown in Scheme 1. Scheme 1 indicates that CocH-catalyzed hydrolyses of (+)-cocaine and (–)-cocaine share the common deacylation stage. Since the detailed reaction pathway for CocH-catalyzed hydrolysis of (–)-cocaine has already been elucidated in our previous study⁶⁰, the present study is focused on the acylation stage for CocH-catalyzed hydrolysis of (+)-cocaine. Based on the computational results, the second reaction step of the acylation is associated with the highest free energy barrier for CocH-catalyzed hydrolysis of (+)-cocaine, which is remarkably different from the (+)-cocaine hydrolysis catalyzed by CocE (bacterial

cocaine esterase) in which the first reaction step of the deacylation is rate-determining. The overall free energy barrier calculated for the acylation stage of CocH-catalyzed hydrolysis of (+)-cocaine is in good agreement with the experimental kinetic data.

Computational Methods

Structure preparation

The initial structure of the Michaelis–Menten complex (**ES**) of CocH binding with (+)-cocaine was constructed on the basis of the QM/MM-optimized geometry of the corresponding complex of (–)-cocaine in our previous study.⁶⁰ The system was solvated in an orthorhombic box of TIP3P water molecules⁶⁴ with a minimum solute-wall distance of 10 Å. After full energy minimization, the prepared system was equilibrated *via* the gradual increase in temperature from 10 to 298.15 K during molecular dynamics (MD) simulation. Then, the production MD simulation was performed for ~2 ns at 298.15 K. The MD simulation was performed by using the SANDER module of AMBER9^{65, 66} in the present study. During the MD simulation, the time step was set as 2 fs with a cutoff value of 10 Å for non-bonded interactions. The SHAKE algorithm^{67, 68} was used to constrain all covalent bonds involving hydrogen atoms, and the particle mesh Ewald (PME) method was employed to treat long-range electrostatic interactions.

QM/MM-FE Simulation

The general QM/MM-FE methodology has been described in detail in the literature.^{33, 35, 36, 43} Here we only briefly discuss the part most relevant to the present work. The initial structure for QM/MM calculations was prepared from the snapshot close to the average structure of the MD trajectory. The pseudobond QM/MM method^{33, 35} implemented recently in a revised version²⁵ of Gaussian03⁶⁹ and AMBER8⁷⁰ programs was used for all of the QM/MM calculations. The pseudobond approach was used to treat the QM-MM interface, where each boundary atom of MM part is replaced by a seven-valence electron atom with an effective core potential, forming a pseudobond with the boundary atom of QM part. In QM/MM calculation, all atoms of (+)-cocaine and the side chains of S198, H438, and E325 residues were considered as the QM atoms, whereas other atoms were treated as MM atoms (Figure 1). The QM/MM calculations were carried out using an iterative minimization procedure³⁶ at the B3LYP/6-31G*:AMBER level, *i.e.* the QM calculations at the B3LYP/6-31G* level and the MM calculations using the AMBER force field implemented in the AMBER8 program. For calculations on the QM subsystem, the geometry optimization convergence criterion followed the original Gaussian03 default criteria; for calculations on the MM subsystem, the convergence criterion for geometry optimization was that the root-mean-square deviation (RMSD) value of the energy gradient $< 0.1 \text{ kcal}\cdot\text{mol}^{-1}\cdot\text{\AA}^{-1}$. The minimum-energy path was then determined by using an iterative restrained optimization procedure, which was repeatedly applied to the different points along the reaction coordinate. Full QM/MM geometry-optimizations and vibrational frequency analyses at the B3LYP/6-31G*:AMBER level were performed to characterize the reactant, intermediates, and transition states. The contribution of the QM subsystem fluctuation to the free energy change was then evaluated with the obtained vibrational frequencies using the

harmonic approximation. In addition, single-point energy calculations were performed at the QM/MM(MP2/6-31+G*:AMBER) level for each geometry along the minimum-energy path.

The free energy changes associated with the QM-MM interaction were then more reasonably determined by the free energy perturbation (FEP) method^{36, 71} implemented in a revised version⁴³ of the AMBER8 program. In the FEP calculations, the time step was 2 fs, and bond lengths involving hydrogen atoms were constrained. The QM subsystem was frozen at different points along the reaction path in sampling of the MM subsystem at 298.15 K.³⁶ Each FEP calculation consisted of 50 ps of equilibration and 300 ps of sampling, which is a reliable protocol validated in the previous computational studies.^{12, 72, 73}

The MD simulation and QM/MM-FE calculations were performed on a supercomputer (*e.g.* IBM X-series Cluster with 340 nodes or 1,360 processors) at University of Kentucky Center for Computational Sciences. The other less-time-consuming modeling and computations were carried out on SGI Fuel workstations and a 34-processor IBM x335 Linux cluster in our own lab.

Results and Discussion

BChE-(+)-Cocaine Binding Structure

A ~2 ns MD simulation was performed on the **ES** complex. The MD-simulated RMSD values for the C^α atoms (depicted in Figure 1) *versus* the simulation time were very stable, showing that the simulated structure of **ES** complex was in a stable equilibrium state. The trace D1 is the internuclear distance between the hydroxyl oxygen (O^γ) of S198 residue and the carbonyl carbon (C^δ) of (+)-cocaine benzoyl ester. The trace D2 is the internuclear distance between the hydroxyl hydrogen (H^γ) of S198 residue and the nitrogen (N^ε) of H438 residue. The traces D3, D4, and D5 represent the internuclear distances between the carbonyl oxygen (O^η) of (+)-cocaine benzoyl ester and the NH hydrogen atom of G117, NH hydrogen atom of S199, and hydroxyl hydrogen of S199, respectively. In the MD simulation, the average value of D1 was $\sim 3.45 \pm 0.13$ Å, showing that (+)-cocaine was located at an appropriate position for the nucleophilic attack on the carbonyl carbon (C^δ) of (+)-cocaine by the hydroxyl oxygen O^γ of S198 residue. The average value of D2 was $\sim 1.87 \pm 0.10$ Å, indicating that H438 residue of the BChE catalytic triad was well positioned to facilitate the nucleophilic attack by accepting a proton from S198 residue. The average values of D3, D4, and D5 were 2.61 ± 0.15 Å, 2.40 ± 0.18 Å, 1.89 ± 0.14 Å, respectively, and did not significantly change over the trajectory, indicating that the carbonyl oxygen O^η of (+)-cocaine forms stable hydrogen bonds with the oxyanion hole.

Fundamental Reaction Pathway

According to our pseudobond QM/MM reaction-coordinate calculations at the B3LYP/6-31G*:AMBER level, the acylation stage of CocH-catalyzed hydrolysis of (+)-cocaine consists of two reaction steps. The first step is the nucleophilic attack on the carbonyl carbon (C^δ) of (+)-cocaine benzoyl ester by the O^γ atom of catalytic S198, and the second step is the dissociation between the benzoyl ester and ecgonine methyl ester of (+)-cocaine.

Step 1: Nucleophilic attack on the benzoyl carbonyl carbon (C^{ζ}) by the hydroxyl oxygen (O^{γ}) of S198—In the nucleophilic attack process, the O^{γ} atom of S198 side chain attacks the carbonyl carbon (C^{ζ}) of (+)-cocaine benzoyl ester, while the H^{γ} proton on the S198 side chain transfers to the nitrogen (N^e) atom on the H438 side chain. Since the break of $O^{\gamma}-H^{\gamma}$ bond and the formations of both $C^{\zeta}-O^{\gamma}$ and N^e-H^{γ} bonds are involved in this reaction step, the nature of this chemical reaction step can be reflected by the distances between O^{γ} and H^{γ} atoms ($R_{O^{\gamma}-H^{\gamma}}$), between C^{ζ} and O^{γ} atoms ($R_{C^{\zeta}-O^{\gamma}}$), and between N^e and H^{γ} atoms ($R_{N^e-H^{\gamma}}$). Therefore, $R_{O^{\gamma}-H^{\gamma}} - R_{C^{\zeta}-O^{\gamma}} - R_{N^e-H^{\gamma}}$ was set as the reaction coordinate for the QM/MM reaction-coordinate calculations for this reaction step. The reactant, intermediate, and transition state were characterized by full geometry optimizations at the B3LYP/6-31G*:AMBER level followed by the harmonic vibrational frequency analysis. Their geometries are shown in Figure 2, and important geometric parameters are summarized in Table 1. During the reaction process of the nucleophilic attack, in which the carbonyl C^{ζ} atom of (+)-cocaine benzoyl ester is attacked by the hydroxyl O^{γ} atom of S198, $R_{C^{\zeta}-O^{\gamma}}$ is shortened from 2.64 Å in the reactant (**ES**) to 1.54 Å in the intermediate (**INT1**), indicating that a covalent bond between the C^{ζ} and O^{γ} atoms is formed. The C^{ζ} atom, which is sp^2 -hybridized and in a planar geometry with its three attached groups in **ES**, gradually changes into a tetrahedral geometry with the center on the sp^3 -hybridized C^{ζ} atom in **INT1** through the transition state (**TS1**). $R_{N^e-H^{\gamma}}$ is shortened from 1.67 Å in **ES** to 1.03 Å in **INT1** and $R_{O^{\gamma}-H^{\gamma}}$ is elongated from 1.02 Å in **ES** to 1.98 Å in **INT1**, illustrating that the proton H^{γ} has been transferred to the N^e atom of H438 from the hydroxyl group of S198. In addition, the distance between the H^{δ} atom of H438 and the O^{δ} atom of E325 is shortened from 1.62 Å in **ES** to 1.42 Å in **INT1**, implying that the hydrogen bond ($N^{\delta}-H^{\delta}\cdots O^{\delta}$) involving the H^{δ} and O^{δ} atoms is stronger in **INT1** than that in **ES** and, thus, plays an important role in stabilizing the developed positive charge on the protonated H438 in **INT1**.

Step 2: Dissociation of (+)-Cocaine Benzoyl Ester—In this reaction step, the ecgonine group of (+)-cocaine gradually departs from the (+)-cocaine benzoyl ester group through the break of the benzoyl ester bond $C^{\zeta}-O^{\zeta}$ while the proton (H^{γ}) of the protonated H438 residue gradually transfers to the benzoyl ester oxygen atom (O^{ζ}) of (+)-cocaine. The changes of the distances of $R_{C^{\zeta}-O^{\zeta}}$, $R_{O^{\zeta}-H^{\gamma}}$ and $R_{N^e-H^{\gamma}}$ reflect the nature of this dissociation process of (+)-cocaine benzoyl ester. Thus the reaction coordinate for the current reaction step was chosen as $R_{C^{\zeta}-O^{\zeta}} + R_{N^e-H^{\gamma}} - R_{O^{\zeta}-H^{\gamma}}$. The intermediates and transition states were characterized through full geometry optimizations at QM/MM(B3LYP/6-31G*:AMBER) level followed by the harmonic vibrational frequency analysis. Their geometries are shown in Figure 3, and important geometric parameters are summarized in Table 1. Two transition states were observed on the minimum-energy path of the potential energy surface for the current reaction step. This observation is the same as that in the Coch-catalyzed hydrolysis of (–)-cocaine. These two transition states and the intermediate between the two transition states are labeled as **TS2**, **TS2'**, and **INT1'**, respectively. In **INT1**, a strong hydrogen bond $N^e-H^{\gamma}\cdots O^{\gamma}$ is formed between S198 terminal oxygen and H438 side chain ($R_{O^{\gamma}-H^{\gamma}}$ is 1.98 Å). Although the H^{γ} atom is about to be transferred to the O^{ζ} atom, the interaction between H^{γ} and O^{ζ} atoms in **INT1** is very weak ($R_{O^{\zeta}-H^{\gamma}}$ is 3.33 Å), indicating an unfavorable environment for proton (H^{γ}) transfer

from N^e atom of H438 to the leaving ester oxygen (O⁵) atom. In changing from **INT1** to **INT1'**, the only remarkable structural difference is R_{C⁵-O⁵}, which is elongated to be 2.86 Å in **INT1'** from 1.52 Å in **INT1**. The structural changes clearly show the break of the covalent bond C⁵-O⁵ and the leave of the ecgonine group of (+)-cocaine. The C⁵ atom, which is *sp*³-hybridized with a tetrahedral geometry in **INT1**, gradually changes into a planar geometry centering on the *sp*²-hybridized C⁵ atom in **INT1'** through the transition state (**TS2**). In **INT1'**, H438 keeps protonated as in **INT1**. From **INT1'** to **INT2**, R_{O⁵-H^γ} is shortened to be 0.98 Å from 3.46 Å and R_{N^e-H^γ} is elongated to be 2.25 Å from 1.02 Å. Based on these two major differences between **INT1'** and **INT2**, the proton (H^γ) transfers from the N^e atom of the protonated H438 to the leaving ester oxygen (O⁵) atom, and the protonated H438 in **INT1'** changes back to the neutral state in **INT2**. The distance between the H^δ of H438 and the O^δ atom of E325 gradually changes from 1.42 Å in **INT1** to 1.51 Å in **INT1'** and then to 1.66 Å in **INT2**, indicating that the hydrogen bond (N^δ-H^δ...O^δ) between the H^δ and O^δ atoms becomes weaker and the gradual increase of the distance is consistent with the gradual change in the protonation state of H438.

Energetics

In order to accurately calculate the energetics, we carried out further QM/MM single-point energy calculations at the QM/MM(MP2/6-31+G*:AMBER) level by using geometries optimized at the QM/MM(B3LYP/6-31G*:AMBER) level. For each geometry along the minimum-energy path, the ESP charges on QM atoms were determined from the QM/MM single-point energy calculation, and used in subsequent FEP simulations for estimating the free energy changes along the reaction path. Depicted in Figure 4 is the energy profile determined by the QM/MM-FE calculations excluding the zero-point and thermal corrections for the QM subsystem. The values in parentheses are the corresponding relative free energies with the zero-point and thermal corrections for the QM subsystem. The free energy barrier for the first reaction step is ~0.8 kcal/mol. For the second reaction step, *i.e.* the dissociation of cocaine benzyl ester, two transition states (**TS2** and **TS2'**) were found on the minimum-energy path. The free energy barrier for the two reaction processes associated with **TS2** and **TS2'** are ~11.1 and ~5.8 kcal/mol, respectively. Since the energy of **TS2'** is ~4.9 kcal/mol higher than that of **TS2**, the overall free energy barrier for the second reaction step is ~16.0 kcal/mol, which is the free energy change from **INT1** to **TS2'**. It is significantly higher than that of the first reaction step (~0.8 kcal/mol). Therefore, the second reaction step, *i.e.* the dissociation of cocaine benzyl ester, is rate-determining for the acylation stage.

As mentioned above, CocH-catalyzed (+)- and (-)-cocaine hydrolyses share the common deacylation stage. The free energy barrier was calculated to be ~10.9 kcal/mol for the deacylation stage.⁶⁰ Clearly, the second reaction step in the acylation stage with the free energy barrier of ~16.0 kcal/mol is the rate-determining step for the CocH-catalyzed hydrolysis of (+)-cocaine. Notably, the rate-determining step for CocH-catalyzed hydrolysis of (+)-cocaine is remarkably different from that for bacterial CocE-catalyzed hydrolysis of (+)-cocaine. For bacterial CocE-catalyzed hydrolysis of (+)-cocaine, the highest free energy barrier (17.9 kcal/mol) is associated with the first step of the deacylation stage according to the QM/MM-FE calculations at the same level.⁷⁴ Compared to bacterial CocE-catalyzed

hydrolysis of (+)-cocaine, the free energy barrier (~10.9 kcal/mol) calculated for the deacylation stage of CocH-catalyzed hydrolysis of (+)/(-)-cocaine is significantly lower (by ~7 kcal/mol), and the free energy barrier (~16.0 kcal/mol) calculated for the acylation stage of CocH-catalyzed hydrolysis of (+)-cocaine is significantly higher (by ~7 kcal/mol), as seen in Table 2. As a result, the overall free energy barrier of ~16.0 kcal/mol for CocH-catalyzed hydrolysis of (+)-cocaine is ~1.9 kcal/mol lower than that for bacterial CocE-catalyzed hydrolysis of (+)-cocaine.

To validate the computational data, we carried out experimental kinetic analysis on the CocH-catalyzed hydrolysis of (+)-cocaine;⁷⁵ in fact, the computational studies described in this report were actually completed far before the experimental study⁷⁵, but the submission of this report for publication was delayed considerably due to some unexpected reason. The experimental kinetic analysis revealed that catalytic rate constant ($k_{\text{cat}} = 8990 \text{ min}^{-1}$)⁷⁵ for CocH-catalyzed hydrolysis of (+)-cocaine is indeed significantly larger than that for bacterial CocE-catalyzed hydrolysis of (+)-cocaine ($k_{\text{cat}} = \sim 1078 \text{ min}^{-1}$)⁷⁴ determined in the room temperature. Further, based on the experimentally determined kinetic data ($k_{\text{cat}} = 8990 \text{ min}^{-1}$), the free energy barrier for CocH-catalyzed hydrolysis of (+)-cocaine should be ~14.5 kcal/mol according to the conventional transition state theory.⁷⁶ The calculated free energy barrier of ~16.0 kcal/mol is reasonably close to the experimentally determined free energy barrier of ~14.5 kcal/mol.

Conclusion

In the present study, first-principles pseudobond quantum mechanical/molecular mechanical (QM/MM)-free energy (FE) calculations have been performed to elucidate the reaction mechanism for (+)-cocaine hydrolysis catalyzed by a highly efficient cocaine hydrolase (CocH, which is the A199S/F227A/S287G/A328W/Y332G mutant of human BChE). During the acylation stage, the hydroxyl oxygen (O^γ) of S198 side chain initiates the nucleophilic attack on carbonyl carbon (C^ζ) of (+)-cocaine benzoyl ester, which leads to formation of a tetrahedral intermediate. The developing negative charge of carbonyl oxygen (O^η) in the tetrahedral intermediate is stabilized by oxyanion hole through strong hydrogen-bonding interactions. Meanwhile, a proton (H^γ) gradually transfers from the hydroxyl group of S198 side chain to the N^ϵ atom of the H438 side chain. Then, the benzoyl ester bond $\text{C}^\zeta\text{--O}^\zeta$ of (+)-cocaine is broken and the protonated H438 transfers the proton (H^γ) to the ester oxygen (O^ζ) of the leaving ecgonine group, completing the acylation stage. The second reaction step of the acylation stage is identified as the rate-determining step of the CocH-catalyzed hydrolysis of (+)-cocaine. The uncovered reaction mechanism for CocH-catalyzed hydrolysis of (+)-cocaine is remarkably different from that for bacterial CocE-catalyzed hydrolysis of (+)-cocaine in which the first reaction step of the deacylation is rate-determining. The overall free energy barrier (~16.0 kcal/mol) calculated for CocH-catalyzed hydrolysis of (+)-cocaine is significantly lower than that (~17.9 kcal/mol) calculated for bacterial CocE-catalyzed hydrolysis of (+)-cocaine, which is consistent with the relative values of the experimental rate constant ($k_{\text{cat}} = 8990 \text{ min}^{-1}$ for CocH-catalyzed hydrolysis of (+)-cocaine and $k_{\text{cat}} = \sim 1078 \text{ min}^{-1}$ for bacterial CocE-catalyzed hydrolysis of (+)-cocaine) at the room temperature. In addition, the calculated free energy barrier of ~16.0 kcal/mol is close to the experimentally derived free energy barrier of ~14.5 kcal/mol,

suggesting that the obtained computational insights into the catalytic mechanism are reasonable.

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Figure 1. Division of the QM/MM system for simulating Coch-catalyzed (+)-cocaine hydrolysis (upper) and plots of RMSD and key internuclear distances (D1 to D5) vs the simulation time in the MD simulation on the enzyme-substrate complex (middle and bottom). Atoms in blue are treated by QM method. Three boundary carbon atoms in red are treated with the improved pseudobond parameters.³³ All other atoms belong to the MM subsystem.

Figure 2.

Key states for the first reaction step, the nucleophilic attack by O γ atom of S198 side chain. The geometries were optimized at QM/MM(B3LYP/6-31G*:AMBER) level. The key distances in the figure are in Å. Carbon, oxygen, nitrogen, and hydrogen atoms are colored in green, red, blue, and white, respectively. The backbone of the protein is rendered as cartoon and colored in orange. The QM atoms are represented as ball and stick, and the surrounding residues rendered as sticks.

Figure 3.

Key states except INT1 for the second reaction step, the dissociation of (+)-cocaine benzoyl ester. The geometries were optimized at QM/MM(B3LYP/6-31G*:AMBER) level. The structure of INT1 is given in Figure 2C. This figure is represented using the same scheme as that for Figure 2.

Figure 4.

Free energy profile determined by the MP2/6-31+G*:AMBER QM/MM-FE calculations excluding the zero-point and thermal corrections for the QM subsystem. The values in parentheses are relative free energies including the zero-point and thermal corrections for the QM subsystem.

Scheme 1.

The reaction mechanisms for CocH-catalyzed hydrolyses of (+)- and (-)-cocaine.

Important internuclear distances ($\text{\AA}$) in the optimized geometries of the key states involved in the reaction process

Table 1

	C-O ₅	ΔH-O ₄	O ₅ -O ₄	O ₅ -H ₇	H ₇ -N ₃	N ₃ -H ₈	H ₈ -O ₃	O ₃ -H ₉	H ₉ -O ₂	O ₂ -H ₁	O ₂ -H ₂
ES	2.64	1.02	1.35	3.49	1.67	1.06	1.62	2.10	1.79	2.35	
TS1	2.15	1.46	1.39	3.18	1.13	1.09	1.49	2.08	1.76	2.24	
INT1	1.54	1.98	1.52	3.33	1.03	1.12	1.42	2.15	1.67	2.09	
TS2	1.41	2.29	1.95	3.74	1.02	1.08	1.55	2.11	1.67	1.98	
INT1'	1.34	1.98	2.86	3.46	1.02	1.09	1.51	2.07	1.60	1.91	
TS2'	1.34	2.28	2.83	1.60	1.08	1.07	1.62	2.19	1.58	1.89	
INT2	1.33	2.95	3.02	0.98	2.25	1.05	1.66	2.18	1.59	1.92	

Table 2

Summary of the calculated free energy barriers (kcal/mol) for the acylation and deacylation stages of the enzymatic (+)-cocaine hydrolysis reactions in comparison with experimentally determined catalytic rate constants.

Enzyme	Calculated free energy barriers			k_{cat} (min ⁻¹) ^b
	Individual stage		Overall	
CocH	Acylation	16.0	16.0	8990
	Deacylation	10.9		
Bacterial CocE ^a	Acylation	9.1	17.9	1078
	Deacylation	17.9		

^aThe data for bacterial CocE were reported in ref.⁷⁴

^bThe experimental catalytic rate constant determined in the room temperature for CocH-catalyzed hydrolysis of (+)-cocaine⁷⁵ and bacterial CocE-catalyzed hydrolysis of (+)-cocaine.⁷⁴