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Reaction pathways and free energy profiles for cholinesterase-catalyzed hydrolysis of 6-monoacetylmorphine

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Abstract

As the most active metabolite of heroin, 6-monoacetylmorphine (6-MAM) can penetrate into the brain for the rapid onset of heroin effects. The primary enzymes responsible for the metabolism of 6-MAM to the less potent morphine in humans are acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The detailed reaction pathways for AChE- and BChE-catalyzed hydrolysis of 6-MAM to morphine have been explored, for the first time, in the present study by performing first-principles quantum mechanical/molecular mechanical free energy calculations. It has been demonstrated that the two enzymatic reaction processes follow the similar catalytic reaction mechanism, and the whole catalytic reaction pathway for each enzyme consists of four reaction steps. According to the calculated results, the second reaction step associated with the transition state TS2^a/TS2^b should be rate-determining for the AChE/BChE-catalyzed hydrolysis, and the free energy barrier calculated for the AChE-catalyzed hydrolysis (18.3 kcal/mol) is 2.5 kcal/mol lower than that for the BChE-catalyzed hydrolysis (20.8 kcal/mol). The free energy barriers calculated for the AChE- and BChE-catalyzed reactions are in good agreement with the experimentally derived activation free energies (17.5 and 20.7 kcal/mol for the AChE- and BChE-catalyzed reactions, respectively). Further structural analysis reveals that the aromatic residues Phe295 and Phe297 in the acyl pocket of AChE (corresponding to Leu286 and Val288 in BChE) contribute to the lower energy of TS2^a relative to TS2^b. The obtained structural and mechanistic insights could be valuable for use in future rational design of a novel therapeutic treatment of heroin abuse.

Introduction

Heroin (3,6-diacetylmorphine) is a well-known illegal and highly addictive opiate drug, synthesized from morphine, a natural product extracted from the seed pod of Asian opium poppy plant.¹ Heroin produces euphoria or pleasurable feelings, followed by drowsy feeling for several hours. The drowsy feeling is caused by depression of the central nervous system

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Supplementary Information Available. An additional figure for the plots of the key internuclear distances vs the simulation time in the MD-simulated BChE-6-MAM binding structure.

(CNS). In addition, heroin can cause heart failure, liver failure, suicidal thoughts, and other problems. Heroin is recognized as the most abused one of the opiates.¹ For example, in 2011, 4.2 million Americans reported using heroin at some time in their lives, with 23% of the individuals were classified with dependence on or abuse of heroin.¹ Heroin abuse can result in serious health and social problems. The medical and social consequences of heroin abuse have a devastating impact on society and cost billions of dollars per year, which has made a high priority the development of an effective pharmacological treatment of heroin abuse.

Heroin is also known as the most rapidly acting of the opiates.^{1–3} Once injected, heroin is very rapidly transformed to 6-monoacetylmorphine (6-MAM) through an enzymatic hydrolysis pathway consisting of four reaction steps⁴ and then to morphine (see Scheme 1) at a relatively lower rate.^{5,6} 6-MAM is the most active metabolite of heroin, and it has been demonstrated that heroin acts principally *via* 6-MAM.^{7–11} 6-MAM can readily cross the blood-brain barrier (BBB) and be rapidly concentrated in the brain.^{5,6,12} Moreover, 6-MAM has a higher μ -opioid receptor affinity than its precursor heroin and its metabolite morphine.¹¹ For this reason, accelerating the chemical transformation of the highly active 6-MAM into the less potent morphine by administration of an efficient exogenous enzyme would be a promising, alternative method to reduce the health hazards of heroin addiction.

In order to develop a possible enzyme therapy for heroin abuse treatment (the long-term goal of our investigation), we are particularly interested in understanding the reaction mechanism of 6-MAM metabolism concerning how 6-MAM is hydrolyzed to morphine. It has been known that several endogenous enzymes, including carboxylesterases 1 and 2 (hCE-1 and hCE-2) in liver, serum butyrylcholinesterase (BChE) in plasma, and erythrocyte acetylcholinesterase (AChE) in red blood cells, can catalyze hydrolysis of 6-MAM to morphine.^{13,14} Concerning that blood is the major site for 6-MAM production^{9,15} and 6-MAM can readily cross BBB,^{5,6,12} erythrocyte AChE and serum BChE in plasma should be the major enzymes for the hydrolysis of 6-MAM to morphine in human body. Further, AChE has a higher catalytic activity for the hydrolysis of 6-MAM to morphine compared to BChE,^{13,14} although BChE has a higher catalytic activity for the hydrolysis of heroin to 6-MAM compared to AChE. A detailed understanding of the metabolic mechanism of the drug could provide valuable mechanistic base for the structure-and-mechanism-based rational design of a novel therapeutic treatment of heroin abuse, similar to what we have accomplished in development of a novel enzyme therapy for cocaine abuse treatment.^{16–23}

In the present study, the fundamental reaction pathways for AChE- and BChE-catalyzed hydrolysis of 6-MAM to morphine (shown in Schemes 2 and 3) have been explored, for the first time, by performing molecular dynamics (MD) simulations and first-principles quantum mechanical/molecular mechanical (QM/MM)-free energy (QM/MM-FE) calculations. For convenience of the discussion below (including all Schemes and Figures), we use superscript “a” to represent the stationary states associated with the AChE-catalyzed hydrolysis and superscript “b” represent the stationary states corresponding to the BChE-catalyzed hydrolysis. In the QM/MM-FE calculations, first-principles QM/MM reaction-coordinate calculations were followed by free energy perturbation (FEP) calculations to account for the dynamic effects of the protein environment on the free energy profile for the

catalytic reaction process. Our QM/MM calculations are based on the pseudobond first-principles QM/MM approach,^{24–27} which has been demonstrated to be a powerful tool in simulating a variety of enzymes,^{19,28–35} and some theoretical predictions have been subsequently confirmed by experimental studies.^{19,32} The computational results clearly reveal the detailed reaction pathways and the corresponding free energy profiles for AChE- and BChE-catalyzed hydrolysis of 6-MAM. Based on the calculated free energy profile for the enzymatic reaction process, the rate-determining step is identified, and the roles of essential residues are discussed on the basis of the QM/MM-optimized geometries.

Computational Methods

Preparation of the initial structures of the reaction systems

The initial enzyme-substrate (ES) structures were constructed from the X-ray crystal structures of human AChE (PDB ID: 1B41)³⁶ and BChE (PDB ID: 2XQF)³⁷ and the quantum mechanically (QM) optimized structure of 6-MAM. The water molecules were removed from the crystal structures. Initially, the geometry of 6-MAM was optimized at the HF/6-31G* level using the Gaussian03 program.³⁸ Then, the optimized geometry of 6-MAM was used to calculate the electrostatic potential distribution on the molecular surface at the same HF/6-31G* level. The calculated electrostatic potential distribution was used to determine the partial atomic charges by using the standard restrained electrostatic potential (RESP) fitting procedure^{39,40} implemented in the Antechamber module of the AMBER11 program.⁴¹ The determined RESP charges of the 6-MAM atoms were used in the MD simulation. Then, 6-MAM was docked into the crystal structures of AChE and BChE, i.e., the acetyl group of 6-MAM was positioned in the oxyanion hole consisting of Gly121, Gly122, and Ala204 in AChE (corresponding to Gly116, Gly117, and Ala199 in BChE), and the positively charged amino-group was placed in the choline-binding site nearby Trp86 in AChE (corresponding to Trp82 in BChE). The constructed enzyme-substrate complex was then solvated in an orthorhombic box of TIP3P water molecules⁴² with a minimum solute-wall distance of 10 Å and neutralized by adding chloride ions. The solvated system was refined by performing a ~2 ns MD simulation. As seen in Schemes 2 and 3, there are two reaction stages (acylation and deacylation) in the enzymatic hydrolysis of 6-MAM. The reaction product morphine leaves the system after acylation stage. Consequently, we constructed the structure of INT2^a of AChE (and INT2^b of BChE) by removing morphine from the QM/MM-optimized INT2^a of AChE (and INT2^b of BChE) structure. The constructed INT2^a and INT2^b structures were then relaxed by performing MD simulations (each for ~2 ns) for which the system was also solvated in an orthorhombic box of TIP3P water molecules,⁴² with a minimum solute-wall distance of 10 Å.

For both the acylation and deacylation stages, the last snapshots of the MD simulations were used as the initial structures to prepare the pseudobond first-principles QM/MM calculations because the structures of the last snapshots were close to the average structures simulated. Since we are mainly interested in the reaction center, the water molecules beyond 50 Å of the hydroxyl oxygen (O^y) of Ser203 of AChE were removed, leaving the QM/MM system with 3,881 water molecules and a total of 19,941 atoms for the acylation stage, and 4,731 water molecules and a total of 22,451 atoms for the deacylation stage of AChE. Similarly,

the water molecules beyond 50 Å of the hydroxy oxygen (O^γ) of Ser198 of BChE were removed, leaving the QM/MM system with 3,404 water molecules and a total of 18,623 atoms for the acylation stage, and 4,232 water molecules and a total of 21,065 atoms for the deacylation stage of BChE. The QM/MM interface was treated by using a pseudobond approach.^{24–27} The used boundary of the QM/MM system for the whole reaction system is indicated in the figures to be discussed below. Prior to the QM/MM geometry optimization, the initial structure of the reaction system was energy-minimized with the MM method by using the AMBER11 program,⁴¹ and the convergence criterion for energy gradient of 0.1 kcal·mol⁻¹·Å⁻¹ was achieved.

Minimum-energy path of the reaction

With a reaction-coordinate driving method and an iterative energy minimization procedure,²⁵ the enzymatic reaction path was determined by the pseudobond QM/MM calculations at the B3LYP/6-31G*:AMBER level, in which the QM calculations were performed with the B3LYP functional^{43–45} and 6-31G* basis set by using a modified version¹⁹ of Gaussian03 program³⁸ and the MM calculations were performed by using a modified version of the AMBER8 program.⁴⁶ The main changes made in the Gaussian03 program include reading and using information about the MM atoms (atomic coordinates and topology, atomic charges, and the contributions of the MM atoms to the energies and gradients of the QM system) from the modified AMBER8 program in the geometry optimization of the QM system in the MM environment. The MM calculations using the modified AMBER8 program were carried out in the presence of the electrostatic potential (ESP) charges of the frozen QM atoms (the information from the modified Gaussian03 program). The QM and MM calculations were performed iteratively until the mutual convergence is achieved. Normal mode analysis was performed to characterize the reactant, intermediates, transition states, and the final product of the enzymatic reaction process. The QM/MM-optimized geometries were then used to perform single-point QM/MM energy calculations with the QM part performed at the B3LYP/6-31++G** level. This method has been used extensively in our previous QM/MM calculations on enzymatic reaction systems^{4,30,35,47} and the calculated results are generally in good agreement with the experimental data. Throughout the QM/MM calculations, the boundary carbon atoms were treated with improved pseudobond parameters.^{24–27} No cutoff for non-bonded interactions was used in the QM/MM calculations. For the QM subsystem, the convergence criterion for geometry optimizations followed the original Gaussian03 defaults. For the MM subsystem, the geometry optimization convergence criterion was when the root-mean-square deviation (rmsd) of energy gradient is 0.1 kcal·mol⁻¹·Å⁻¹. The atoms within 20 Å of O^γ atom in the side chain of Ser203 of AChE (Scheme 2) (or Ser198 of BChE in Scheme 3) were allowed to move while all the other atoms outside this range were frozen in all the QM/MM calculations. During the QM/MM geometry optimization, the QM and MM subsystems were energy-minimized iteratively. For each step of the iteration, the MM subsystem was energy-minimized when the QM subsystem was kept frozen, whereas the QM subsystem was energy-minimized when the MM subsystem was kept frozen.

Free energy perturbation

Free energy perturbation (FEP) can be performed to evaluate free energy change caused by a small structural change.^{48–51} The FEP method, in combination with the MD or Monte Carlo (MC) simulation, has been used to study organic reactions,^{48,51–53} protein-ligand interactions,^{54–59} and protein stability.^{60,61} After the minimum-energy path was determined by the QM/MM calculations, the free energy changes associated with the QM/MM interactions were determined by using the free energy perturbation (FEP) method.²⁵ In the FEP calculations, sampling of the MM subsystem was carried out with the QM subsystem frozen at each state along the reaction path. The point charges on the frozen QM atoms used in the FEP calculations were determined by fitting the electrostatic potential (ESP) in the QM part of the QM/MM single-point calculations. The total free energy difference between the transition state and the reactant was calculated with the same procedure as used in our previous work on other reaction systems.^{28–34} The FEP calculations (using our own version of the implementation³⁴) enabled us to more reasonably determine the relative free energy changes due to the QM/MM interactions. Technically, the final (relative) free energy determined by the QM/MM-FE calculations was the QM part of the QM/MM energy (excluding the Coulombic interaction energy between the point charges of the MM atoms and the ESP charges of the QM atoms) plus the relative free energy change determined by the FEP calculations. In the FEP calculations, the used time step was 2 fs, and all bond lengths involving hydrogen atoms were constrained. In sampling of the MM subsystem by MD simulations, the temperature was maintained at 298.15 K. Each FEP calculation consisted of 50 ps of equilibration and 300 ps of sampling.

The MD simulations and QM/MM-FE calculations were performed on a supercomputer (*i.e.* the Dell X-series Cluster with 384 nodes or 4,768 processors) at the University of Kentucky's Computer Center. Some other modeling and computations were carried out on SGI Fuel workstations in our own laboratory at the University of Kentucky.

Results and Discussion

1. AChE-catalyzed hydrolysis of 6-MAM

1. 1 AChE-6-MAM binding structure from MD simulation—Depicted in Figure 1 is a representative AChE-6-MAM binding structure derived from the MD simulation. As shown in Figure 1, the active site of AChE consists of Ser203, His447, and Glu334, and 6-MAM fits well in the deep and narrow active-site gorge of AChE. Three aromatic residues (Phe295, Phe297, and Phe338) constitute the acyl pocket and encompass the acetyl group of 6-MAM. The carbonyl oxygen of the acetyl group of 6-MAM is located in the oxyanion hole formed by Gly121, Gly122, and Ala204. The carbonyl carbon of the acetyl group of 6-MAM is positioned to form a covalent bond with O γ of Ser203 side chain. The positively charged amine group is positioned in the putative anionic binding site nearby Trp86. In addition, aromatic residue Tyr337 forms hydrophobic interaction with 6-MAM. Depicted in Figure 2 are the key internuclear distances *vs* the simulation time in the MD-simulated ES complex. Traces D1, D2, and D3 represent the internuclear distances between the carbonyl oxygen O1 of the acetyl group of 6-MAM and the NH hydrogen atoms of residues Gly121, Gly122, and Ala204, respectively. Trace D4 is the internuclear distance between O γ of

Ser203 side chain and carbonyl carbon C¹ of the acetyl group of 6-MAM. Trace D5 is hydrogen bond distance between H^γ of Ser203 side chain and N^ε atom of His447.

As one can seen in Figure 2B, in the MD-simulated ES structure, the average values for D1, D2, and D3 are 2.31, 1.94, and 3.06 Å, respectively, indicating that only two hydrogen bonds are formed between the carbonyl oxygen of 3-acetyl group of 6-MAM and the oxyanion hole in the initial ES structure. The average value of 3.28 Å for D4 indicates that the O^γ of Ser203 is at an appropriate position to nucleophilic attack on the C1 of 6-MAM. The average value of D5 is 1.83 Å, showing that a strong hydrogen bond exists between Ser203 hydroxyl group and the N^ε atom of His447.

1.2 Fundamental reaction pathway for AChE-catalyzed hydrolysis of 6-MAM to morphine—As seen in Figure 2B and 2C, the MD simulation led to a dynamically stable ES structure. The QM/MM reaction-coordinate calculations at the B3LYP/6-31G*:AMBER level starting from the MD-simulated ES structure revealed that the AChE-catalyzed 6-MAM hydrolysis reaction pathway consists of four reaction steps. The reaction is initiated by the nucleophilic attack of O^γ of Ser203 side chain on the carbonyl carbon C¹ of 6-MAM, followed by the dissociation between acetyl-enzyme intermediate and morphine. Next step, a water molecule initiates the nucleophilic attack on the carbonyl carbon (C¹) of the acylated Ser203. Finally, the dissociation of the acetic acid regenerates Ser203 of AChE. Depicted in Figures 3 and 4 are the QM/MM-optimized structures of the reactant, intermediates, transition states, and final product. Below we discuss each of the reaction steps in detail.

Nucleophilic attack on the carbonyl carbon C¹ of 6-MAM by the O^γ atom of Ser203

The initial ES complex was first optimized at the QM/MM(B3LYP/6-31G*:AMBER) level prior to the reaction-coordinate calculations. As shown in Figure 3B for the QM/MM-optimized ES structure, the carbonyl oxygen O¹ of 6-MAM forms two strong hydrogen bonds with the backbone NH groups of Gly121 and Gly122 in the oxyanion hole, which are 1.95 and 1.79 Å respectively. In comparison, the distance between the backbone NH of Ala204 and the carbonyl oxygen O¹ of 6-MAM is longer (2.41 Å), indicating that the hydrogen bond interaction between them is very weak.

For the first step of the reaction, the hydroxyl oxygen O^γ of Ser203 may gradually approach the carbonyl carbon C¹ of 6-MAM for the nucleophilic attack. Meanwhile, the hydroxyl hydrogen H^γ of Ser203 gradually transfers to the N^ε atom of His447 side chain. As shown in Scheme 2, this reaction step involves the breaking of the H^γ–O^γ bond and the formation of both the H^γ–N^ε and C¹–O^γ bonds. So, the changes of the distances H^γ–O^γ (R_{H^γ-O^γ}), H^γ–N^ε (R_{H^γ-N^ε}), and C¹–O^γ (R_{C¹-O^γ}) can reflect the nature of reaction step 1. Therefore, R_{H^γ-O^γ}–R_{H^γ-N^ε}–R_{C¹-O^γ} was set as the reaction coordinate in the QM/MM reaction-coordinate calculations for this reaction step. As shown in Figure 3, R_{C¹-O^γ} shortens from 2.46 Å in ES^a to 1.82 Å in TS1^a and then to 1.53 Å in INT1^a. Simultaneously, R_{H^γ-O^γ} elongates from 0.99 Å in ES^a to 1.36 Å in TS1^a and then to 1.67 Å in INT1^a, while R_{H^γ-N^ε} shortens from 1.69 Å in ES^a to 1.17 Å in TS1^a and then to 1.06 Å in INT1^a. These structural changes indicate that the C¹–O^γ bond has gradually formed and the proton (H^γ) has gradually transferred to N^ε atom of His447 in intermediate INT1^a. In our previous theoretical studies on cholinesterases,

the oxyanion hole is always identified to stabilize the transition state and play a catalytic role in the hydrolysis reaction.^{4,29,31,33} Here, we also tracked the changes of the key hydrogen-bond distances between O¹ of 6-MAM and oxyanion hole. As shown in Figure 3, the distance D1 (between the hydrogen of NH group in Gly121 and O¹ of 6-MAM) is elongated from 1.95 Å in ES^a to 1.97 Å in TS1^a and then shortened to 1.90 Å in INT1^a, the distance D2 (between the hydrogen of NH group in Gly122 and O¹ of 6-MAM) is elongated from 1.79 Å in ES^a to 1.86 Å in TS1^a and then shortened to 1.85 Å in INT1^a, and D3 (between the hydrogen of NH group in Ala204 and O¹ of 6-MAM) is shortened from 2.41 Å in ES^a to 2.22 Å in TS1^a and then to 2.12 Å in INT1^a. Thus, the interaction between the oxyanion hole and the negatively charged carbonyl oxygen (O¹) of 6-MAM is strengthened in TS1^a and INT1^a, especially in INT1^a.

Dissociation of morphine from the enzyme

In the second reaction step, the C¹–O² bond gradually breaks while the proton (H^γ) gradually transfers from N^ε of His447 to O², resulting in the dissociation of morphine from the enzyme. This reaction step involves the breaking of the N^ε–H^γ and C¹–O² bonds and the formation of the O²–H^γ bond. The nature of this reaction step can be represented by the changes of the N^ε–H^γ distance (R_{N^ε-H^γ}), the C¹–O² distance (R_{C¹-O²}), and the O²–H^γ distance (R_{O²-H^γ}). Thus, the reaction coordinate in the QM/MM calculations for this step was chosen as R_{N^ε-H^γ}+R_{C¹-O²}-R_{O²-H^γ}. In the INT1^a structure, the proton (H^γ) of Ser203 side chain has transferred to the N^ε of His447 in reaction step 1 while the distance R_{O^γ-H^γ} between the O^γ atom of Ser203 and the H^γ atom of His447 is 1.67 Å, indicating a very strong hydrogen bond (N^ε–H^γ...O^γ) between the oxygen of Ser203 side chain and the NH group of His447 side chain. The distance (R_{O²-H^γ}) between the H^γ atom and the leaving ester oxygen O² atom to which the proton (H^γ) is about to be transferred is 2.44 Å. However, accompanied by the elongation of R_{C¹-O²} (1.54 Å in INT1^a and 1.95 Å in TS2^a), R_{O^γ-H^γ} is gradually elongated (1.67 Å in INT1^a and 2.23 Å in TS2^a), and R_{O²-H^γ} is gradually shortened (2.44 Å in INT1^a and 1.54 Å in TS2^a). The reaction-coordinate calculations reveal that it is a one-step reaction process consisting of two substeps. In the first substep, the C¹–O² bond is broken. In the second substep, the proton (H^γ) gradually transfers to O². It should be noted that the interaction between the oxyanion hole and the negatively charged carbonyl oxygen (O¹) of 6-MAM is enhanced from ES^a to TS2^a, indicating that the oxyanion hole plays an important role in stabilization of the transition state TS2^a.

Nucleophilic attack on the carbonyl carbon by a water molecule

In the third reaction step, the generated morphine was removed from the above-discussed QM/MM-optimized geometry of INT2^a to construct the initial structure of INT2^a which was then relaxed by performing the MD simulation. A water molecule close to the carbonyl carbon (C¹) of the substrate was selected as the nucleophile and was included in the QM part of the QM/MM calculation. As shown in Figure 4, in the QM/MM-optimized geometry of INT2^a, two strong hydrogen bonds are formed between the carbonyl oxygen (O¹) and the oxyanion hole.

The current nucleophilic attack process involves the breaking of the $O^{\omega}-H^{\omega}$ bond and the formation of the C^1-O^{ω} and $N^{\varepsilon}-H^{\omega}$ bonds (Scheme 2). Thus, the distances $R_{O^{\omega}-H^{\omega}}$, $R_{C^1-O^{\omega}}$, and $R_{N^{\varepsilon}-H^{\omega}}$ were chosen to represent the reaction coordinate as $R_{O^{\omega}-H^{\omega}}-R_{C^1-O^{\omega}}-R_{N^{\varepsilon}-H^{\omega}}$ in the QM/MM calculations for the current reaction step. During this reaction process, the distance $R_{O^{\omega}-H^{\omega}}$ elongates from 0.99 Å in INT2^a to 1.49 Å in TS3^a and then to 2.27 Å in INT3^a, while the distances $R_{C^1-O^{\omega}}$ and $R_{N^{\varepsilon}-H^{\omega}}$ respectively shorten from 2.85 and 1.80 Å in INT2^a to 1.76 and 1.10 Å in TS3^a and then to 1.48 and 1.07 Å in INT3^a. These distance variations demonstrate that in processing from INT2^a to INT3^a, the nucleophilic water dissociates into a hydroxide ion attaching to C^1 atom and a proton (H^{ω}) attaching to N^{ε} atom.

Dissociation between the Acetic Acid and Ser203 of AChE

As seen from Scheme 2, this final reaction step (step 4) involves the breaking of the C^1-O^{γ} and $N^{\varepsilon}-H^{\omega}$ bonds and the formation of the $O^{\gamma}-H^{\omega}$ bond. Because the changes in the distances $R_{C^1-O^{\gamma}}$, $R_{N^{\varepsilon}-H^{\omega}}$, and $R_{O^{\gamma}-H^{\omega}}$ reflect the nature of this reaction step, the reaction coordinate $R_{C^1-O^{\gamma}}+R_{N^{\varepsilon}-H^{\omega}}-R_{O^{\gamma}-H^{\omega}}$ was set in the QM/MM calculations. In the formed intermediate INT3^a, the distance $R_{O^{\gamma}-H^{\omega}}$ is 1.57 Å, indicating that a strong hydrogen bond has formed between the oxygen of Ser203 side chain and the H^{ω} atom. As shown in Figure 4, the distances $R_{C^1-O^{\gamma}}$ and $R_{N^{\varepsilon}-H^{\omega}}$ respectively elongate from 1.61 and 1.07 Å in INT3^a to 1.79 and 1.15 Å in TS4^a and then to 2.43 and 1.71 Å in PD^a, while the distance $R_{O^{\gamma}-H^{\omega}}$ shortens from 1.57 Å in INT3^a to 1.35 Å in TS4^a and then to 1.00 Å in PD^a.

2. BChE-catalyzed hydrolysis of 6-MAM to morphine

2.1 BChE-6-MAM binding structure from MD simulation—Depicted in Figure 5 is a representative BChE-6-MAM binding structure derived from the MD simulation. In general, BChE-6-MAM binding interaction is similar to the AChE-6-MAM binding interaction. The catalytic triad in the active site of BChE, which consists of Ser198, His438, and Glu325, is very similar to that of AChE. The oxyanion hole formed by Gly116, Gly117, and Ala199 in BChE is also hydrogen-bonded with the carbonyl oxygen of the acetyl group of 6-MAM. In addition, residue Trp82 in BChE is positioned well to form a cation- π interaction with the positively charged amine group of 6-MAM. However, BChE has a larger binding pocket than AChE and confers more flexibility for 6-MAM. Noteworthy, the acyl pocket of BChE which encompasses the acetyl group of 6-MAM is very different from that of AChE. The aromatic residues Phe295 and Phe297 in the acyl pocket of AChE are replaced by the smaller hydrophobic residues Leu286 and Val288 in BChE, which confer more flexibility of the acetyl group of 6-MAM and render the interaction between acetyl oxygen of 6-MAM and oxyanion hole weaker than that in AChE (demonstrated by the longer hydrogen-bond distances shown in Figure S1B). In addition, aromatic residue Tyr337 in AChE is also replaced by Ala328 in BChE, which weakens the hydrophobic interaction with 6-MAM. The weakened interactions between BChE and 6-MAM render the average internuclear distance 3.59 Å between O^{γ} of Ser198 side chain and the carbonyl carbon C^1 of the acetyl group of 6-MAM (shown in Figure S1C of the Supporting Information) relatively longer than that in the AChE-6-MAM binding structure.

2.2 Fundamental reaction pathway for BChE-catalyzed hydrolysis of 6-MAM to morphine

As shown in Scheme 3, BChE-catalyzed hydrolysis of 6-MAM to morphine proceeds following the mechanism similar to that of AChE-catalyzed hydrolysis of 6-MAM (Scheme 2). The acylation stage is initiated by the nucleophilic attack of O^γ of Ser198 side chain on the carbonyl carbon C^1 of 6-MAM, while the proton (H^γ of Ser198) gradually transfers to the N^ϵ atom of His438 side chain *via* transition state TS1^b. Similarly, $R_{H^\gamma-O^\gamma}-R_{H^\gamma-N^\epsilon}-R_{C^1-O^\gamma}$ was set as the reaction coordinate in the QM/MM reaction-coordinate calculations for this reaction step. The second step in acylation stage is the dissociation between acetyl-enzyme intermediate and morphine *via* transition state TS2^b, and $R_{N^\epsilon-H^\gamma}+R_{C^1-O_2}-R_{O_2-H^\gamma}$ was set as the reaction coordinate for this step. In the third reaction step, the deacylation is initiated with the nucleophilic attack of a water molecule on the carbonyl carbon (C^1) of the acylated Ser198 *via* transition state TS3^b, and $R_{O_\omega-H_\omega}-R_{C^1-O_\omega}-R_{N^\epsilon-H_\omega}$ was set as the reaction coordinate for this step. Finally, the dissociation of the acetic acid regenerates Ser198 of BChE *via* transition state TS4^b, and the reaction coordinate for this step was set as $R_{C^1-O^\gamma}+R_{N^\epsilon-H_\omega}-R_{O^\gamma-H_\omega}$. The QM/MM-optimized structures of the reactant, intermediates, transition states, and products are depicted in Figures 6 and 7.

3 Free energy profiles

As discussed above, the QM/MM reaction-coordinate calculations at the B3LYP/6-31G*:AMBER level have revealed that there are four reaction steps (steps 1 and 2 in the acylation stage, and steps 3 and 4 in the deacylation stage) in the AChE/BChE-catalyzed hydrolysis of 6-MAM to morphine. In order to determine the free energy profile for the reaction pathway, we performed the single-point QM/MM energy calculations with the QM part treated at the B3LYP/6-31++G** level for each QM/MM optimized geometry along the minimum-energy path. For each geometry along the reaction path, the ESP charges determined in the QM part of the QM/MM single-point energy calculation were used in the subsequent FEP simulations to estimate the free energy changes. Depicted in Figure 8 are the free energy profiles determined by the QM/MM-FE calculations at the B3LYP/6-31++G*:AMBER level without the zero-point and thermal corrections for the QM subsystem. The values in parentheses are the corresponding relative free energies including the zero-point and thermal corrections for the QM subsystem.

As seen in Figure 8(A) and 8(B) for the AChE-catalyzed hydrolysis of 6-MAM to morphine, the calculated results reveal that the relative energy of TS2^a is higher than that of TS1^a, indicating that the overall free energy barrier for the entire acylation stage is the free energy change from ES^a to TS2^a. Similarly, the higher energy of TS3^a relative to TS4^a indicates that the overall free energy barrier for the deacylation is the free energy change associated with the structural transformation from INT2^a to TS3^a. According to the results, the calculated overall free energy barrier for the acylation (18.3 kcal/mol) is higher than that for the deacylation (15.4 kcal/mol), demonstrating that the second step associated with the transition state TS2^a is rate-determining for the whole catalytic reaction process. Noteworthy, the energy barrier of the fourth step (*i.e.* the dissociation of the acetic acid and Ser203) is only ~0.7 kcal/mol on the potential energy surface determined at the QM/MM(B3LYP/6-31G*:AMBER) level. This negligible energy barrier disappears according to

the final free-energy calculations at the B3LYP/6-31++G**:*AMBER* QM/MM-FE level, suggesting that the final reaction step of the enzymatic reaction is actually barrier-less.

Depicted in Figure 8(C) and 8(D) are the calculated free energy profile for BChE-catalyzed hydrolysis of 6-MAM to morphine. Similar to AChE, the calculated overall free energy barrier for the acylation (20.8 kcal/mol) is also higher than that for the deacylation (13.0 kcal/mol), demonstrating that the second step associated with the transition state TS2^b is also rate-determining for the whole catalytic reaction process. Likewise, the energy barrier of the fourth step, *i.e.* the dissociation of the acetic acid and Ser198, is only ~0.2 kcal/mol on the potential energy surface determined at the QM/MM(B3LYP/6-31G*:*AMBER*) level. This negligible energy barrier disappears according to the final free-energy calculations at the B3LYP/6-31++G**:*AMBER* QM/MM-FE level, suggesting that the final reaction step of the enzymatic reaction is also barrier-less.

4 Comparison with experimental results

It is important to compare the calculated energetic results with available experimental kinetic data. Experimental kinetic analysis has been reported for the enzymatic reactions of 6-MAM to morphine,¹⁴ although the experimental catalytic rate constant (k_{cat}) for AChE-catalyzed hydrolysis of 6-MAM to morphine was not given explicitly in the original report. In fact, according to the time-dependent product changes shown in the original report (specifically the Figure 1(b)),¹⁴ we may estimate that the catalytic rate constant for AChE-catalyzed hydrolysis of 6-MAM should be about one sixth of the catalytic rate constant (351 min⁻¹) for AChE-catalyzed hydrolysis of heroin. Thus, we may have $k_{\text{cat}} = 58.5 \text{ min}^{-1}$ for AChE-catalyzed hydrolysis of 6-MAM. According to the conventional transition state theory (CTST),⁶² a rate constant of 58.5 min⁻¹ is associated with an activation free energy of 17.5 kcal/mol. Our calculated free energy barrier of 18.3 kcal/mol is in good agreement with the experimentally derived activation free energy of 17.5 kcal/mol.

For BChE-catalyzed hydrolysis of 6-MAM to morphine, the experimental catalytic rate constant was reported to be 0.25 min⁻¹,¹³ corresponding to an activation free energy of 20.7 kcal/mol according to the CTST.⁶² The calculated free energy barrier of 20.8 kcal/mol is in excellent agreement with the experimentally derived activation free energy of 20.7 kcal/mol, suggesting that the computational results are reasonable.

We should also note that the free energy barrier calculated for AChE-catalyzed hydrolysis of 6-MAM (18.3 kcal/mol) is 2.5 kcal/mol lower than that for BChE-catalyzed hydrolysis of 6-MAM (20.8 kcal/mol). The calculated energy barrier difference between the two enzymatic reaction processes is attributed to the different characteristics of the binding pockets of the two enzymes (AChE and BChE). As discussed above, the aromatic residues Phe295 and Phe297 in the acyl pocket of AChE are replaced by residues Leu286 and Val288 in BChE, which renders the internuclear distance between O^γ of Ser198 side chain and carbonyl carbon C¹ of the acetyl group of 6-MAM relatively longer than that in the AChE-6-MAM binding structure ($R_{\text{C1-O}\gamma} = 2.46 \text{ \AA}$ in ES^a and 2.62 $\text{\AA}$ in ES^b). This means that the structural change from ES^a to TS2^a ($R_{\text{C1-O}\gamma} = 2.46 \text{ \AA}$ in ES^a and 1.40 $\text{\AA}$ in TS2^a) is smaller than that from ES^b to TS2^b ($R_{\text{C1-O}\gamma} = 2.62 \text{ \AA}$ in ES^b and 1.41 $\text{\AA}$ in TS2^b). The relatively smaller structural change from ES^a to TS2^a makes the free energy barrier of AChE-catalyzed

reaction process relatively lower than BChE-catalyzed reaction process. In addition, we should also notice that the small acyl pocket in AChE restrains the hydrogen-bond distances between acetyl oxygen of 6-MAM and oxyanion hole and make the hydrogen-bond distances relatively shorter than that in BChE (in ES^a, D1=1.95 Å, D2=1.79 Å, and D3=2.41 Å, in ES^b, D1=2.34 Å, D2=1.85 Å, and D3=2.51 Å). Moreover, from ES^a to TS2^a, the hydrogen-bond interactions between the oxyanion hole and carbonyl oxygen of 6-MAM are strengthened more significantly (from D1=1.95 Å, D2=1.79 Å, and D3=2.41 Å in ES^a to D1=1.93 Å, D2=1.85 Å, and D3=1.94 Å in TS2^a) compared to that from ES^b to TS2^b (from D1=2.34 Å, D2=1.85 Å, and D3=2.51 Å in ES^b to D1=2.21 Å, D2=2.05 Å, and D3=2.01 Å in TS2^b). The stronger hydrogen-bond interactions between the oxyanion hole and carbonyl oxygen of 6-MAM in TS2^a make it more stable than TS2^b.

Conclusion

The detailed reaction pathways for the AChE- and BChE-catalyzed hydrolysis of 6-MAM to morphine have been explored using first-principles QM/MM-FE calculations in this study. Based on the results from the QM/MM calculations, the two enzymatic reaction processes share the similar reaction mechanism, and the reaction pathway for each enzyme consists of four reaction steps. The enzymatic reaction is initiated by the nucleophilic attack of hydroxyl oxygen (O^γ) atom of Ser203/198 side chain of AChE/BChE on the carbonyl carbon (C¹) of the acetyl group of 6-MAM, accompanied by the proton (H^γ) transfer from the Ser203/198-O^γ atom of AChE/BChE to the His447/438-N^ε atom of AChE/BChE. The tetrahedral intermediate INT1^a/INT1^b of AChE/BChE is thus formed, in which the negatively charged carbonyl oxygen (O¹) is stabilized by the backbone NH groups of Gly121/116, Gly122/117, and Ala204/199 of AChE/BChE in the oxyanion hole. Subsequently, the C¹–O² bond is gradually broken while the proton (H^γ) transfers from His447/438-N^ε of AChE/BChE to the O² atom of 6-MAM, leading to the formation of morphine and the acyl-enzyme intermediate. The third reaction step is initiated by the nucleophilic attack of a water molecule on the carbonyl carbon (C¹) atom of the acyl-enzyme intermediate. This process is coupled with a proton (H^ω) transfer from the water oxygen (O^ω) to His447/438-N^ε of AChE/BChE. Finally, the acetyl group is released with the regeneration of Ser203/198 of AChE/BChE.

The corresponding free energy profile indicates that the second reaction step associated with the transition state TS2^a/TS2^b of AChE/BChE should be rate-determining, and the free energy barriers are 18.3 and 20.8 kcal/mol for the AChE- and BChE-catalyzed reactions, respectively. Further structural analysis on the key transitional states reveals that the relatively lower free energy barrier for AChE-catalyzed hydrolysis of 6-MAM compared to BChE-catalyzed hydrolysis of 6-MAM is due to the different characteristics of their binding pockets, especially the acyl pocket. The obtained structural and mechanistic insights could be valuable for use in future rational design of a novel therapeutic strategy which aims to treat heroin abuse. In particular, it would be interesting to design an AChE/BChE mutant with a significantly improved catalytic activity for hydrolysis of 6-MAM to morphine, because such a mutant enzyme could be used an exogenous enzyme to quickly change the highly potent 6-MAM into the less potent morphine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Oldendorf WH, Hyman S, Braun L, Oldendorf SZ. *Science*. 1972; 178:984–986. [PubMed: 5084666]
2. Inturrisi CE, Schultz M, Shin S, Umans JG, Angel L, Simon EJ. *Life Sci*. 1983; 33:773–776. [PubMed: 6319928]
3. Strandberg JJ, Kugelberg FC, Alkass K, Gustavsson A, Zahlén K, Spigset O, Druid H. *Toxicol Lett*. 2006; 166:11–18. [PubMed: 16793228]
4. Qiao Y, Han K, Zhan CG. *Biochemistry*. 2013; 52:6467–6479. [PubMed: 23992153]
5. Way EL, Kemp JW, Young JM, Grassetti DR. *J Pharmacol Exp Ther*. 1960; 129:144–154. [PubMed: 13843200]
6. Way EL, Young JM, Kemp JW. *B Narcotics*. 1965; 17:25–33.
7. Eddy NB, Howes HA. *J Pharmacol Exp Ther*. 1935; 53:430–439.
8. Wright CI, Barbour FA. *J Pharmacol Exp Ther*. 1935; 54:25–33.
9. Lockridge O, Mottershaw-Jackson N, Eckerson HW, La Du BN. *J Pharmacol Exp Ther*. 1980; 215:1–8. [PubMed: 7452476]
10. Andersen JM, Ripel A, Boix F, Normann PT, Morland J. *J Pharmacol Exp Ther*. 2009; 331:153–161. [PubMed: 19541908]
11. Selley DE, Cao CC, Sexton T, Schwegel JA, Martin TJ, Childers SR. *Biochem Pharmacol*. 2001; 62:447–455. [PubMed: 11448454]
12. Goldberger BA, Cone EJ, Grant TM, Caplan YH, Levine BS, Smialek JE. *J Anal Toxicol*. 1994; 18:22–28. [PubMed: 8127080]
13. Kamendulis LM, Brzezinski MR, Pindel EV, Bosron WF, Dean RA. *J Pharmacol Exp Ther*. 1996; 279:713–717. [PubMed: 8930175]
14. Salmon AY, Goren Z, Avissar Y, Soreq H. *Clin Exp Pharmacol Physiol*. 1999; 26:596–600. [PubMed: 10474772]
15. Boix F, Andersen JM, Morland J. *Addict Biol*. 2013; 18:1–7. [PubMed: 21481103]
16. Pan Y, Gao D, Yang W, Cho H, Yang G, Tai HH, Zhan CG. *Proc Natl Acad Sci*. 2005; 102:16656–16661. [PubMed: 16275916]
17. Pan Y, Gao D, Yang W, Cho H, Zhan CG. *J Am Chem Soc*. 2007; 129:13537–13543. [PubMed: 17927177]
18. Pan Y, Gao D, Zhan CG. *J Am Chem Soc*. 2008; 130:5140–5149. [PubMed: 18341277]
19. Zheng F, Yang WC, Ko MC, Liu JJ, Cho H, Gao DQ, Tong M, Tai HH, Woods JH, Zhan CG. *J Am Chem Soc*. 2008; 130:12148–12155. [PubMed: 18710224]
20. Yang W, Pan Y, Zheng F, Cho H, Tai HH, Zhan CG. *Biophys J*. 2009; 96:1931–1938. [PubMed: 19254552]
21. Xue L, Ko MC, Tong M, Yang W, Hou S, Fang L, Liu J, Zheng F, Woods JH, Tai HH, Zhan CG. *Mol Pharmacol*. 2011; 79:290–297. [PubMed: 20971807]
22. Xue L, Hou S, Tong M, Fang L, Chen X, Jin Z, Tai HH, Zheng F, Zhan CG. *Biochem J*. 2013; 453:447–454. [PubMed: 23849058]

23. Zheng F, Yang W, Xue L, Hou S, Liu J, Zhan CG. *Biochemistry*. 2010; 49:9113–9119. [PubMed: 20886866]
24. Zhang YK, Lee TS, Yang WT. *J Chem Phys*. 1999; 110:46–54.
25. Zhang YK, Liu HY, Yang WT. *J Chem Phys*. 2000; 112:3483–3492.
26. Zhang YK. *J Chem Phys*. 2005; 122:024114. [PubMed: 15638579]
27. Zhang YK. *Theor Chem Acc*. 2006; 116:43–50.
28. Li D, Huang X, Lin J, Zhan CG. *Dalton Trans*. 2013; 42:3812–3820. [PubMed: 23303461]
29. Yao Y, Liu J, Zhan CG. *Biochemistry*. 2012; 51:8980–8992. [PubMed: 23092211]
30. Wei DH, Lei BL, Tang MS, Zhan CG. *J Am Chem Soc*. 2012; 134:10436–10450. [PubMed: 22697787]
31. Chen X, Fang L, Liu J, Zhan CG. *Biochemistry*. 2012; 51:1297–1305. [PubMed: 22304234]
32. Chen X, Zhao X, Xiong Y, Liu J, Zhan CG. *J Phys Chem B*. 2011; 115:12208–12219. [PubMed: 21973014]
33. Chen X, Fang L, Liu J, Zhan CG. *J Phys Chem B*. 2010; 115:1315–1322. [PubMed: 21175195]
34. Liu JJ, Hamza A, Zhan CG. *J Am Chem Soc*. 2009; 131:11964–11975. [PubMed: 19642701]
35. Wei D, Huang X, Liu J, Tang M, Zhan CG. *Biochemistry*. 2013; 52:5145–5154. [PubMed: 23862626]
36. Kryger G, Harel M, Giles K, Toker L, Velan B, Lazar A, Kronman C, Barak D, Ariel N, Shafferman A, Silman I, Sussman JL. *Acta Crystallogr, Sect D: Biol Crystallogr*. 2000; 56:1385–1394. [PubMed: 11053835]
37. Wandhammer M, Carletti E, Van der Schans M, Gillon E, Nicolet Y, Masson P, Goeldner M, Noort D, Nachon F. *J Biol Chem*. 2011; 286:16783–16789. [PubMed: 21454498]
38. Frisch, MJ.; Trucks, GW.; Schlegel, HB.; Scuseria, GE.; Robb, MA.; Cheeseman, JR.; Montgomery, J.; Vreven, JAT.; Nudin, K.; Burant, JC.; Millam, JM.; Syengar, S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, AG.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Shida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, JE.; Hratchian, HP.; Cross, JB.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, RE.; Yazyev, O.; Austin, AJ.; Cammi, R.; Pomelli, C.; Ochterski, JW.; Ayala, PY.; Morokuma, K.; Voth, GA.; Salvador, P.; Dannenberg, JJ.; Zakrzewski, VG.; Dapprich, S.; Daniels, AD.; Strain, MC.; Farkas, O.; Malick, DK.; Rabuck, AD.; Raghavachari, K.; Foresman, JB.; Ortiz, JV.; Cui, Q.; Baboul, AG.; Clifford, S.; Cioslowski, J.; Stefanov, BB.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, RL.; Fox, DJ.; Keith, T.; Al-Laham, MA.; Peng, CY.; Nanayakkara, A.; Challacombe, M.; Gill, PMW.; Johnson, B.; Chen, W.; Wong, MW.; Gonzalez, C.; Pople, JA. Version C.02 ed. Gaussian, Inc; Wallingford, CT: 2004.
39. Bayly CI, Cieplak P, Cornell WD, Kollman PA. *J Phys Chem*. 1993; 97:10269–10280.
40. Cieplak P, Cornell WD, Bayly C, Kollman PA. *J Comput Chem*. 1995; 16:1357–1377.
41. Case, DA.; Darden, TA.; Cheatham, TE.; Simmerling, CL.; Wang, J.; Duke, RE.; Luo, R.; Merz, KM.; Wang, B.; Pearlman, DA.; Crowley, M.; Brozell, S.; Tsui, V.; Gohlke, H.; Mongan, J.; Hornak, V.; Cui, G.; Beroza, P.; Schafmeister, C.; Caldwell, JW.; Ross, WS.; Kollman, PA. University of California; San Francisco: 2010.
42. Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. *J Chem Phys*. 1983; 79:926–935.
43. Lee C, Yang W, Parr RG. *Phys Rev B*. 1988; 37:785–789.
44. Becke AD. *J Chem Phys*. 1993; 98:5648–5652.
45. Stephens PJ, Devlin FJ, Chabalowski CF, Frisch MJ. *J Phys Chem*. 1994; 98:11623–11627.
46. Case, DA.; Darden, TA.; Cheatham, TE.; Simmerling, CL.; Wang, J.; Duke, RE.; Luo, R.; Merz, KM.; Wang, B.; Pearlman, DA.; Crowley, M.; Brozell, S.; Tsui, V.; Gohlke, H.; Mongan, J.; Hornak, V.; Cui, G.; Beroza, P.; Schafmeister, C.; Caldwell, JW.; Ross, WS.; Kollman, PA. University of California; San Francisco: 2004.
47. Wei D, Fang L, Tang M, Zhan CG. *J Phys Chem B*. 2013; 117:13418–13434. [PubMed: 24111489]
48. Chandrasekhar J, Smith SF, Jorgensen WL. *J Am Chem Soc*. 1984; 106:3049–3050.

49. Kollman P. *Chem Rev.* 1993; 93:2395–2417.
50. Beveridge DL, DiCapua FM. *Annu Rev Biophys Biophys Chem.* 1989; 18:431–492. [PubMed: 2660832]
51. Acevedo O, Jorgensen WL. *Acc Chem Res.* 2010; 43:142–151. [PubMed: 19728702]
52. Acevedo O, Jorgensen WL. *J Am Chem Soc.* 2006; 128:6141–6146. [PubMed: 16669683]
53. Acevedo O, Jorgensen WL. *J Am Chem Soc.* 2005; 127:8829–8834. [PubMed: 15954791]
54. Zhang W, Hou TJ, Qiao XB, Huai S, Xu XJ. *J Mol Model.* 2004; 10:112–120. [PubMed: 14986176]
55. Jarmula A, Cieplak P, Les A, Rode W. *J Comput Aided Mol Des.* 2003; 17:699–710. [PubMed: 15068368]
56. Udier-Blagovic M, Tirado-Rives J, Jorgensen WL. *J Med Chem.* 2004; 47:2389–2392. [PubMed: 15084137]
57. Guimaraes CR, Boger DL, Jorgensen WL. *J Am Chem Soc.* 2005; 127:17377–17384. [PubMed: 16332087]
58. Guimaraes CR, Udier-Blagovic M, Jorgensen WL. *J Am Chem Soc.* 2005; 127:3577–3588. [PubMed: 15755179]
59. Alexandrova AN, Rothlisberger D, Baker D, Jorgensen WL. *J Am Chem Soc.* 2008; 130:15907–15915. [PubMed: 18975945]
60. Danciulescu C, Nick B, Wortmann FJ. *Biomacromolecules.* 2004; 5:2165–2175. [PubMed: 15530030]
61. Funahashi J, Sugita Y, Kitao A, Yutani K. *Protein Eng.* 2003; 16:665–671. [PubMed: 14560052]
62. Alvarez-Idaboy JR, Galano A, Bravo-Perez G, Ruiz ME. *J Am Chem Soc.* 2001; 123:8387–8395. [PubMed: 11516288]

Figure 1.

Representative AChE-6-MAM binding structure derived from the MD simulation. AChE enzyme is represented as cartoon. Substrate 6-MAM is shown in balls and sticks. The catalytic triad and oxyanion hole are shown in sticks. Other residues within 5 Å around 6-MAM are shown in lines.

Figure 2.
Key internuclear distances vs the simulation time in the MD-simulated AChE-6-MAM binding structure.

Figure 3.

(A) Division of the QM/MM system. Atoms in blue color belong to the QM subsystem. The boundary carbon atoms colored in red were treated with the improved pseudobond parameters. All of the other atoms were considered as the MM subsystem. (B-F) Optimized geometries for the key states during the acylation stage of AChE-catalyzed hydrolysis of 6-MAM. The geometries were optimized at the QM/MM(B3LYP/6-31G*:AMBER) level. The key distances in the figures are in Å. Carbon, oxygen, nitrogen, and hydrogen atoms are colored in green, red, blue, and white, respectively. The QM atoms are represented as balls and sticks and the surrounding residues are rendered as sticks. The protein is rendered as cartoon. Figures to be discussed below are represented using the same method.

Figure 4.

(A) Division of the QM/MM system. Atoms in blue color belong to the QM subsystem. The boundary carbon atoms colored in red were treated with the improved pseudobond parameters. All of the other atoms were considered as the MM subsystem. (B-F) Optimized geometries for the key states of the reaction system in the deacylation stage of human AChE-catalyzed hydrolysis of 6-MAM. The geometries were optimized at the QM/MM(B3LYP/6-31G*:AMBER) level. The color scheme is the same as that of Figure 3.

Figure 5.

Representative BChE-6-MAM binding structure derived from MD simulation. BChE enzyme is represented as carton. 6-MAM is shown in balls and sticks. The catalytic triad and oxyanion hole are shown in stick. Other residues within 5 Å around 6-MAM are shown in lines.

Figure 6.

(A) Division of the QM/MM system. Atoms in blue color belong to the QM subsystem. The boundary carbon atoms colored in red were treated with the improved pseudobond parameters. All of the other atoms were considered as the MM subsystem. (B-F) Optimized geometries for the key states of the reaction system in the acylation stage of human BChE-catalyzed hydrolysis of 6-MAM. The geometries were optimized at the QM/MM(B3LYP/6-31G*:AMBER) level. The color scheme is the same as that of Figure 3.

Figure 7.

(A) Division of the QM/MM system. Atoms in blue color belong to the QM subsystem. The boundary carbon atoms colored in red were treated with the improved pseudobond parameters. All of the other atoms were considered as the MM subsystem. (B-F) Optimized geometries for the key states of the reaction system in the deacylation stage of human BChE-catalyzed hydrolysis of 6-MAM. The geometries were optimized at the QM/MM(B3LYP/6-31G*:AMBER) level. The color scheme is the same as that of Figure 3.

Figure 8.

Free energy profiles determined by the QM/MM-FE calculations for the acylation of AChE (A), deacylation of AChE (B), acylation of BChE (C), and deacylation of BChE (D) stages of the enzymatic reaction processes. The relative free energies were determined first without the zero-point and thermal corrections, and then corrected with the zero-point and thermal corrections for the QM subsystem (values in parentheses). The QM/MM-FE calculations were performed at the B3LYP/6-31++G**:*AMBER* level for each QM/MM optimized geometry along the reaction path.

Scheme 1.

The metabolic pathway of heroin to morphine.

Scheme 2.

Fundamental reaction pathway for AChE-catalyzed hydrolysis of 6-MAM to morphine. Atoms colored in blue belong to the QM subsystem in the pseudobond first-principles QM/MM calculations. Atoms colored in red are treated with the improved pseudobond parameters. All other atoms belong to the MM subsystem.

Scheme 3.

Fundamental reaction pathway for BChE-catalyzed hydrolysis of 6-MAM to morphine. Atoms colored in blue belong to the QM subsystem in the pseudobond first-principles QM/MM calculations. Atoms colored in red are treated with the improved pseudobond parameters. All other atoms belong to the MM subsystem.