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Molecular Dynamics Simulation of Cocaine Binding with Human Butyrylcholinesterase and Its Mutants

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Abstract

Molecular dynamics (MD) simulations were carried out to study cocaine binding with wild-type human butyrylcholinesterase (BChE) and its mutants based on a recently reported X-ray crystal structure of human BChE. For each BChE-cocaine system, we simulated both the non-prereactive and prereactive complexes in water. To our knowledge, this is the first time for using an X-ray crystal structure of BChE to perform a MD simulation of cocaine binding with a BChE and the first time for carrying out MD simulation of prereactive complexes between cocaine and BChE mutant. Despite the significant difference found at the acyl binding pocket, the simulated structures confirm the fundamental structural and mechanistic insights obtained from earlier computational studies of wild-type BChE with cocaine based on a homology model, *e.g.* the rate-determining step for BChE-catalyzed hydrolysis of biologically active (–)-cocaine is the (–)-cocaine rotation in the active site from the non-prereactive BChE-(–)-cocaine complex to the prereactive complex. It has been demonstrated that the MD simulations on both the non-prereactive and prereactive BChE-cocaine complexes can clearly reveal whether specific mutations produce the desired BChE-(–)-cocaine binding structures in which the (–)-cocaine rotation is less hindered while the required prereactive BChE-(–)-cocaine binding is maintained. Based on the MD simulations, both A328W/Y332A and A328W/Y332G BChE's are expected to have catalytic activity for (–)-cocaine hydrolysis higher than that of wild-type BChE and the activity of A328W/Y332G BChE should be slightly higher than that of A328W/Y332A BChE due to the less-hindered (–)-cocaine rotation in the mutant BChE's. However, the less-hindered (–)-cocaine rotation is only a necessary condition for a higher-activity mutant BChE. The (–)-cocaine rotation is also less hindered in A328W/Y332A/Y419S BChE, but (–)-cocaine binds with A328W/Y332A/Y419S BChE in a way that is not suitable for the catalysis. Thus, A328W/Y332A/Y419S BChE is expected to lose the catalytic activity. The computational predictions were confirmed by experimental kinetic data, demonstrating that the MD simulation-based computational protocol used in this study is reliable in prediction of the catalytic activity of BChE mutants for (–)-cocaine hydrolysis.

Introduction

Cocaine abuse is a major medical and public health problem that continues to defy treatment.^{1,2} The disastrous medical and social consequences of cocaine addiction, such as violent crime, loss in individual productivity, illness and death, have made the development of an effective pharmacological treatment a high priority.^{3,4} However, cocaine mediates its reinforcing and toxic effects by blocking neurotransmitter reuptake and the classical pharmacodynamic approach has failed to yield small-molecule receptor antagonists due to the difficulties inherent in blocking a blocker.^{1–4} An alternative to receptor-based approaches is to interfere with the delivery of cocaine to its receptors and accelerate its metabolism in the body.⁴ An ideal

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molecule for this purpose should be a potent enzyme, or catalytic antibody (artificial enzyme),^{5,6} which can catalyze the hydrolysis of cocaine into biologically inactive metabolites.

The dominant pathway for cocaine metabolism in primates is butyrylcholinesterase (BChE)-catalyzed hydrolysis at the benzoyl ester group (Chart 1).^{4,7} Only 5% of the cocaine is deactivated through oxidation by the liver microsomal cytochrome P450 system.⁸ Cocaine hydrolysis at benzoyl ester group yields ecgonine methyl ester, whereas the oxidation produces norcocaine.⁹ The metabolite ecgonine methyl ester is biologically inactive metabolite, whereas the metabolite norcocaine is hepatotoxic and a local anesthetic. Clearly, BChE-catalyzed hydrolysis of cocaine at the benzoyl ester is the pathway most suitable for amplification and, therefore, we focus on the improvement of the catalytic activity of the primary cocaine-metabolizing enzyme. BChE is synthesized in the liver and widely distributed in the body, including plasma, brain, and lung.¹⁰ Extensive experimental studies in animals and humans demonstrate that enhancement of BChE activity by administration of exogenous enzyme substantially decreases cocaine half-life.^{11,12,13,14} So, enhancement of cocaine metabolism by administration of BChE has been recognized to be a promising pharmacokinetic approach for treatment of cocaine abuse and dependence.⁴ However, the catalytic activity of this plasma enzyme is three orders-of-magnitude lower against the naturally occurring (–)-cocaine than that against the biologically inactive (+)-cocaine enantiomer. (+)-cocaine can be cleared from plasma in seconds and prior to partitioning into the central nervous system (CNS), whereas (–) cocaine has a plasma half-life of ~ 45 – 90 min, long enough for manifestation of the CNS effects which peak in minutes.^{15,16} Hence, BChE mutants with high activity against (–)-cocaine are highly desired for use in humans.

Recently reported studies^{17,18,19,20} suggested that, for both (–)-cocaine and (+)-cocaine, the BChE-substrate binding involves two different types of complexes: non-prereactive and prereactive BChE-substrate complexes. Whereas the non-prereactive BChE-cocaine complexes were first reported by Sun *et al.*,¹⁹ we were the first reporting the prereactive BChE-cocaine complexes and reaction coordinate calculations.²⁰ It was demonstrated²⁰ that (–)/(+) cocaine first slides down the substrate-binding gorge to bind to W82 and stands vertically in the gorge between D70 and W82 (non-prereactive complex) and then rotates to a position in the catalytic site within a favorable distance for nucleophilic attack and hydrolysis by S198 (prereactive complex). In the prereactive complex, cocaine lies horizontally at the bottom of the gorge. The main structural difference between the BChE-(–)-cocaine complexes and the corresponding BChE-(+)-cocaine complexes exists in the relative position of the cocaine methyl ester group.²⁰ Reaction coordinate calculations²⁰ revealed that the rate-determining step of BChE-catalyzed hydrolysis of (+)-cocaine is the chemical reaction process, whereas for (–)-cocaine the change from the non-prereactive complex to the prereactive complex is rate determining. A further analysis of the structural changes from the non-prereactive complex to the prereactive complex reveals specific amino acid residues hindering the structural changes, providing initial clues for the rational design of BChE mutants with improved catalytic activity for (–)-cocaine.²⁰

Previous molecular dynamics (MD) simulations of prereactive BChE-cocaine binding were limited to wild-type BChE.²⁰ Even for the non-prereactive BChE-cocaine complex, only one mutant (A328W/Y332A) BChE binding with (–)-cocaine was simulated and its catalytic activity for (–)-cocaine was reported.¹⁹ No MD simulation was performed on any prereactive enzyme-substrate complex for (–)- or (+)-cocaine binding with a mutant BChE. In addition, all previous computational studies^{19,20} of BChE interacting with cocaine were performed based on a homology model of BChE when three-dimensional (3D) X-ray crystal structure was not available for BChE. Nicolet *et al.*²¹ recently reported 3D X-ray crystal structures of BChE. As expected, the structure of BChE is similar to a previously published theoretical model of this enzyme and to the structure of acetylcholinesterase. The main difference between

the experimentally determined BChE structure and its model was found at the acyl binding pocket (acyl loop) that is significantly bigger than expected.²¹ Does the structural difference at the acyl binding pocket significantly affect BChE binding with (–)-cocaine and (+)-cocaine? It is fundamentally important for rational design of high-activity BChE mutants to examine whether using the X-ray crystal structure, instead of the homology model, in the computational studies will change our previous conclusion²⁰ concerning the non-prereactive and prereactive BChE-cocaine binding and catalytic mechanisms. Although previous MD simulations of cocaine binding with wild-type BChE and the reaction coordinate calculations point to some amino acid residues that might need to be mutated for the purpose of improving the catalytic activity for (–)-cocaine hydrolysis, further MD simulations of both non-prereactive and prereactive mutant BChE-cocaine complexes are clearly necessary to theoretically examine whether BChE mutants proposed simply based on the cocaine binding with wild-type BChE really have a higher catalytic activity for (–)-cocaine.

In the present study, we have performed a detailed computational study of cocaine binding with wild-type and mutant BChE's starting from the available X-ray crystal structure of wild-type BChE. The simulated mutants include A328W/Y332G, A328W/Y332A, and A328W/Y332A/Y419S, as our MD simulations of (–)-cocaine binding with wild-type BChE suggest that these mutations could be important for changing the (–)-cocaine rotation from the non-prereactive complex to the prereactive complex (see below for discussion of the results). We have also carried out wet experimental tests on the catalytic activity of these mutants for (–)-cocaine in order to verify the computational predictions. All of the obtained results clearly demonstrate that molecular modeling and MD simulations of cocaine binding with BChE mutants provide a reliable computational approach to the rational design of high-activity mutants of BChE for the (–)-cocaine hydrolysis.

Materials and methods

3D model of BChE

The initial coordinates of human BChE used in our computational studies came from the X-ray crystal structure²¹ deposited in the Protein Data Bank (pdb code: 1POP).²² The missing residues (D2, D3, E255, D378, D379, N455, L530, E531, and M532) in the X-ray crystal structure were built using the automated homology modeling tool Modeler^{23,24}/InsightII software (Accelrys, Inc.) with the default parameters.

Molecular docking

Molecular docking was performed for each non-prereactive protein-ligand binding complex. The binding site was defined as a sphere with an approximately 15 Å radius around the active site residue S198. The amino acid residues included in the binding site model are not contiguous in the protein. Cocaine, considered as a ligand, was initially positioned at 17 Å in front of S198 of the binding site. Each BChE-cocaine binding complex was energy-minimized by using the steepest descent algorithm first until the maximum energy derivative is smaller than 4 kcal/mol/Å and then the conjugated gradient algorithm until the maximum energy derivative is smaller than 0.001 kcal/mol/Å. The energy minimization was followed by a 300 ps molecular dynamics (MD) simulation at T = 298 K with a time step of 1 fs. During the energy minimization and MD simulation, only cocaine and the residues of BChE included in the binding site were allowed to move, while the remaining part of the protein was fixed. The energy-minimization and MD simulation for these processes were performed by using the Amber force field implemented in the Discover_3/InsightII calculation engine.²⁵ The non-bonded cut-off method and the dielectric constant were set up to group based (12 Å cut-off distance) and distance dependent, respectively ($\epsilon = 4r$).²⁶

Molecular dynamic simulation in water

The initial coordinates used in the MD simulation of the non-prereactive complexes were determined by using the molecular docking procedure described above, whereas the initial coordinates used in the MD simulation of the prereactive complexes were obtained from superimposing backbone of the X-ray crystal structure to that of our previously simulated prereactive complex²⁰ between cocaine and a homology model of wild-type BChE. Each BChE-cocaine binding complex was neutralized by adding two chloride counterions and was solvated in a rectangular box of TIP3P water molecules²⁷ with a minimum solute-wall distance of 10 Å. The general procedure for carrying out the MD simulations in water is similar to that used in our previously reported other computational studies.^{20,28} These simulations were performed by using the Sander module of Amber7 program.²⁹ The solvated system was optimized prior to the MD simulation. First of all, the protein-ligand was frozen and the solvent molecules with counterions were allowed to move during a 5000-step minimization with the conjugate gradient algorithm and a 5 ps MD run at T = 300 K. After full relaxation and the entire solvated system was energy-minimized, the system was slowly heated from T = 10 K to T = 300 K in 30 ps before the production MD simulation for 500 ps. The full minimization and equilibration procedure was repeated for each mutant. The MD simulations were performed with a periodic boundary condition in the NPT ensemble at T = 300 K with Berendsen temperature coupling³⁰ and constant pressure (P = 1 atm) with isotropic molecule-based scaling.³⁰ The SHAKE algorithm³¹ was applied to fix all covalent bonds containing a hydrogen atom, a time step of 2 fs was used, and the non-bond pair list was updated every 10 steps. The pressure was adjusted by isotropic position scaling. The particle mesh Ewald (PME) method³² was used to treat long-range electrostatic interactions. A residue-based cutoff of 10 Å was applied to the noncovalent interactions. During the 500 ps production MD simulation, the coordinates of the simulated complex were saved every 1 ps.

Molecular docking and MD simulation procedures described above were performed to study cocaine binding with wild-type BChE and three mutants, *i.e.* A328W/Y332A, A328W/Y332A/Y419S, and A328W/Y332G. For each protein system (wild-type or mutant BChE), we considered the protein binding with cocaine in both the non-prereactive and prereactive enzyme-substrate complexes.

Most of the MD simulations in water were performed on a supercomputer, Superdome, at the Center for Computational Sciences, University of Kentucky. The other computations were carried out on SGI Fuel workstations and a 34-processors IBM x335 Linux cluster in our own lab.

Experimental procedure

Site-directed mutagenesis of human BChE cDNA was performed by the QuikChange method.³³ Mutations were generated from wild-type human BChE in a pRc/CMV expression plasmid. Using plasmid DNA as template and primers with specific base-pair alterations, mutations were made by polymerase chain reaction with *Pfu* DNA polymerase, for replication fidelity. The PCR product was treated with *Dpn I* endonuclease to digest the parental DNA template. Modified plasmid DNA was transformed into *Escherichia coli*, amplified, and purified. The DNA sequences of the mutants were confirmed by DNA sequencing. BChE mutants were expressed in human embryonic kidney cell line 293T/17. Cells were grown to 80–90% confluence in 6-well dishes and then transfected by Lipofectamine 2000 complexes of 4 µg plasmid DNA per each well. Cells were incubated at 37 °C in a CO₂ incubator for 24 hours and cells were moved to 60-mm culture vessel and cultured for four more days. The culture medium [10% fetal bovine serum in Dulbecco's modified Eagle's medium (DMEM)] was harvested for a BChE activity assay. To measure cocaine and benzoic acid, the product of cocaine hydrolysis by BChE, we used sensitive radiometric assays based on toluene extraction

of [^3H]cocaine labeled on its benzene ring.^{19b} In brief, to initiate reactions, 100 nCi of [^3H]cocaine was mixed with 100 μl of culture medium. Reactions proceeded at 37 °C for varying times. Reactions were stopped by adding 300 μl of 0.02 M HCl, which neutralized the liberated benzoic acid while ensuring a positive charge on the residual cocaine. [^3H]benzoic acid was extracted by 1 ml of toluene and measured by scintillation counting. Finally, the measured time-dependent radiometric data were fitted to the kinetic equation so that the catalytic efficiency ($k_{\text{cat}}/K_{\text{M}}$) was determined.

Results and discussion

Depicted in Figure 1 are plots of some important distances in the MD-simulated (–)cocaine binding with A328W/Y332G BChE *versus* the simulation time, along with root-mean-square deviation (RMSD) of the coordinates of backbone atoms in the simulated structure from those in the X-ray crystal structure. MD trajectories for other complexes were similar to these two in Figure 1, although the simulated average distances are different. Summarized in Table 1 are the average values of some important geometric parameters in the simulated complexes.

(–)- and (+)-cocaine binding with wild-type BChE

Figure 2 shows the binding structures of the simulated non-prereactive and prereactive complexes of wild-type BChE binding with the two enantiomers of cocaine. In the non-prereactive complexes with (–)- and (+)-cocaine, the methyl ester group of cocaine is positioned at the top of the H438 backbone, while the cocaine benzoyl ester moiety is quasi-parallel to the C-O γ side chain of S198 with a dihedral angle Θ of -8° and 140° , respectively. Here, Θ refers to the dihedral angle formed by S198 O γ and the plane of carboxylate group of the cocaine benzoyl ester (see Chart 2). The simulated internuclear distances between the carbonyl oxygen of cocaine benzoyl ester group and the NH hydrogen of G116, G117, and A199 are comparable for the two enantiomers. The simulated average distances between the carbonyl carbon of the benzoyl ester and S198 O γ are 5.60 Å and 5.18 Å for (–)- and (+)-cocaine, respectively. Comparing the simulated protein backbone structures to the X-ray crystal structure,²¹ one can see from Figure 1 that the RMSD values are all smaller than 1.3 Å for the whole protein structures.

The MD simulations of the prereactive complexes reveal that wild-type BChE binding with (–)-cocaine is essentially the same as the binding with (+)-cocaine in the binding site, except for the different positions of methyl ester group of the substrates. The simulated average distances between the carbonyl carbon of the benzoyl ester and S198 O γ are 3.27 and 3.69 Å for (–)-cocaine and (+)-cocaine, respectively. Moreover, the (+)-cocaine is stabilized more effectively by the formation of strong hydrogen bonds with the backbone NH of residues G116, G117, and A199 (see Table 1). The cocaine benzoyl ester moiety is positioned quasi-perpendicular to S198 C-O γ with a dihedral angle Θ of $\sim 67^\circ$ and $\sim 61^\circ$ for (–)- and (+)-cocaine, respectively.

We compared our currently simulated structures of the BChE-cocaine binding with those simulated previously by using a homology model of BChE²⁰ and we noted two major differences between the two sets of structures. By using the X-ray crystal structure,²¹ the acyl loop is positioned on the top of the cocaine benzoyl ester moiety of the cocaine, whereas the acyl loop is far from the cocaine benzoyl ester moiety in the structure simulated starting from the homology model.²⁰ The RMSD of the coordinates of backbone atoms in the previously simulated prereactive BChE-(–)-cocaine complex from those in the X-ray crystal structure of BChE is ~ 2.0 Å for the entire protein and ~ 3.0 Å for the acyl loop. The RMSD value became ~ 2.4 Å for the entire protein and ~ 3.3 Å for the acyl loop, when the X-ray crystal structure was replaced by the MD-simulated prereactive BChE-(–)-cocaine complex starting from the X-ray crystal structure. Despite these structural differences, the benzoyl ester group of the

ligand is still close to the key residues (S197, G116, and G117) in the BChE binding site. Some significant differences are associated with the distances between the S198 O γ atom and the carbonyl carbon of the cocaine benzoyl ester in non-prereactive complexes. The average values of this distance in the non-prereactive complexes were ~ 9.5 and ~ 8.5 Å for (–)- and (+)-cocaine, respectively,²⁰ when a homology model was used. The corresponding average values became ~ 5.6 and ~ 5.2 Å, respectively, when we used the X-ray crystal structure in the current study. So, both (–)- and (+)-cocaine became closer to the binding site when the homology model was replaced by the X-ray crystal structure. However, we did not observe significant changes of the binding in the prereactive complexes when the used homology model was replaced by the X-ray crystal structure. The average values of the simulated distance between the S198 O γ atom and the carbonyl carbon of the cocaine benzoyl ester in the prereactive complexes are always close to ~ 3.5 Å for both (–)- and (+)-cocaine no matter whether the X-ray crystal structure or homology model of BChE was used as the starting structure. The similar computational results obtained from the use of the X-ray crystal structure and homology model of BChE suggest that the fundamental structural and mechanistic insights obtained from our previous computational studies²⁰ are reliable, despite the previous simulations were performed by using the homology model when the X-ray crystal structure was not available.

Further, we superimposed the simulated structures of the non-prereactive BChE-cocaine complexes with the corresponding prereactive complexes. As shown in Figure 3, the (–)-cocaine rotation in the BChE active site from the non-prereactive complex to the prereactive complex is hindered by some residues as the positions of Y332, A328, and F329 residues in the non-prereactive complex are significantly different from the corresponding positions in the prereactive complex, whereas none of these residues hinders the (+)-cocaine rotation in the BChE active site from the non-prereactive complex to the prereactive complex because these residues stay in nearly the same positions in the two BChE-(+)-cocaine complexes.

(–)-cocaine binding with BChE mutants

Now that the (–)-cocaine rotation from the non-prereactive complex to the prereactive complex has been known to be the rate-determining step of the BChE-catalyzed hydrolysis of (–)-cocaine,²⁰ useful BChE mutants should be designed to specifically accelerate the change from the non-prereactive BChE-(–)-cocaine complex to the prereactive complex. The question is whether MD simulation can be performed to help design BChE mutants that have higher catalytic activity for (–)-cocaine hydrolysis.

In the simulated non-prereactive complex, the average distance between the carbonyl carbon of cocaine benzoyl ester and S198 O γ is 7.6 Å for A328W/Y332A BChE and 7.1 Å for A328W/Y332G BChE, as seen in Table 1. In the simulated prereactive complex, the average values of this important internuclear distance become 3.87 and 3.96 Å for A328W/Y332A and A328W/Y332G BChE's, respectively. Compared to the simulated wild-type BChE-(–)-cocaine prereactive complex, the average distances between the carbonyl carbon of the cocaine benzoyl ester and S198 O γ in the prereactive complex of (–)-cocaine with A328W/Y332A and A328W/Y332G BChE's are all slightly longer, whereas the average distances between the carbonyl oxygen of the cocaine benzoyl ester and the NH of G116, G117, and A199 residues are all shorter. This suggests that (–)-cocaine more strongly bind with A328W/Y332A and A328W/Y332G BChE's in the prereactive complexes. More importantly, the (–)-cocaine rotation in the active site of A328W/Y332A and A328W/Y332G BChE's from the non-prereactive complex to the prereactive complex did not cause considerable changes of the positions of A332 (or G332), W328, and F329 residues as seen in Figure 4, compared to the (–)-cocaine rotation in the active site of wild-type BChE. These results suggest that A328W/Y332A and A328W/Y332G BChE's should be associated with lower energy barriers than the wild-type for the (–)-cocaine rotation from the non-prereactive complex to the prereactive complex.

Further, (–)-cocaine binding with A328W/Y332G BChE is very similar to the binding with A328W/Y332A BChE, but the position change of F329 residue caused by the (–)-cocaine rotation was significant only in A328W/Y332A BChE, thus suggesting that the energy barrier for the (–)-cocaine rotation in A328W/Y332G BChE should be slightly lower than that in A328W/Y332A BChE.

Concerning (–)-cocaine binding with A328W/Y332A/Y419S BChE, Y419 stays deep inside the protein and does not directly contact with the cocaine molecule. We tested the Y419S mutation because we initially expected this mutation to further increase the free space of the active site pocket so that the (–)-cocaine rotation could be easier. However, as seen in Table 1, the average distance between the carbonyl carbon of cocaine benzoyl ester and S198 O^γ atom in the simulated prereactive complex was as long as 5.84 Å. The average distances between the carbonyl oxygen of the cocaine benzoyl ester and the NH hydrolysis atoms of G116, G117, and A199 residues are between 4.56 and 6.97 Å; no any hydrogen bond between them. In addition to the internuclear distances, another interesting geometric parameter is the dihedral angle, Θ , formed by S198 O^γ and the plane of the carboxylate group of the cocaine benzoyl ester. As seen in Table 1, the Θ values in the prereactive complexes of cocaine with wild-type BChE and all of the BChE mutants other than A328W/Y332A/Y419S BChE all slightly deviate from the ideal value of 90° for the nucleophilic attack of S198 O^γ at the carbonyl carbon of cocaine. The Θ value in the prereactive complex of (–)-cocaine with A328W/Y332A/Y419S BChE is 164°, which is considerably different from the ideal value of 90°.

Catalytic activity

The aforementioned discussion suggests that the energy barriers for the (–)-cocaine rotation in A328W/Y332A and A328W/Y332G BChE's from the non-prereactive complex to the prereactive complex, the rate-determining step for the BChE-catalyzed hydrolysis of (–)-cocaine, should be lower than that in the wild-type BChE. Thus, the MD simulations predict that both A328W/Y332A and A328W/Y332G BChE's should have a higher catalytic activity than the wild-type BChE for (–)-cocaine hydrolysis. Further, the MD simulations also suggest that the energy barrier for the (–)-cocaine rotation in A328W/Y332G BChE should be slightly lower than that in A328W/Y332A BChE and, therefore, the catalytic activity of A328W/Y332G BChE for the (–)-cocaine hydrolysis should be slightly higher than the activity of A328W/Y332A BChE. In addition, the MD simulations predict that A328W/Y332A/Y419S BChE should have no catalytic activity, or have a considerably lower catalytic activity than the wild-type, for (–)-cocaine hydrolysis because (–)-cocaine binds with the mutant BChE in a way that is not suitable for the catalysis.

The catalytic efficiency ($k_{\text{cat}}/K_{\text{M}}$) of A328W/Y332A BChE for (–)-cocaine hydrolysis was reported^{19c} to be $8.56 \times 10^6 \text{ M min}^{-1}$, which is 9.39 times of the $k_{\text{cat}}/K_{\text{M}}$ value ($9.11 \times 10^5 \text{ M min}^{-1}$) of the wild-type BChE. To examine our theoretical predictions of the relative activity for A328W/Y332G and A328W/Y332A/Y419S BChE's, we produced A328W/Y332A, A328W/Y332G, and A328W/Y332A/Y419S BChE's through site-directed mutagenesis. To minimize the possible systematic experimental errors of the kinetic data, we performed kinetic studies with all of three mutants under the same condition and compared the catalytic efficiency of the A328W/Y332G and A328W/Y332A/Y419S to that of the A328W/Y332A for (–)-cocaine hydrolysis at benzoyl ester group. Based on the kinetic analysis of the measured time-dependent radiometric data, the ratio of the $k_{\text{cat}}/K_{\text{M}}$ value of A328W/Y332G BChE to the $k_{\text{cat}}/K_{\text{M}}$ value of A328W/Y332A BChE for the (–)-cocaine hydrolysis was determined to be 2.08, or A328W/Y332G BChE has a $k_{\text{cat}}/K_{\text{M}}$ value of $1.78 \times 10^7 \text{ M min}^{-1}$ for the (–)-cocaine hydrolysis. The radiometric data show no significant catalytic activity for A328W/Y332A/

Y419S BChE. These experimental data are consistent with the theoretical predictions based on the MD simulations.

Conclusion

Molecular modeling, molecular docking, and molecular dynamics (MD) simulations were performed to study cocaine binding with human butyrylcholinesterase (BChE) and its mutants, including A328W/Y332A, A328W/Y332G, and A328W/Y332A/Y419S BChE's, based on a recently reported X-ray crystal structure of human BChE. We simulated both the non-prereactive and prereactive BChE-cocaine complexes. The MD simulations of cocaine binding with wild-type BChE led to average BChE-cocaine binding structures similar to those obtained recently from the MD simulations based on a homology model of BChE, despite the significant difference found at the acyl binding pocket. This confirms the fundamental structural and mechanistic insights obtained from our earlier computational studies based on a homology model of BChE, *e.g.* the rate-determining step for BChE-catalyzed hydrolysis of biologically active (–)-cocaine is the (–)-cocaine rotation in the BChE active site from the non-prereactive BChE-(–)-cocaine complex to the prereactive complex.

The MD simulations further reveal that the (–)-cocaine rotation in the active site of wild-type BChE from the non-prereactive complex to the prereactive complex is hindered by some residues such that the positions of Y332, A328, and F329 residues in the non-prereactive complex are significantly different from those in the prereactive complex. Compared to (–) cocaine binding with wild-type BChE, (–)-cocaine more strongly bind with A328W/Y332A and A328W/Y332G BChE's in the prereactive complexes. More importantly, the (–)-cocaine rotation in the active site of A328W/Y332A and A328W/Y332G BChE's from the non-prereactive complex to the prereactive complex did not cause considerable changes of the positions of A332 or G332, W328, and F329 residues. These results suggest that A328W/Y332A and A328W/Y332G BChE's should be associated with lower energy barriers than wild-type BChE for the (–)-cocaine rotation from the non-prereactive complex to the prereactive complex. Further, (–)-cocaine binding with A328W/Y332G BChE is very similar to the binding with A328W/Y332A BChE, but the position change of F329 residue caused by the (–)-cocaine rotation was significant only in A328W/Y332A BChE, thus suggesting that the energy barrier for (–)-cocaine rotation in A328W/Y332G BChE should be slightly lower than that in A328W/Y332A BChE. It has also been demonstrated that (–)-cocaine binds with A328W/Y332A/Y419S BChE in a way that is not suitable for the catalysis.

Based on the computational results, both A328W/Y332A and A328W/Y332G BChE's are expected to have catalytic activity for (–)-cocaine hydrolysis higher than that of wild-type BChE and the activity of A328W/Y332G BChE should be slightly higher than that of A328W/Y332A BChE, whereas A328W/Y332A/Y419S BChE is expected to lose the catalytic activity. The computational predictions are completely consistent with the experimental kinetic data, suggesting that the used computational protocol, including molecular modeling, molecular docking, and MD simulations, is reliable in prediction of the catalytic activity of BChE mutants for (–)-cocaine hydrolysis.

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Figure 1.

Plots of the key internuclear distances (in Å) *versus* the simulation time in the simulated non-prereactive complex (A) and the prereactive complex (B) of $(-)$ -cocaine with A328W/Y332G mutant BChE. Trace D1 represents the distance between the carbonyl carbon of the cocaine benzoyl ester and Ser198 O γ . Traces D2, D3, and D4 refer to the distances from the carbonyl oxygen to the NH hydrogen atoms of G116, G117, and A199 residues, respectively. RMSD represents the root-mean-square deviation (in Å) of the simulated positions of BChE backbone atoms from those in the X-ray crystal structure.

Figure 2.

The BChE binding site in the simulated non-prereactive complex of (-)-cocaine with wild-type BChE (A), the prereactive complex of (-)-cocaine with wild-type BChE (B), non-prereactive complex of (+)-cocaine with wild-type BChE (C), and prereactive complex of (+)-cocaine with wild-type BChE (D).

Figure 3. Relative positions of cocaine and some residues in the simulated non-prereactive and prereactive wild-type BChE-($-$)-cocaine (A) and BChE-($+$)-cocaine (B) complexes. The red and black refer to the non-prereactive and prereactive complexes, respectively. The positions of S198 in the non-prereactive and prereactive complexes were essentially the same; the highlighted S198 came from the prereactive complex.

Figure 4.

Relative positions of (-)-cocaine and some residues in the simulated non-prereactive and prereactive complexes of (-)-cocaine with A328W/Y332A BChE (A), A328W/Y332G BChE (B), and A328W/Y332A/Y419S BChE (C). The red and yellow refer to the non-prereactive and prereactive complexes, respectively. The positions of S198 in the non-prereactive and prereactive complexes were essentially the same; the highlighted S198 came from the prereactive complex.

Chart 1.
Hydrolysis of (-)-cocaine and (+)-cocaine

Chart 2.

Four important internuclear distances (D1, D2, D3, and D4) between (-)-cocaine and the residues interacting with (-)-cocaine. Θ is the dihedral angle formed by the S198 O^γ atom and the plane of the carboxylate group of the cocaine benzoyl ester.

Table 1

The time-averaged values of some key geometric parameters (Å and degree) in the simulated non-prereactive and prereactive BChE-cocaine complexes.

BChE-cocaine binding ^a	Average values of the geometric parameters ^c						RMSD ^d	
	<D1> _{non}	<D1>	<D2>	<D3>	<D4>	<Θ>	nonpre	pre
wild-type	5.60	3.27	5.77	2.71	3.37	67	1.14	1.27
wild-type with (+)-cocaine ^b	7.64	3.69	2.88	2.30	2.83	61	1.15	1.13
A328W/Y332A	7.11	3.87	3.30	2.14	3.01	51	1.58	1.65
A328W/Y332G	7.06	3.96	2.28	2.52	2.42	60	1.20	1.35
A328W/Y332A/Y419S	5.18	5.84	5.64	4.56	6.97	164	2.66	2.62

^aRefers to (–)-cocaine binding with wild-type human BChE or (–)-cocaine binding with a mutant BChE, unless indicated otherwise.

^bRefers to (+)-cocaine binding with wild-type human BChE.

^c<D1>_{non} and <D1> represent the average distances between the S198 O^γ atom and the carbonyl carbon of the cocaine benzoyl ester in the simulated non-prereactive and prereactive BChE-cocaine complexes, respectively. <D2>, <D3>, <D4> refer to the average values of the simulated distances of the cocaine benzoyl ester to the NH hydrogen atoms of G116, G117, and A199 residues, respectively. <Θ> is the average value of the dihedral angle formed by the S198 O^γ atom and the plane of the carboxylate group of the cocaine benzoyl ester. See Chart 2.

^dThe root-mean-square deviation (RMSD) of the coordinates of backbone atoms in the simulated structure from those in the X-ray crystal structure of BChE. “nonpre” and “pre” refer to the non-prereactive and prereactive BChE-cocaine complexes, respectively.