Computational redesign of human butyrylcholinesterase for anticocaine medication

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Molecular dynamics was used to simulate the transition state for the first chemical reaction step (TS1) of cocaine hydrolysis catalyzed by human butyrylcholinesterase (BChE) and its mutants. The simulated results demonstrate that the overall hydrogen bonding between the carbonyl oxygen of (-)-cocaine benzyol ester and the oxyanion hole of BChE in the TS1 structure for (-)-cocaine hydrolysis catalyzed by A199S/S287G/A328W/Y332G BChE should be significantly stronger than that in the TS1 structure for (-)-cocaine hydrolysis catalyzed by the WT BChE and other simulated BChE mutants. Thus, the transition-state simulations predict that A199S/S287G/A328W/Y332G mutant of BChE should have a significantly lower energy barrier for the reaction process and, therefore, a significantly higher catalytic efficiency for (-)-cocaine hydrolysis. The theoretical prediction has been confirmed by wet experimental tests showing an (~45 ± 41)-fold improved catalytic efficiency of A199S/S287G/A328W/Y332G BChE against (-)-cocaine. This is a unique study to design an enzyme mutant based on transition-state simulation. The designed BChE mutant has the highest catalytic efficiency against cocaine of all of the reported BChE mutants, demonstrating that the unique design approach based on transition-state simulation is promising for rational enzyme redesign and drug discovery.

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Abbreviations: TS1, transition state for the first chemical reaction step; BChE, butyrylcholinesterase; MD, molecular dynamics; HBE, hydrogen-bonding energy; ES, prereactive enzyme–substrate complex.

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reaction process. Further, recently reported computational modeling also suggests that the formation of the ES is hindered mainly by the bulky side chain of Y332 residue in WT BChE but that the hindering can be removed by the Y332A or Y332G mutation (27). Therefore, starting from the A328W/Y332A or A328W/Y332G mutant, the rational design of further mutation(s) to improve the catalytic efficiency of BChE against (−)-cocaine can aim to decrease the energy barrier for the first reaction step without significantly affecting the ES formation and other chemical reaction steps.

Here, we report rational design of a high-activity mutant of BChE against (−)-cocaine based on detailed computational modeling of the transition state for the rate-determining step (i.e., the first step of the chemical reaction process). Molecular dynamics (MD) simulations were performed to model the protein environmental effects on the stabilization of the transition-state structure for BChE-catalyzed hydrolysis of (−)-cocaine. The simulated results indicate that the transition-state structure can be much better stabilized by the protein environment in A199S/S287G/A328W/Y332G BChE than that in WT BChE and in other BChE mutants examined. The computational modeling led to a prediction of the higher catalytic efficiency for the A199S/S287G/A328W/Y332G mutant against (−)-cocaine. The prediction has been confirmed by wet experimental tests showing that the A199S/S287G/A328W/Y332G mutant has a remarkably improved catalytic efficiency against (−)-cocaine. All of the obtained results clearly demonstrate that directly modeling the transition-state structure provides a reliable computational approach to the rational design of a high-activity mutant of BChE against (−)-cocaine.

Materials and Methods

MD Simulations. We must address a critical issue before describing how we performed any MD simulation on a transition state. In principle, MD simulation using a classical force field (molecular mechanics) can only simulate a stable structure corresponding to a local minimum on the potential energy surface, whereas a transition state during a reaction process is always associated with a first-order saddle point on the potential energy surface. Hence, MD simulation using a classical force field cannot directly simulate a transition state without any restraint on the geometry of the transition state. Nevertheless, if we can technically remove the freedom of imaginary vibration in the transition-state structure, then the number of vibrational freedoms (normal vibration modes) for a nonlinear molecule will decrease from 3N−6 to 3N−7 or less. The transition-state structure is associated with a local minimum on the potential energy surface within a subspace of the reduced vibrational freedoms, although it is associated with a first-order saddle point on the potential energy surface with all of the 3N−6 vibrational freedoms. Theoretically, the vibrational freedom associated with the imaginary vibrational frequency in the transition-state structure can be removed by appropriately freezing the reaction coordinate. The reaction coordinate corresponding to the imaginary vibration of the transition-state structure is generally parameterized by a combination of some key geometric parameters. These key geometric parameters are bond lengths of the forming and breaking covalent bonds for BChE-catalyzed hydrolysis of cocaine, as seen in Fig. 3. Thus, we just need to maintain the bond lengths of the forming and breaking covalent bonds during the MD simulation on a transition state. Technically, we can maintain the bond lengths of the forming and breaking covalent bonds by simply fixing all atoms within the reaction center, by using some constraints on the forming and breaking covalent bonds, or by redefining the forming and breaking covalent bonds. It should be pointed out that the only purpose of performing these types of MD simulations on a transition state is to examine the dynamic change of the protein environment surrounding the reaction center and the interaction between the reaction center and the protein environment. We are interested only in the simulated structures, because the total energies calculated in this way are meaningless. Transition-state modeling with empirical force fields was previously performed to study various organic reactions, and the theoretical justification of the transition-state modeling was discussed in detail by Eksterowicz and Houk (29).

The initial BChE structures used in the MD simulations were prepared based on our previous MD simulation (27) on the ES for WT BChE with (−)-cocaine in water using the AMBER 7 package (30). Our previous MD simulations (27) on the ES started from the x-ray crystal structure (31) deposited in the Protein Data Bank (PDB ID code 1P0P) (32). The present MD simulation on the transition state for the first chemical reaction step (TS1) was performed in such a way that bond lengths of the partially formed and partially broken covalent bonds in the transition state were all constrained to be the same as those obtained from our previous ab initio reaction coordinate calculations on the model reaction system of WT BChE (24). For convenience, the partially formed and partially broken covalent bonds in the transition state will be called “transition” bonds (33, 34). A sufficiently long MD simulation with the transition bonds constrained should lead to a reasonable protein environment stabilizing the reaction center in the simulated transition-state structure. Further, the simulated TS1 structure for WT BChE with (−)-cocaine was used to build the initial structures of TS1 for the examined BChE mutants with (−)-cocaine; only the side chains of mutated residues needed to be changed.

The partial atomic charges for the nonstandard residue atoms, including cocaine atoms, in the TS1 structures were calculated by using the RESP protocol implemented in the antechamber module of the AMBER 7 package following electrostatic potential (ESP) calculations at ab initio HF/6-31G* level using the GAUSSIAN 03 program (35). The geometries used in the ESP calculations came from those obtained from the previous ab initio reaction coordinate calculations (26), but the functional groups representing the oxygen hole were removed. Thus, residues G116, G117, and A199 were the standard residues as supplied by AMBER 7 in the MD simulations. The general procedure for carrying out the MD simulations in water is essentially the same as that used in our previously reported computational studies (26, 27, 36–39). Each aforesaid model of starting TS1 structure was neutralized by adding chloride counterions and was solvated in a rectangular box of TIP3P water molecules (40) with a minimum solute-wall distance of 10 Å. The total number of atoms in the solvated protein structures for the MD simulations is nearly 70,000, although the total number of atoms of BChE and (−)-cocaine is only 8,417 (for the WT BChE). All of the MD simulations were performed by using the sander module of the AMBER 7 package. The solvated systems were carefully equilibrated and fully energy-minimized. These systems were gradually heated from T = 10 K to T = 298.15 K in 30 ps before running the MD simulation at T = 298.15 K for 1 ns or longer, making sure that we obtained a stable MD trajectory for each of the simulated TS1 structures. The time step used for the MD simulations was 2 fs. Periodic boundary conditions in the NPT ensemble at T = 298.15 K with Berendsen temperature coupling (41) and P = 1 atm with isotropic molecule-based scaling (41) were applied. The SHAKE algorithm (42) was used to fix all covalent bonds containing hydrogen atoms. The nonbonded pair list was updated every 10 steps. The PME (particle mesh Ewald) method (43) was used to treat long-range electrostatic interactions. A residue-based cutoff of 10 Å was used for the noncovalent interactions. The coordinates of the simulated systems were collected every 1 ps during the production MD stages.

The above-described MD procedure was performed first for the TS1 structures of the WT, A328W/Y332A, and A328W/Y332G BChEs. Starting from the simulated TS1 structure for the A328W/Y332G mutant, we hoped to identify a mutant (with additional mutations) that possibly has a more stable TS1 structure. For this
purpose, we particularly focused on the possible enhancement of the hydrogen bonding between the carbonyl oxygen of (−)-cocaine and the oxyanion hole of the enzyme, which made it necessary to examine the possible mutations on the amino acid residues within and nearby the oxyanion hole of the enzyme. The initial candidate mutants were chosen by simple geometric consideration of the possible modification of the TS1 structure: only an energy minimization was carried out in the simple geometric consideration of each possible mutant. Then, the MD simulations were performed only for the candidate mutants whose energy-minimized TS1 structures clearly suggested possibly stronger hydrogen bonding between the carbonyl oxygen of (−)-cocaine and the oxyanion hole of the enzyme. Only the most promising mutants identified by the MD simulations were tested by wet experimental studies (see below for the experimental procedure).

Most of the MD simulations were performed in parallel on a Hewlett-Packard supercomputer (a Superdome with 256 shared-memory processors) at the Center for Computational Sciences (University of Kentucky). Some of the computations were carried out on a 34-processor x335 Linux cluster (IBM, White Plains, NY) and SGI Fuel workstations (Silicon Graphics, Mountain View, CA) in our own laboratory.

**Experimental Materials.** Cloned pfu DNA polymerase and DpnI endonuclease were obtained from Stratagene. [3H]- (−)-cocaine [50 Ci/mmoll (1 Ci = 37 GBq)] was purchased from PerkinElmer. The expression plasmid pRC/CMV was a gift from O. Lockridge (University of Nebraska Medical Center, Omaha). All oligonucleotides were synthesized by Integrated DNA Technologies and the University of alville, IA). The QIAprep Spin Plasmid Miniprep Kit, plasmid purification kit, and QIAquick PCR purification kit were obtained from Qiagen (Valencia, CA). Human embryonic kidney 293T cells were from American Type Culture Collection. DMEM was from Sigma. Anti-BChe (mouse monoclonal antibody, product no. HAH002-01) was purchased from AntibodyShop (Gen-tact, Denmark), and goat anti-mouse IgG horseradish peroxidase conjugate complex diluted to a final 1:3,000 dilution and were incubated at room temperature for 1.5 h, followed by washing four times. The enzyme reactions were started by addition of 100 µl of substrate (3,5,5′-tetramethylbenzidine) solution (51). The reactions were stopped after 15 min by the addition of 100 µl of 2 M sulfuric acid, and the absorbance was read at 460 nm by using a Bio-Rad ELISA plate reader.

**Results and Discussion**

**Hydrogen Bonding.** In the design of a high-activity mutant of BChE against (−)-cocaine, we aimed to predict some possible mutations that can lower the energy of TS1 and, therefore, lower the energy barrier for this critical reaction step. Apparently, a mutant associated with the stronger hydrogen bonding between the carbonyl oxygen of (−)-cocaine benzoyl ester and the oxyanion hole of the BChE mutant in the TS1 structure may potentially have a more stable TS1 structure and, therefore, a higher catalytic activity for (−)-cocaine hydrolysis. Hence, the hydrogen bonding with the oxyanion hole in the TS1 structure is a crucial factor affecting the transition-state stabilization and the catalytic activity. The possible effects of some mutations on the hydrogen bonding were examined by performing MD simulations on the TS1 structures for (−)-cocaine hydrolysis catalyzed by WT BChE and its various mutants.

The MD simulation in water was performed for 1 ns or longer to make sure we obtained a stable MD trajectory for each simulated TS1 structure with WT or mutant BChE. The MD trajectories actually became stable quickly, as did the H–O distances involved in the potential hydrogen bonds between the carbonyl oxygen of (−)-cocaine and the oxyanion hole of BChE. Depicted in Fig. 1 are plots of four important H–O distances in the MD-simulated TS1 structure versus the simulation time for (−)-cocaine hydrolysis catalyzed by A199S/S287G/A328W/Y332G BChE, along with the rms deviation of the simulated positions of backbone atoms from those in the corresponding initial structure. The H–O distances in the simulated TS1 structures for WT BChE and its three mutants are summarized in Table 1.

As seen in Table 1, the simulated H–O distance D1 is always too
long for the peptidic NH of G116 to form a N–H–O hydrogen bond with the carbonyl oxygen of (−)-cocaine in all of the simulated TS1 structures. In the simulated TS1 structure for WT BChE, the carbonyl oxygen of (−)-cocaine formed a firm N–H–O hydrogen bond with the peptidic NH hydrogen atom of the A199 residue; the simulated H–O distance (D3) was 1.61–2.35 Å, with an average D3 value of 1.92 Å. Meanwhile, the carbonyl oxygen of (−)-cocaine also had a partial N–H–O hydrogen bond with the peptidic NH hydrogen atom of the G117 residue; the simulated H–O distance (D2) was 1.97–4.14 Å (the average D2 value was 2.91 Å). The average D2 and D3 values became 2.35 and 1.95 Å, respectively, in the simulated TS1 structure for the A328W/Y332A mutant. These distances suggest a slightly weaker N–H–O hydrogen bond with A199 but a stronger N–H–O hydrogen bond with G117 in the simulated TS1 structure for the A328W/Y332A mutant than the corresponding N–H–O hydrogen bonds for the WT. The average D2 and D3 values (2.25 and 1.97 Å, respectively) in the simulated TS1 structure for the A328W/Y332G mutant are close to the corresponding distances for the A328W/Y332A mutant. The overall strength of the hydrogen bonding between the carbonyl oxygen of (−)-cocaine and the oxyanion hole of the enzyme is not expected to change considerably when WT BChE is replaced by the A328W/Y332A or A328W/Y332G mutant.

However, the story for the simulated TS1 structure for the A199S/Y332G/A328W/Y332G mutant was remarkably different. As one can see from Table 1 and Figs. 1 and 2, when residue 199 becomes a serine (i.e., S199), the hydroxyl group on the side chain of S199 can also hydrogen-bond to the carbonyl oxygen of (−)-cocaine to form an O–H–O hydrogen bond, in addition to the two N–H–O hydrogen bonds with the peptidic NH of G117 and S199. The simulated average H–O distances with the peptidic NH hydrogen of G117, the peptidic NH hydrogen of S199, and the hydroxyl hydrogen of S199 are 2.60, 2.01, and 1.76 Å, respectively. Because of the additional O–H–O hydrogen bond, the overall strength of the hydrogen bonding with the modified oxyanion hole of A199S/Y332G/A328W/Y332G BChE should be significantly stronger than that of WT, A328W/Y332A, and A328W/Y332G BChEs.

To better represent the overall strength of hydrogen bonding between the carbonyl oxygen of (−)-cocaine and the oxyanion hole in a MD-simulated TS1 structure, we estimated the hydrogen-bonding energy (HBE) associated with each simulated H–O distance by using the empirical HBE equation implemented in the AUTODOCK 3.0 program suite (52). Based on the general HBE equation, we have $HBE(r) = 0.5 \sigma_0^2 / r^2 - 6 \sigma_0^2 / r^6$, in which $r$ is the H–O distance in the considered hydrogen bond and $\sigma_0$ is the minimum value of the H–O distance for which the HBE equation can be used (33). We used $\sigma_0 = 1.52$ Å because it is the shortest H–O distance found in all of our MD simulations. The $\sigma$ value was determined by using the condition that $HBE(r) = -5.0$ kcal/mol when $r = 1.90$ Å. Specifically, for each hydrogen bond with the

### Table 1. Summary of the MD-simulated key distance (in Å) and the calculated total HBEs (in kcal/mol) between the oxyanion hole and the carbonyl oxygen of (−)-cocaine benzyol ester in TS1

<table>
<thead>
<tr>
<th>Transition state</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>Total HBE†</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS1 structure for (−)-cocaine hydrolysis catalyzed by WT BChE</td>
<td>4.59</td>
<td>2.91</td>
<td>1.92</td>
<td>0.00</td>
<td>−5.5 (−4.6)</td>
</tr>
<tr>
<td>Average Maximum Minimum Fluctuation</td>
<td>5.73</td>
<td>4.14</td>
<td>2.35</td>
<td>0.00</td>
<td>—</td>
</tr>
<tr>
<td>TS1 structure for (−)-cocaine hydrolysis catalyzed by A328W/Y332A mutant of BChE</td>
<td>3.62</td>
<td>2.35</td>
<td>1.95</td>
<td>0.00</td>
<td>−6.2 (−4.9)</td>
</tr>
<tr>
<td>Average Maximum Minimum Fluctuation</td>
<td>4.35</td>
<td>3.37</td>
<td>3.02</td>
<td>0.00</td>
<td>—</td>
</tr>
<tr>
<td>TS1 structure for (−)-cocaine hydrolysis catalyzed by A328W/Y332G mutant of BChE</td>
<td>2.92</td>
<td>1.78</td>
<td>1.61</td>
<td>0.00</td>
<td>—</td>
</tr>
<tr>
<td>Average Maximum Minimum Fluctuation</td>
<td>2.92</td>
<td>1.78</td>
<td>1.61</td>
<td>0.00</td>
<td>—</td>
</tr>
<tr>
<td>TS1 structure for (−)-cocaine hydrolysis catalyzed by A199S/Y332G/A328W/Y332G mutant of BChE</td>
<td>4.39</td>
<td>2.60</td>
<td>2.01</td>
<td>1.76</td>
<td>−14.0 (−12.0)</td>
</tr>
<tr>
<td>Average Maximum Minimum Fluctuation</td>
<td>5.72</td>
<td>4.42</td>
<td>2.68</td>
<td>2.50</td>
<td>—</td>
</tr>
<tr>
<td>TS1 structure for (−)-cocaine hydrolysis catalyzed by A199S/Y332G/A328W/Y332G mutant of BChE</td>
<td>2.89</td>
<td>1.77</td>
<td>1.62</td>
<td>1.48</td>
<td>—</td>
</tr>
<tr>
<td>Average Maximum Minimum Fluctuation</td>
<td>2.89</td>
<td>1.77</td>
<td>1.62</td>
<td>1.48</td>
<td>—</td>
</tr>
</tbody>
</table>

†D1, D2, and D3 represent the internuclear distances between the carbonyl oxygen of cocaine benzoyl ester and the NH hydrogen of residues 116 (i.e., G116), 117 (i.e., G117), and 199 (i.e., A199 or S199) of BChE, respectively. D4 is the internuclear distance between the carbonyl oxygen of cocaine benzyol ester and the hydroxyl hydrogen of the S199 side chain in the A199S/Y332G/A328W/Y332G BChE. RMSD is the rms deviation (in Å) of the simulated positions of the protein backbone atoms from those in the initial structure.
carbonyl oxygen of (−)-cocaine, a HBE value can be evaluated with each snapshot of the MD-simulated structure. The final HBE of the MD-simulated hydrogen bond is considered to be the average HBE value of all snapshots taken from the stable MD trajectory. The estimated total HBE value for the hydrogen bonds between the carbonyl oxygen of (−)-cocaine and the oxygen hole in each simulated TS1 structure is also listed in Table 1.

We also estimated the HBE for each hydrogen bond by using the MD-simulated average H–O distance. As seen in Table 1, the total HBEs (i.e., −4.6, −4.9, −5.0, and −12.0 kcal/mol for the WT, A328W/Y332A, A328W/Y332G, and A199S/S287G, respectively) estimated in this way are systematically higher (i.e., less negative) than the corresponding total HBEs (i.e., −5.5, −6.2, −6.4, and −14.0 kcal/mol) estimated in the aforementioned way. However, the two sets of total HBE values are qualitatively consistent with each other in terms of the relative hydrogen-bonding strengths in the three simulated TS1 structures. In particular, the two sets of total HBE values consistently reveal that the overall strength of the hydrogen bonding between the carbonyl oxygen of (−)-cocaine and the oxygen hole in the estimated TS1 structure for A199S/S287G/A328W/Y332G BChEs is significantly higher than that for WT, A328W/Y332A, and A328W/Y332G BChEs.

### Catalytic Activity

The computational results discussed above suggest that the TS1 of (−)-cocaine hydrolysis catalyzed by the A199S/S287G/A328W/Y332G mutant should be significantly more stable than that by the A328W/Y332A or A328W/Y332G mutant because of the significant increase of the overall hydrogen bonding between the carbonyl oxygen of (−)-cocaine and the oxygen hole of the enzyme in the TS1 structure. The aforementioned analysis of the literature (24–26, 28) also indicates that the first chemical reaction step associated with TS1 should be the rate-determining step of (−)-cocaine hydrolysis catalyzed by BChE mutants including the Y332A or Y332G mutation, although the formation of the ES is the rate-determining step for (−)-cocaine hydrolysis catalyzed by WT BChE. This mechanistic insight suggests a clear correlation between the TS1 stabilization and the catalytic activity of A328W/Y332A, A328W/Y332G, and A199S/S287G/A328W/Y332G BChEs for (−)-cocaine hydrolysis: the more stable the TS1 structure, the lower the energy barrier and the higher the catalytic activity. Thus, the MD simulations predict that A199S/S287G/A328W/Y332G BChE should have a higher catalytic activity than A328W/Y332A or A328W/Y332G BChE for (−)-cocaine hydrolysis.

The catalytic efficiency (k_{cat}/K_m) of A328W/Y332A BChE for (−)-cocaine hydrolysis was reported to be ~8.6 × 10^4 M min⁻¹ (25), which is ~9.4 times the k_{cat}/K_m value (~9.1 × 10^4 M min⁻¹) of WT BChE for (−)-cocaine hydrolysis. The catalytic efficiency of A328W/Y332G BChE was found to be slightly higher than that of A328W/Y332A BChE for (−)-cocaine hydrolysis (27). To examine our theoretical prediction of the higher catalytic activity for A199S/S287G/A328W/Y332G BChE, we produced the A328W/Y332A and A199S/S287G/A328W/Y332G mutants of BChE through site-directed mutagenesis. To minimize the possible systematic experimental errors of the kinetic data, we performed kinetic studies with the two mutants and WT BChE under the same conditions and compared the catalytic efficiency of A328W/Y332A and A199S/S287G/A328W/Y332G BChEs to that of the WT for (−)-cocaine hydrolysis at the benzoyl ester group. Based on the kinetic analysis of the measured time-dependent radiometric data and the ELISA data, the ratio of the k_{cat}/K_m value of A328W/Y332A BChE to the k_{cat}/K_m value of WT BChE for (−)-cocaine hydrolysis was determined to be ~8.6. The determined catalytic efficiency ratio of ~8.6 is in good agreement with the ratio of ~9.4 determined by Sun et al. (25). Further, by using the same experimental protocol, the ratio of the k_{cat}/K_m value of A199S/S287G/A328W/Y332G BChE to the k_{cat}/K_m value of A328W/Y332A BChE for (−)-cocaine hydrolysis was determined to be ~50.6. These data indicate that the ratio of the k_{cat}/K_m value of A199S/S287G/A328W/Y332G BChE to the k_{cat}/K_m value of WT BChE for (−)-cocaine hydrolysis should be ~50.6 × 8.6 = ~435 or ~50.6 × 9.4 = ~476. Thus, we can conclude that A199S/S287G/A328W/Y332G BChE has an ~456 ± 41-fold improved catalytic efficiency against (−)-cocaine compared with the WT or that A199S/S287G/A328W/Y332G BChE has a k_{cat}/K_m value of (4.15 ± 0.37) × 10^4 M min⁻¹ for (−)-cocaine hydrolysis. The catalytic efficiency of A199S/S287G/A328W/Y332G BChE against (−)-cocaine is significantly higher than that of AME-359 (i.e., F227A/S287G/A328W/Y332G BChE, for which k_{cat}/K_m = 3.1 × 10^4 M min⁻¹ and whose catalytic efficiency against (−)-cocaine is the highest of all of the previously reported BChE mutants) (28), which has an ~34-fold improved catalytic efficiency against (−)-cocaine compared with WT BChE.

By using the designed A199S/S287G/A328W/Y332G BChE as an exogenous enzyme in humans, when the concentration of this mutant is kept the same as that of the WT BChE in plasma, the half-life of (−)-cocaine in plasma should be reduced from ~45–90 min to only ~6–12 s, considerably shorter than the time required for cocaine crossing the blood–brain barrier to reach the CNS. Hence, the outcome of this study could eventually result in a valuable, efficient anticocaine medication.

### Conclusion

The transition-state simulations demonstrate that the overall hydrogen bonding between the carbonyl oxygen of (−)-cocaine benzoyl ester and the oxygen hole of BChE in the TS1 structure for (−)-cocaine hydrolysis catalyzed by A199S/S287G/A328W/Y332G BChE should be significantly stronger than that in the TS1 structure for (−)-cocaine hydrolysis catalyzed by the WT BChE and the other simulated BChE mutants. Thus, the MD simulations predict that A199S/S287G/A328W/Y332G BChE should have a significantly lower energy barrier for the chemical reaction process and, therefore, a significantly higher catalytic efficiency (k_{cat}/K_m) for (−)-cocaine hydrolysis. The theoretical prediction has been confirmed by wet experimental tests that show an ~456 ± 41-fold improved catalytic efficiency for A199S/S287G/A328W/Y332G BChE against (−)-cocaine compared with the WT BChE. The k_{cat}/K_m value determined for A199S/S287G/A328W/Y332G BChE is much higher than the k_{cat}/K_m value for AME-359 (i.e., F227A/S287G/A328W/Y332G BChE, whose catalytic efficiency against (−)-cocaine is the highest of all of the previously reported BChE mutants) (28), which has an ~34-fold improved catalytic efficiency against (−)-cocaine compared with the WT BChE. The encouraging outcome of this study suggests that the transition-state simulation is a promising, unique approach for rational enzyme redesign and drug discovery.

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