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Active Site Gating and Substrate Specificity of Butyrylcholinesterase and Acetylcholinesterase: Insights from Molecular Dynamics Simulations

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Abstract

Butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) are highly homologous proteins with distinct substrate preferences. In this study we compared the active sites of monomers and tetramers of *human* BChE and *human* AChE after performing molecular dynamics (MD) simulations in water-solvated systems. By comparing the conformational dynamics of gating residues of AChE and BChE, we found that the gating mechanisms of the main door of AChE and BChE are responsible for their different substrate specificities. Our simulation of the tetramers of AChE and BChE indicates that both enzymes could have two dysfunctional active sites due to their restricted accessibility to substrates. The further study on catalytic mechanisms of multiple forms of AChE and BChE would benefit from our comparison of the active sites of the monomers and tetramers of both enzymes.

Introduction

Butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) are the two subfamilies of cholinesterases distinguished by their substrate preferences.¹ AChE is considered to be specific to its natural substrate acetylcholine (ACh).² By rapidly catalyzing the hydrolysis of ACh, this enzyme is responsible for the termination of cholinergic neurotransmission at neuronal and neuromuscular synapses.^{3–5} AChE was found to exist in multiple forms.^{6–7} So far, X-ray crystal structures of the catalytic domains of different species have been identified. These include *human* AChE (*hAChE*),⁸ *mouse* AChE (*mAChE*),⁹ and *Torpedo California* AChE (*TcAChE*).¹⁰ Previous studies have shown that the catalytic domains of AChE in various species overlap with one another well.⁸ Identical dimers have been observed among different species of AChE with two opposite-positioned subunits associating with each other to form a four helix bundle,^{8,11} causing the active sites of the two subunits to face in opposite directions. Recently, the model of an AChE tetramer complexed with collagen-tail protein (ColQ) was built by docking of the tetramerization domain of an AChE tetramer on the catalytic domains of the *Electrophorus electricus* AChE (*EeAChE*) tetramer.⁷ Kinetic data have shown that AChE is one of the most efficient enzymes, with its catalytic efficiency almost reaching the diffusion limit.⁴ However, crystal structures of AChE indicate that the active site of this enzyme is buried at the bottom of a deep and narrow gorge lined with a few aromatic residues.^{8–10} Many studies^{4,12–22} have

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Supporting Information Available. Two figures (S1 and S2) showing the overall and detailed view of different entrances to the active site of AChE and BChE. This material is available free of charge *via* the Internet at <http://pubs.acs.org>.

been reported to search for the cause for the high catalytic efficiency. The most likely cause is that there may be some alternative doors in addition to the main door that can lead to the active site. These alternative doors can be channels for small molecules such as water and ions, so that the entering of the reactants and the leaving of products can be much smoother. So far, three alternative doors have been proposed, including the back door, the side door, and the acyl loop door. The back door was first proposed by Sussman *et al.* in 1993¹³ and supported by a short MD simulation (119 ps) done by McCammon *et al.* in 1994.¹⁴ Later, it was also supported by longer MD simulations.^{16–19} This back door is basically formed by four residues, including Trp 86, Gly 448, Tyr 449 and Ile 451 (*hAChE* residue numbering). The gating mechanism is described as the temporary side chain (indole ring) movement of Trp 86. Later in 1997, a side door was found by McCammon *et al.* using the same method.^{4,12} This side door is mainly formed by six residues, including Asp 74, Thr 75, Leu 76, Thr 83, Glu 84, and Asn 87. The gating mechanism of this door was also mainly due to the fluctuations of the side chains of these residues. Based on previous studies, this side door was only found in the AChE complex, either with small molecule or with protein. As explained in one of the studies done by McCammon *et al.*,¹⁶ the substrate here may help to induce the structure change of the residues that form the side door. In 1998, another alternative door was proposed by Brooks *et al.*²¹ The method they used is different from the method used by McCammon *et al.*¹⁴ In the paper, they studied two MD trajectories by using the umbrella sampling method.²³ They found this door formed by residues Arg 247, Phe 297, and Trp 236 could be a better exit for the acetic acid and acetate ion releasing from AChE. No further information (such as the size and open frequency of this door) has been released on this door in AChE. But a study on BChE in 2005 found evidence for the existence of an analogous door²⁰ and investigated the size of the door, although the size of the door is very small, with the average radius of 0.8 ± 0.2 Å during the whole simulation time.

Compared with AChE, the natural function of BChE is unclear;²⁴ however, it has been shown that this enzyme is the principal cocaine hydrolase in human plasma.^{25–26} Thus, this enzyme could be a promising option to solve the problems of cocaine addiction and cocaine abuse. Unfortunately, the catalytic efficiency of wild-type BChE is very low ($k_{\text{cat}} = 4.1 \text{ min}^{-1}$, $K_M = 4.5 \text{ } \mu\text{M}$).²⁷ In order to improve the catalytic efficiency of BChE, various mutants were designed by our group based on our understanding of the mechanism of cocaine hydrolysis using the integrated computational-experimental approach.^{26,28–30} One of the mutants (A199S/F227A/S287G/A328W/Y332G BChE) exhibits a ~2000-fold higher catalytic efficiency compared with the wild-type BChE.³⁰ Therefore, high-activity mutants of this enzyme have become promising drug candidates for cocaine abuse, considering their powerful effects of protecting rodents from cocaine toxicity. The first crystal structure of the catalytic domain of *human* BChE (*hBChE*) monomer was identified by Nachon *et al.*^{1,31} Studies have shown that the predominant form of native BChE in plasma is the tetramer.^{32–33} A model of *hBChE* tetramer complexed with a proline-rich peptide was built by our group recently, based on homology modeling of the AChE tetramer followed by MD simulation of the explicit water solvated system.³⁴ In comparison with the high selectivity of AChE, BChE was once called “nonspecific cholinesterase” due to its wide range of substrates, including acetylcholine, butyrylcholine, succinylcholine, organophosphates, and cocaine.² In this study, we compared the main door and alternative doors of the monomers (AChE and BChE) as well as the main door of the tetramers (AChE and BChE) by using MD simulations. We found that the gating residues of BChE and AChE are responsible for their distinct substrate specificities. Our simulations of the tetramers of AChE and BChE indicate that although there are some structural differences, the two active sites of both enzymes facing to the dimer-dimer interface may not function due to their restricted accessibility to substrates.

Methods

Molecular dynamics simulations of AChE and BChE monomers and tetramers

The X-ray crystal structures of the *h*AChE and *h*BChE monomers were from the Protein Data Bank (PDB ID codes: 1B41⁸ and 1P0M,¹ respectively). The structure of ColQ of [AChE]₄-ColQ (PDB ID code: 1VZJ) was truncated to the proline-rich attachment domain (PRAD) consisting of the N-terminal 17 residues to improve the simulation efficiency. Thus, the AChE tetramer (PDB ID code: 1C2O) complex with PRAD sequence is denoted as [AChE]₄-PRAD in this report. The initial structure of *h*BChE tetramer with proline-rich peptide CCLLMPPPPPLFPPPF (denoted as [BChE]₄-PRAD in this report) was built by our group based on the homology modeling of [AChE]₄-ColQ.³⁴

To carry out the MD simulations, the topologic and coordinate files of the initial structures were built with the tLEAP module of Amber 9.³⁵ The Amber force field (ff03) was used for the simulation.³⁶ Two monomers and two tetramers were simulated here. Each structure was neutralized by adding counterions and solvated in a rectangular box of TIP3P water molecules with a minimum solute-wall distance of 10 Å.³⁷ The energy minimization and MD simulation were performed using the SANDER module of Amber 9. First, the solvent molecules of each structure were energy-minimized for 5000 cycles and equilibrated for 10 ps of MD simulation with the enzyme fixed to make sure that they were in an equilibrated condition. Second, the backbone atoms of each enzyme were fixed for 1000 cycles before 1000 cycles of fully relaxed energy minimization. The system was then gradually heated from 10 K to 298.15 K for 40 ps, followed by the MD simulation at 298.15 K for 10 ns. The time step used in the MD simulation was 2 fs. Periodic boundary condition was used in the NPT ensemble with Berendsen temperature coupling and $P = 1$ atm using isotropic molecular-based scaling.³⁸ The SHAKE algorithm³⁹ was used to fix all covalent bonds containing hydrogen atoms. The particle mesh Ewald method⁴⁰ was used to treat long-range electrostatic interactions. The non-bonded cutoff of 10 Å was used.

Method used to evaluate the radii of different doors of AChE and BChE

To measure the radii of different doors of AChE and BChE, the MSMS program⁴¹ was employed here similar to how the method has been used elsewhere. Essentially, the MSMS program will generate a continuous Connolly surface⁴² of the protein by using a probe as shown in Figure 1. If the radius of the door is bigger than the radius of the current probe, then this probe can enter the door and generate the surface of the gorge which will also be part of the continuous Connolly surface of the protein, as in Figure 1(A); otherwise, the probe cannot enter the gorge and no Connolly surface will be generated, as in Figure 1(B). Thus, we can use probes with different radii to detect the radii of different doors including the main doors and the alternative ones. To precisely measure the radius, a series of artificial dummy atoms were used to block the doors other than the one currently being measured. Here we chose several residues as the probing targets, including Gly 122, Glu 202, and Ser 203 (*h*AChE residue numbering). If any of these residues appear in the Connolly surface, that suggests they have a direct contact with the probe; otherwise their Connolly surface area value would be zero.

Results and Discussion

Stability of trajectories

To explore the dynamic stability of the models and to ensure the rationality of the sampling strategy, root mean square deviation (RMSD) values of the protein backbone based on the starting structure along the simulation time were calculated here and plotted in Figure 2 (monomers) and Figure 3 (tetramers).

From Figures 2 and 3, we can see that all the trajectories start with the relatively stable production phase after 2 ns of MD simulation. The RMSD values are all between the ranges of 1.0 Å to 2.5 Å during the entire simulation time. Based on the RMSD values of these trajectories, snapshots after the 2-ns MD simulation can be extracted for later analysis. Considering the large size of AChE tetramer and BChE tetramer, only the catalytic domain of each subunit (without tetramerization domain) was analyzed here. The stable MD trajectories depicted in Figure 3 suggest that the tetramer structures of the enzymes were stabilized during the simulations.

Active site doors of AChE and BChE monomers

We tried to analyze the structures extracted from the MD simulation to investigate the different doors, especially the main door, of the monomers and tetramers. According to our results, there are some similarities and some differences compared with previous studies. In our work here, we find the existence of all of the alternative doors, although the size of the acyl loop back door is relatively smaller. In previous work, two species of AChE (*TcAChE* and *mAChE*) have been studied.^{4,12,14–19} The *hAChE* has not previously been examined. Regarding BChE, *hBChE* is the only species that has been studied.²⁰ But considering that many X-ray crystal structures have been determined and different initial structures can cause different results, here we want to study another X-ray crystal structure - 1POM, which is used by our group for molecular mechanics (MM) and quantum mechanics/molecular mechanics (QM/MM) calculations. For the monomers, we try to calculate all the possible doors; for the tetramers, we focus on the main doors.

Figure 4 graphs the main entrance radius values of AChE monomer and BChE monomer during the MD simulations. The average radius of the main door of AChE monomer is 1.26 Å with the maximum radius of 2.30 Å as shown in Table 1. If we only account for the last 4 ns, the average value would be 1.51 Å. The entrance radius seldom reaches the maximum value. This result suggests that during this 10-ns MD simulation, the main door of AChE will not open spontaneously to allow even small substrates, such as ACh, to enter the active site. This result is similar to that reported by McCammon *et al.* in which the average radius was 1.52 Å with the maximum radius of 2.40 Å.¹⁷ As they concluded, this maximum radius may not be big enough for even small substrates to enter the active site easily, whereas it is also not likely to hinder its binding (within the active site) significantly. It has the “dynamic selectivity” that can prevent the main door from binding much bigger substrates such as cocaine.¹⁸ Figure 4(B) shows the main entrance radius values of the BChE monomer. The range of the radius is from 1.20 Å to 4.00 Å, which is larger than that of the AChE. The average entrance radius of the main door is 2.75 Å with the maximum radius of 4.00 Å. Compared with the “dynamic selectivity” of AChE, BChE can bind not only small substrates such as ACh, but also large substrates such as cocaine. These data are also close to the results calculated by Suárez *et al.* (average radius of 3.1 Å and maximum radius of 3.8 Å).²⁰

The difference between the two enzymes can be explained by the different structures around the main entrance area. In AChE, there are eight aromatic residues associated with the main door. Three of them are located on the entrance of the active site (Tyr 72, Trp 286, and Tyr 341); five of them are located almost the same depth inside the gorge (Tyr 124, Phe 295, Phe 297, Tyr 337, and Phe 338), as show in Figure 5(A). In BChE, however, six of these residues are changed to smaller ones, including Tyr 72 to Asn 68, Tyr 124 to Gln 119, Trp 286 to Ala 277, Phe 295 to Leu 286, Phe 297 to Val 288, and Tyr 337 to Ala 328, with both sets of residues shown in Figure 5(B) and (C). These smaller residues will significantly enlarge the entrance radius, which makes it possible for substrates as large as cocaine to enter the active site.

For the monomers, we also analyzed the alternative doors suggested in previous studies.^{15–17,19} In this study, we found the existence of all these alternative doors, as shown in Figures S1 and S2 in the supporting information. The average radii of these three doors in AChE are all below 1.20 Å, but with different maximum radii and different fractions of opening snapshots (values larger than 1.40 Å will be considered as open). A small fraction (139 out of 8000) of the sampling snapshots showed the opening of the back door. This finding is consistent with a previous study in which 78 out of 10000 snapshots showed the back-door opening event.¹⁷ The flip of the indole ring of Trp 82 is believed to be responsible for this opening. In the current study, the side door has a longer opening time; 3463 out of 8000 sampling snapshots showed the open state. The fluctuations of the side chains of residues that form the side door are responsible for the opening.

After the acyl loop back door was first suggested in 1998 by Brooks *et al.*,²¹ no further studies have been reported to examine this door in AChE. Thus, we also tried to analyze this door in the present study. According to our analysis, 77 out of the 8000 snapshots show the opening of acyl loop back door with the maximum value of 1.50 Å. This is the first time that the existence and the size of the acyl loop back door of AChE were observed and detected by performing MD simulation and the Connolly Surface analysis, although the observed opening snapshots are few and the size of the door is small.

In BChE, the characteristics of these three alternative doors have some similarities but also some differences compared with those of AChE. The calculated average radius of the back door is 1.63 Å with the maximum radius of 2.30 Å. The average radius of the side door is 1.69 Å with the maximum radius of 2.30 Å. Our calculated values of the radii are systematically larger than the corresponding values calculated previously by Suárez *et al.*,²⁰ who reported an average radius of 0.8 Å with a maximum radius of 1.2 Å for the back door and an average radius of 1.1 Å with a maximum radius of 2.2 Å for the side door. One of the possible reasons might be associated with the different versions of the Amber force field. The ff03 was used in our MD simulation with Amber 9 program, whereas the specific Amber force field version used for their MD simulation²⁰ with Amber 7 was not specified.

We also observed the existence of the acyl loop back door in BChE. As in AChE, the calculated average radius for the acyl loop back door is smaller than 1.20 Å. The snapshots with the door opening (defined as the radius being larger than 1.4 Å) are even fewer in BChE. 77 snapshots (out of 8000) show the opening event in AChE, whereas only 9 snapshots (out of 8000) showed the opening event in BChE. In the MD simulation reported previously by Suárez *et al.*,²⁰ no opening event (defined as the radius being larger than 1.4 Å) was observed. All of the computational results suggest that the acyl loop back door in BChE may be insignificant for its catalytic function. Overall, for both AChE and BChE, the acyl loop back door is not as important as the other doors.

To summarize the results calculated for the monomers, there are some similarities but also some differences between the AChE and BChE monomers. BChE has a larger main entrance than AChE, which seems reasonable as BChE can bind substrates as large as cocaine. Two alternative doors (side door and back door in BChE) are larger in size than in AChE. The acyl loop back doors in both enzymes are too small to be significant channels leading to the active sites. Based on these results, we believe that the back door and the side door are more likely and more important alternative doors than the acyl loop back door, as the former ones have larger radii and can exist for longer time. As discussed in a previous study,¹⁵ there is still no direct experimental evidence to prove the function of these alternative doors. However, multiple simulations done by different groups using different forms of enzymes and different force fields have all predicted the existence of these doors^{15–17,19–20} (see Figures S1 and S2 in supporting information for these alternative doors).

Active site doors of AChE and BChE tetramers

Since AChE can exist in the forms of dimer and tetramer and the dominant form of native hBChE in plasma is the tetramer, MD simulations were performed to simulate both [AChE]₄-PRAD and [BChE]₄-PRAD. For the tetramer systems, we mainly focus on the main doors. The overall structures of [AChE]₄-PRAD and [BChE]₄-PRAD are similar, as shown in Figure 6. The parts inside the red squares as labeled in Figure 6(A) and (B) indicate the tetramerization domains, while the others are the catalytic domains of the two enzymes. The four subunits of each enzyme are labeled in four different colors. The tetramers are dimers of the dimers with each dimer oriented vertically. As mentioned in a previous paper,³⁴ the two dimers are oriented in an anti-parallel pattern, so that the subunits of the tetramers are diagonally equivalent. As shown in Figure 6(A) and (B), the two active sites labeled with triangles are fully exposed to the solvent; the other two active sites labeled with circles face to the interface between two dimers (labeled with rectangles).

We tried to examine how many *functional* active sites there are. The data summarized in Table 2 indicate that for the AChE tetramer, the average radii of the main door of each subunit are 1.67 Å, 0.85 Å, 1.65 Å, and 0.80 Å, with the maximum radii of 2.40 Å, 1.30 Å, 2.30 Å, and 1.20 Å, respectively. In the BChE tetramer, these values are 2.07 Å, 1.24 Å, 2.30 Å, and 1.63 Å, with the maximum radii of 3.70 Å, 2.60 Å, 3.40 Å, and 2.80 Å, respectively. In both enzymes, two active sites facing the solvent have a better chance of binding the substrates compared with the two active sites facing to the dimer-dimer interface, which can be seen in Figures 6 and 7. Figure 7(A) shows that if the main door of these enzymes (subunits A and C) that facing to the solvent is opened wide enough, then substrates such as ACh could enter it by following the direction indicated by the white arrow. However, for the subunits B and D (the left part, labeled in multiple colors in Figure 7(B) to (D)) of these two tetramers, the probability of finding a path that can lead to the active site in subunit B/D without being blocked by subunit A/C would be very low, if not impossible; we have accounted for the conformational flexibility of ACh in this analysis. Illustrated in Figure 7(B) to (D) are representative scenarios in which ACh entering the active site of subunit B/D (left part, labeled in multiple colors) is blocked by subunit A/C (the right part, labeled in green).

Further, in order to identify the specific residues in subunit A/C of AChE/BChE tetramer that block ACh entering the active site of the complementary subunit B/D, we carefully examined the distances between the key residues on the main door of subunit B/D and the nearby residues of the complementary subunit A/C in the simulated tetramer structures of AChE and BChE. For convenience of discussion, here we focus on the inter-residue distances between three representative residues on the entrance of the main door in subunit B and residues in complementary subunit A. The three representative residues of AChE are Tyr 72, Trp 286, and Tyr 341, whereas the corresponding residues of BChE are Asn 68, Ala 277, and Tyr 332. The inter-residue distances between subunits C and D are similar to the corresponding inter-residue distances between subunits A and B. For each snapshot of the MD-simulated tetramer structure, we evaluated the distance between the center of mass of each representative residue in subunit B and that of each residue in complementary subunit A. According to the calculated distances, for AChE, the residues in subunit A that are the closet to Tyr 72, Trp 286, and Tyr 341 in subunit B are Asp 320, Phe 321, Gln 322, and Gln 420. The corresponding residues in subunit A of BChE are Leu 309, Gly 310, Gln 311, and Trp 412. The calculated inter-residue distances involving these residues of BChE are depicted in Figure 8; the plots for the corresponding inter-residue distances in AChE (data not shown) are similar to the results obtained for BChE. The inter-residue distances calculated for the snapshots between 2000 and 10000 ps are used to determine the average inter-residue distance for each pair of residues. The determined average inter-residue distances are summarized in Table 3.

We also calculated the van der Waals (vdW) radius of each residue involved in the inter-residue distance calculations in each snapshot, and determined the average vdW radius for the snapshots between 2000 and 10000 ps. The determined average vdW radii are summarized in Table 4. Based on the calculated average inter-residue distances and average vdW radii, we also evaluated another type of distance (which may be called effective inter-residue distance), denoted by ΔR_{AB} :

$$\Delta R_{AB} = R_{AB} - R_A - R_B \quad (1)$$

in which R_{AB} refers to the average inter-residue distance between residues A and B, whereas R_A and R_B are the average vdW radii of residues A and B, respectively. The ΔR_{AB} values, that are also summarized in Table 3 (the values in parentheses), are a better indicator concerning how close the two residues are to each other.

As seen from the ΔR_{AB} values summarized in Table 3, the smallest ΔR_{AB} value is ~ 2.9 Å between Ala 277 in subunit B and Gln 311 in subunit A for the BChE tetramer (see Figure 8(E)). The other two residues (Leu 309 and Gly 310) in subunit A are also close to Ala 277 in subunit B. So, the entrance of the main door in subunit B of the BChE tetramer is blocked mainly by Leu 309, Gly 310, and Gln 311 residues in complementary subunit A, while Trp 412 has only a minor role in the blocking.

For the AChE tetramer, the smallest ΔR_{AB} value is ~ 2.2 Å between Trp 286 in subunit B and Asp 320 in subunit A. The other three residues (Phe 321, Gln 322, and Gln 420) in subunit A are also close to Trp 286 *etc.* in subunit B. So, the entrance of the main door in subunit B of the AChE tetramer is blocked mainly by Asp 320, Phe 321, Gln 322, and Gln 420 residues in complementary subunit A.

To summarize the results obtained from the comparison between the AChE and BChE tetramers, although there are some differences in the size and shape of the active site cavities of the two tetramers, they share some similarity. Two active sites that face the solvent can be the functional active sites, while the other two are not. Considering the door size and the restricted route a substrate could go through to get close to the active site gorge, the possibility of the substrates entering the two interface-facing active sites of both enzymes could be significantly decreased. This observation is also supported by previous experimental studies showing that a possible AChE tetramer with molecular weight of 250 kDa had two effective active sites per molecule.⁴³

Conclusion

The active sites and gating mechanisms of AChE and BChE are compared after we performed MD simulations on the water-solvated systems of the monomers and tetramers of hBChE and AChE. It has been demonstrated that the different gating mechanisms due to the conformational dynamics of gating residues of AChE and BChE are responsible for their different substrate specificities. Our simulations of the tetramers of AChE and BChE indicate that although there are some structural differences, both enzymes could have two dysfunctional active sites due to their special locations. This comparison of the active sites of the monomers and tetramers of AChE and BChE may benefit the future study of the catalysis mechanism of multiple forms of both enzymes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. The continuous Connolly surface of a protein under different probe radii. (A) Probe with smaller radius can enter the cave. (B) Probe with larger radius cannot enter the cave.

Figure 2.

Root-mean-square deviation (RMSD) of the backbone atoms (C, CA, and N) from the starting structure as a function of time, for MD simulations of the AChE (A, top) and the BChE (B, bottom).

Figure 3.

Root-mean-square deviation (RMSD) of the backbone atoms (C, CA, and N) from the starting structure as a function of time, for MD simulations of the AChE tetramer subunits (A, top) and the BChE tetramer subunits (B, bottom). Only catalytic domains were calculated here.

Figure 4.
The main door radii of the monomers: (A, top) AChE; (B, bottom) BChE

Figure 5.

(A, top) The eight residues with aromatic or indole side chains in the gorge of AChE. (B, middle) The eight residues in the gorge of BChE. (C, bottom) Superimposing of the gating residues in AChE and BChE.

Figure 6.

The overall view of AChE tetramer and BChE tetramer. Different subunits are shown clockwise in different colors: subunit A in orange, subunit B in cyan, subunit C in green, and subunit D in blue. Also, the active sites are shown in purple and the proline-rich peptide is shown in red. The red circles show the location of the active sites that are exposed to solvent in each monomer. The triangles show the location of the active sites that face to the interface between two dimers (shown by the rectangle). The squares in the middle show the location of tetramerization domain in each tetramer. Subunit A and subunit B forms one dimer, while subunit C and subunit D forms the other dimer.

Figure 7.

(A) Acetylcholine (ACh) can enter the active site of AChE/BChE monomer or AChE/BChE tetramer subunits A and C when the main door is open. The white arrow shows the entering direction. (B) to (D) The path to active site of AChE/BChE tetramer subunit B or D (left part, labeled in multiple colors) is blocked by subunit A or C (right part, labeled in green). The white crosses mean the clash between ACh and the subunits. Subunits A and D form one dimer, while subunits B and C form the other dimer, in the tetramer. The figure was drawn based on the simulated BChE tetramer structures.

Figure 8.

(A) – (D) Time-dependence of the key inter-residue distances between the center of mass of representative residues in subunit B and that of the main blocking residues in complementary subunit A in the MD-simulated BChE tetramer. (E) A snapshot of the MD-simulated BChE tetramer, showing the relative positions of the residues involved in the inter-residue distance calculations.

Table 1

The average and maximum radii of different doors in monomers.

	Door	Average Radius (Å)	Maximum Radius (Å)
AChE Monomer	main	1.26 ± 0.2	2.30
	back	<1.20	1.80
	side	<1.20	2.10
	acyl loop	<1.20	1.50
BChE Monomer	main	2.75 ± 0.3	4.00
	back	1.63 ± 0.2	2.30
	side	1.69 ± 0.2	2.30
	acyl loop	<1.20	1.70

Table 2

The average and maximum radius of main doors in tetramers.

	Door	Average Radius (Å)	Maximum Radius (Å)
AChE Tetramer	main (subunit A)	1.67 ± 0.3	2.40
	main (subunit B)	0.87 ± 0.1	1.30
	main (subunit C)	1.65 ± 0.2	2.30
	main (subunit D)	0.80 ± 0.1	<1.20
BChE Tetramer	main (subunit A)	2.07 ± 0.3	3.70
	main (subunit B)	1.24 ± 0.1	2.60
	main (subunit C)	2.30 ± 0.2	3.40
	main (subunit D)	1.63 ± 0.2	2.80

Table 3

The inter-residue distances (R_{AB} , in Å) between the center of mass of representative residues in subunit B and that of the main blocking residues in complementary subunit A of AChE and BChE tetramers.

Distance ^a in the AChE tetramer				Distance ^a in the BChE tetramer			
	Tyr72B	Trp286B	Tyr341B		Asn68B	Ala277B	Tyr332B
Asp320A	17.50 (7.02)	12.72 (2.24)	17.28 (6.78)	Leu309A	15.73 (6.42)	9.77 (4.97)	17.70 (7.11)
Phe321A	20.67 (8.98)	16.75 (5.06)	21.00 (9.29)	Gly310A	16.63 (8.14)	12.20 (4.19)	19.63 (9.86)
Gln322A	15.21 (4.21)	14.50 (3.50)	16.94 (5.92)	Gln311A	15.21 (5.69)	11.94 (2.90)	20.70 (9.90)
Gln420A	18.03 (7.30)	15.22 (4.49)	17.96 (7.21)	Trp412A	19.32 (8.91)	15.93 (6.00)	19.56 (7.87)

^aThe distance (R_{AB}) between the center of mass of a residue in subunit B and a residue in complementary subunit A. Given in the parentheses are the corresponding ΔR_{AB} values ($\Delta R_{AB} = R_{AB} - R_A - R_B$, where R_{AB} refers to the average inter-residue distance between residues A and B, and R_A and R_B are the average vdW radii of residues A and B, respectively). The R_A and R_B values used to determine the ΔR_{AB} values are listed in Table 4.

Table 4

The calculated average vdW radii ($\text{\AA}$) of the residues involved in the inter-residue distance calculations for residues in subunits A and B of the AChE and BChE tetramers.

AChE	vdW radius ^a	BChE	vdW radius ^a
Asp320A	4.54	Leu309A	4.80
Phe321A	5.75	Gly310A	3.98
Gln322A	5.06	Gln311A	5.01
Gln420A	4.79	Trp412A	5.90
Tyr72B	5.94	Asn68B	4.51
Trp286B	5.94	Ala277B	4.03
Tyr341B	5.96	Tyr332B	5.79

^aThe vdW radius of a residue is defined as the longest distance between the center of mass of the residue and the vdW surface of the residue. The vdW radius calculations were based on the MD trajectories between 2000 and 10000 ps.