

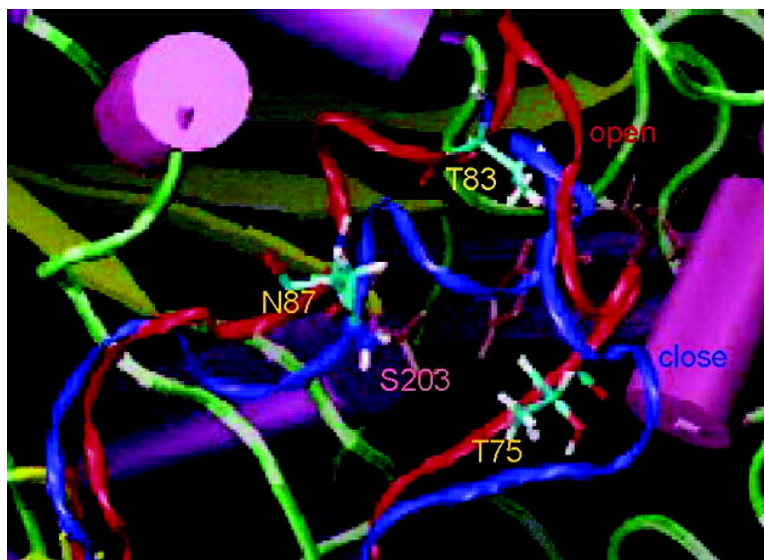
Article

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Acetylcholinesterase: Enhanced Fluctuations and Alternative Routes to the Active Site in the Complex with Fasciculin-2

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Abstract: A 15 ns molecular dynamics simulation is reported for the complex of mouse acetylcholinesterase (mAChE) and the protein neurotoxin fasciculin-2. As compared to a 15 ns simulation of apo-mAChE, the structural fluctuations of the enzyme are substantially increased in magnitude for the enzyme in the complex. Fluctuations of part of the long omega loop (residues 69–96) are particularly enhanced. This loop forms one wall of the active site, and the enhanced fluctuations lead to additional routes of access to the active site.

Introduction

Proteins have been characterized as “screaming and kicking” molecules.¹ A variety of protein motions have been revealed by fluorescence spectroscopy, nuclear magnetic resonance, hydrogen exchange, and Raman scattering.^{2–4} However, understanding the relation between the motions of a protein and its functions has been a challenge. Significant differences in the motions of mouse acetylcholinesterase (mAChE) in the presence and absence of its peptide inhibitor, fasciculin-2 (FAS), have been identified by molecular dynamics (MD) simulations,⁵ which reveal detailed atomic motions. We examine these differences here, with special reference to the structural fluctuations that may allow access to the active site of the enzyme.

Crystal structures^{6–8} have revealed that the surface area of van der Waals contacts at the FAS–mAChE interface extends over 1100 Å² and that no gap is apparent between FAS and the rim of the gorge for substrate entry into mAChE. However, kinetics experiments^{9–12} show some residual catalytic activity

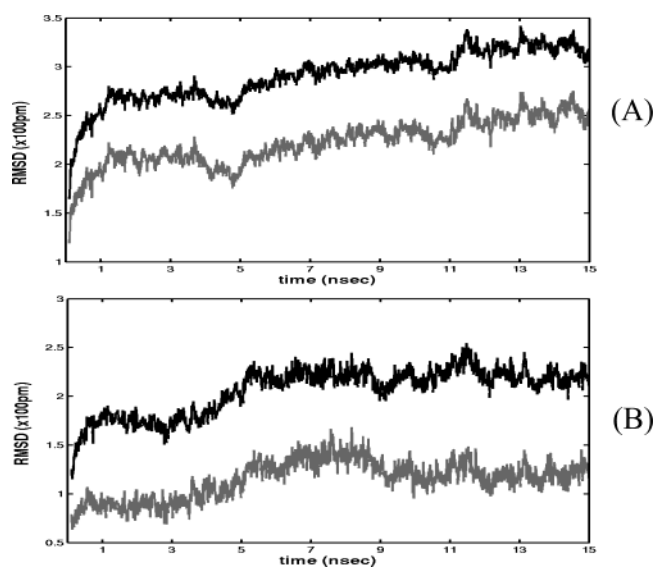


Figure 1. (A) RMSD of bound-AChE and (B) RMSD of bound-FAS. The black line is the RMSD for all heavy atoms, and the gray line is the RMSD for all C_α atoms.

of the FAS-bound enzyme. In addition, these experiments revealed that, for the Trp86Ala mutant of mAChE, FAS became an allosteric activator instead of an inhibitor for certain substrates. Hence, extensive breathing of the complex or a significant conformational change would be expected to accommodate substrate entry or release. Molecular dynamics simulations¹³ of mAChE in the presence of FAS have shown that the fluctuations of mAChE are increased when FAS is bound. The long omega loop defined by the Cys69–Cys96 disulfide bond in the mAChE has been proposed to be important in gating substrate access to the active site.^{14,15} The interface of FAS and mAChE largely comprises those residues of the

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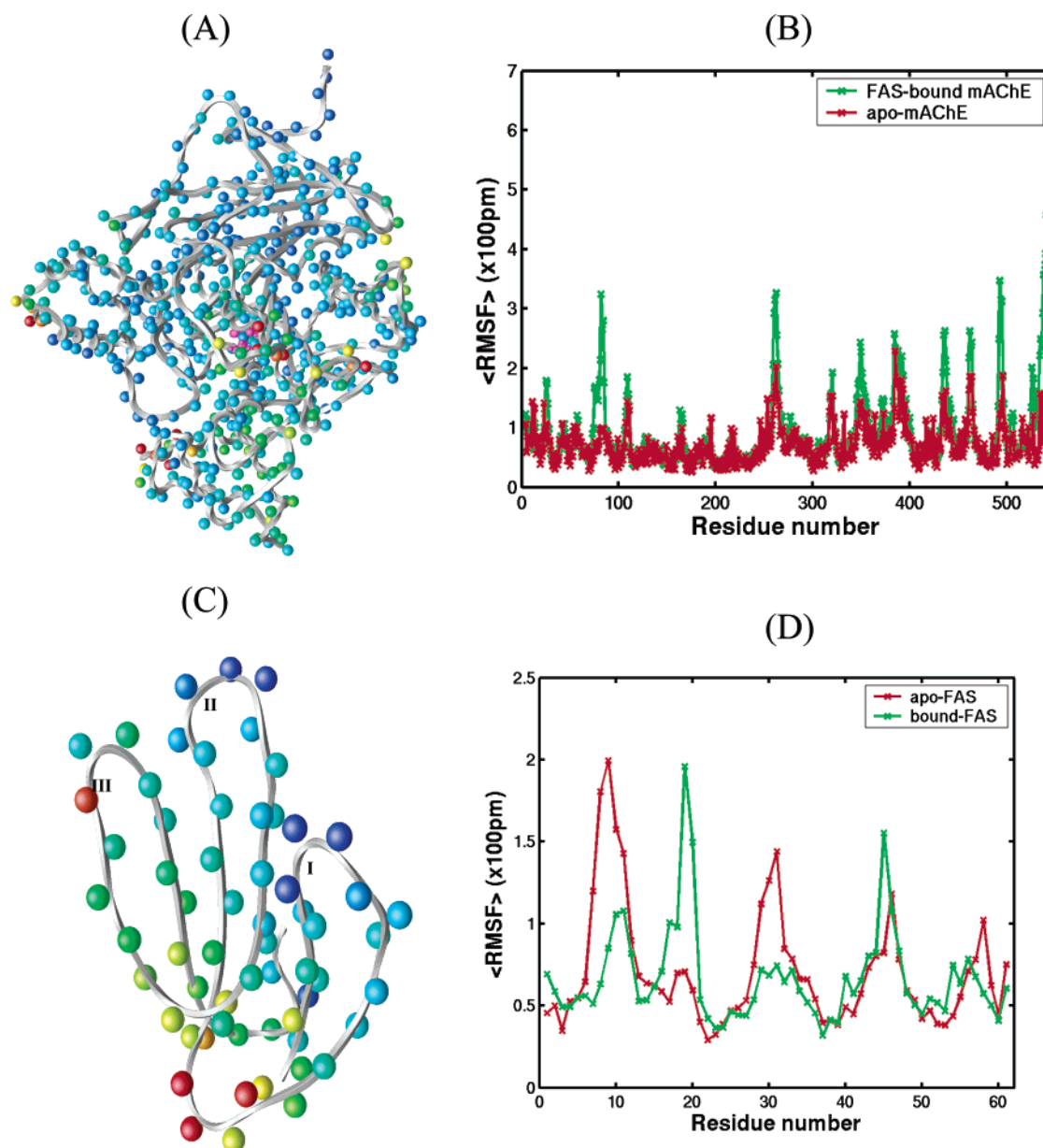


Figure 2. (A) Residue-averaged RMSF ratio of the bound-AChE to those of the apo form (extended from the 10 ns simulation reported by Tai et al.²⁶). Each bead represents a C α atom of a residue. The color gradient is from red with the highest ratio (61.69) to blue (0.29). The viewer looks down the gorge into the active site marked by the magenta Ser203 residue. The N-terminus is at the top of the figure, and the C-terminus is at the lower left. (B) Comparing the residue-averaged RMSFs of the bound-AChE to those of the 15 ns MD apo-AChE. (C) Residue-averaged RMSF ratio of the bound-FAS to those of apo-FAS (unpublished results of a 100 ns MD simulation of FAS). The ratio decreases from red (7.23) to blue (0.122). (D) Comparing the residue-averaged RMSFs of the 15 ns MD bound-FAS to those of the 100 ns MD apo-FAS trajectory.

omega loop that make up the gorge entrance, and loop I and loop II of FAS.^{6,7,11,16} Enhanced motions for the outer portion of the omega loop and other flexible loops near the active site of mACHE are observed in the MD simulations that are described here. These motions may contribute to the residual activity of the complex by creating alternative passages for small molecules to enter and leave the active site.

Computational Details and Analysis Methods

MD Setup. The 5 ns MD simulation of mACHE/FAS¹³ was extended to produce a 15 ns trajectory. The MD setup and simulation parameters

have been described in an earlier report and are summarized here. The 0.25 nm crystal structure of Fas2-mACHe (PDB identification code 1KU6) was used to build the initial structure, with missing residues mended using those from similar structures (PDB codes 1MAA and 1MAH)⁶ and added hydrogen atoms. AMBER 95 force field¹⁷ was used for the solutes. Long-range electrostatic interactions were calculated using particle-mesh Ewald summation.^{18,19} Relaxation and 1 ns of equilibration were carried out before the production run. The MD simulation was performed in the isothermal-isobaric ensemble using the NWChem program.²⁰ The SHAKE algorithm^{19,21} was used to fix the lengths of the covalent bonds involving hydrogen atoms, which

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allowed an integration time step of 2 fs. The simulation system had a total of 116 579 atoms: 8279 atoms of the mAChE, 906 atoms of the FAS, six sodium counterions, and 35 796 SPC/E²² water molecules.

Computed Anisotropy Decay. Time correlation functions, $\rho(\tau)$, of three selected residues of the omega loop, Leu76, Glu81, and Glu84, whose IAEDANS fluorescence anisotropy decays were experimentally measured,¹⁵ were computed in this study. The computed anisotropy of the residues was calculated using the following expression²³

$$\rho(\tau) = \langle P_2[\hat{\mu}(t) \cdot \hat{\mu}(t - \tau)] \rangle_t$$

where P_2 is the second-order Legendre polynomial and $\hat{\mu}$ is a normalized vector that goes from the C_α atom to the atom at the tip of a residue. For the glutamate residues, the average of the two normalized $C_\alpha-O_\epsilon$ vectors was calculated, whereas the normalized $C_\alpha-H_\gamma$ vector was used for Leu76.

Proper Radii of the Channels Leading to the Active Site. The proper radius was defined as the maximum probe size that produced a continuous Connolly surface between the active site and the opening of interest at the surface of the mAChE. The algorithm, described in earlier publications,^{24–26} was used to detect these passages: 1, the main gorge entrance, from the Glu202:OE1 of the active site to the entrance of the gorge Leu76:CD1, Trp286:C β , and Tyr72:OH; 2, the back door, which is formed by Trp86, Gly448, Tyr449, and Ile:451; and 3, the short channel side door, which is formed by residues Asp74, Thr:75, Leu76, Thr83, Glu84, and Asn87. Proper radii were computed using probes that varied in size in increments of 0.1 Å from 0.6 Å up to the largest radius that successfully produced a continuous Connolly surface for each snapshot at 1 ps intervals.

Results and Discussion

Enhanced Flexibility of the FAS/AChE Complex. The root-mean-square deviations (RMSD) of the bound-mAChE and the liganded FAS through the 15 ns FAS–AChE trajectory were computed using the equilibrated crystal structure of the FAS–AChE complex as the reference structure. The moderately large RMSD of the bound-mAChE and, to a lesser extent, the bound-FAS (Figure 1A and B) indicates the existence of conformers that are somewhat different from the crystal structure. To identify the flexible parts of the proteins, the root-mean-square fluctuations (RMSFs) of the heavy atoms were calculated relative to the mean positions of the atoms. The RMSFs of the mAChE of the FAS–mAChE are then compared to those of the 15 ns MD apo-mAChE trajectory to infer the relative internal motions of the bound and unbound enzymes (Figure 2A and B). Surprisingly, not only are the RMSFs of the bound-mAChE mostly larger than those of the apo-AChE, but also many loops of the bound-mAChE show significantly increased motions. The interfacial residues, however, are highly ordered upon the complexation. Of particular interest, residues 81–84 of the long omega loop (residue 69–96), which are in the outer portion of the loop and not part of the gorge that leads to the active site,

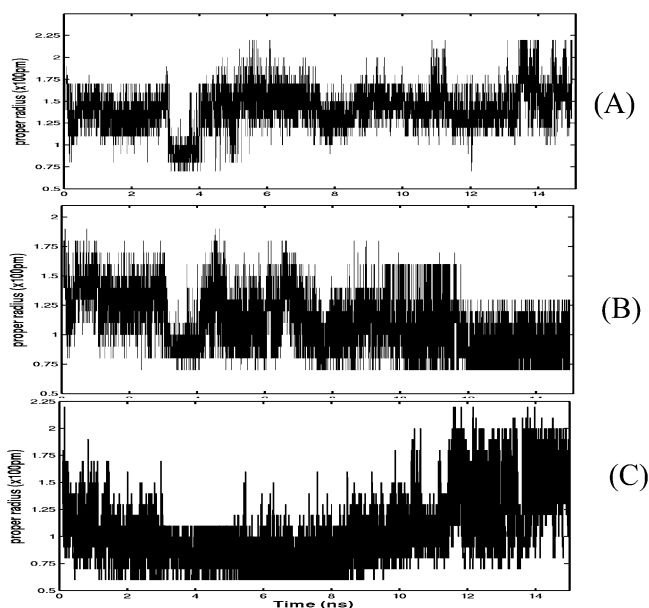


Figure 3. Fluctuations in the width of the channels leading to the active site: (A) the main gorge, (B) the side door, and (C) the back door.

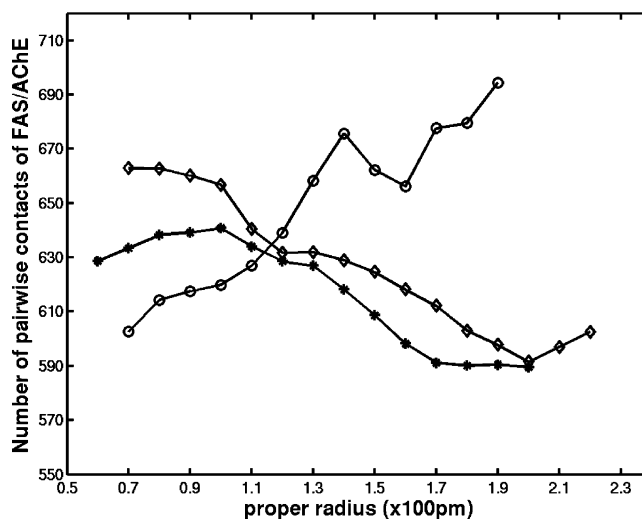


Figure 4. The correlation of the average number of pairwise contacts between heavy atoms of FAS and mAChE with the proper radius of each passage: * is for the main gorge, O denotes the side door, and \diamond is for the back door.

show remarkable flexibility. Flexibility of this loop also been observed experimentally from the measurement of fluorescence anisotropy decays.¹⁵

Similarly, the RMSFs for the bound-FAS show that the tips of loops I and II (Figure 2C and D) are more ordered in comparison to those of the apo-FAS calculated from a 100 ns MD simulation (unpublished results). However, there is a remarkable enhancement in mobility of the turn that connects loops I and II. The tips of these loops are the primary points of contacts with mAChE. This movement can perhaps be thought of as an adaptive linker motion.

Alternative Channels Leading to the Active Site. The enhanced fluctuations of the complex, particularly the motions of the omega loop that constitutes part of the main gorge, indeed create alternative passages large enough for molecules bigger than water to enter or leave the active site. Figure 3 shows the fluctuations of the three channels as functions of time. Many

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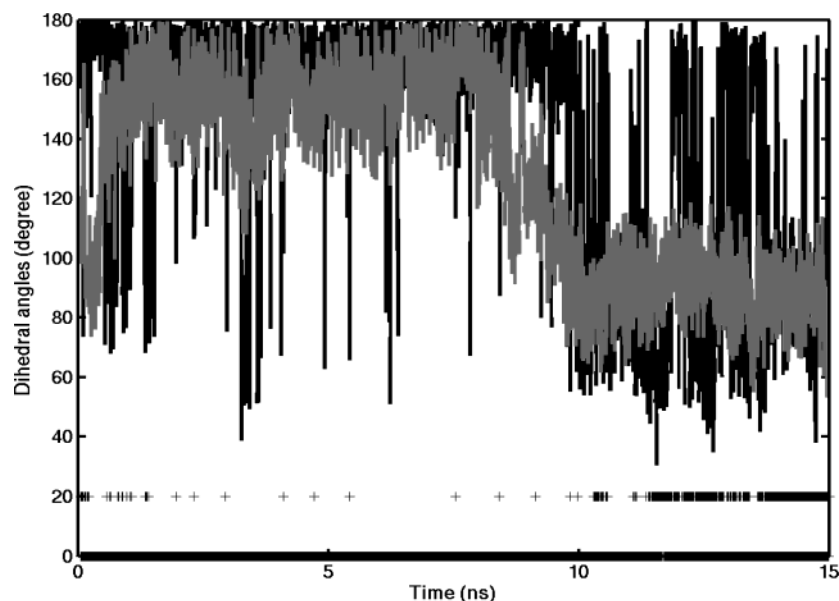


Figure 5. Dihedral angles of the residues that are important to the formation of the back door passage, as a function of time. The gray line is Trp86: χ_1 , the black line is for Ile451 χ_2 , and the plus sign symbols represent conformers that have the back door opened by 1.6 Å or larger.

different conformers in which the back door or the side door was relatively open were visited during the simulation of the complex. To correlate how the binding of FAS enhances these open states, the numbers of pairwise contacts between the FAS and the mAChE were calculated. A contact was considered to exist when the distance between a heavy atom in FAS and another in mAChE was less than 5.0 Å for more than a quarter of 15×10^3 frames. Figure 4 displays the correlation of the width of each passage with the average number of pairwise contacts between FAS and mAChE. Larger numbers of contacts seem to correlate with the closing of the main gorge, but also with the opening of the side door passage. From the crystal structures^{6–8} of the complex, loop II of FAS is plugged into the main gorge entrance and makes multiple contacts around its rim, including parts of the omega loop. The side door is close to the main gorge, and the omega loop runs between them, so that the anticorrelated behavior of the two channels is understandable. This type of backbone movement, coupled with the side chain motions of residues that form the side door passage, creates an opening that connects the active site to the outside of the enzyme. This short side door passage is only open to molecules with radii less than 2.0 Å during this simulation. The channel can therefore presumably serve at least as an alternative way for water molecules and ions to move in and out of the active site. The back door is at a somewhat larger distance from the interaction site, but it also shows some correlation with the number of contacts.

The backbone motions of residues near the back door in the complex include sizable fluctuations in the outer part of the omega loop (residues 81–84); this segment can coil up and form a helical structure, a deviation from the crystal structure (data not shown). Collectively, this affects the backbone motions of the residues nearby, such as Trp86, which moves approximately 2.0 Å relative to the crystal structure. Shifting of the backbone creates a cavity toward the ring that allows it to rotate somewhat. This is reminiscent of other transient packing defect and gating effects.²⁷ Figure 5 shows the large rotation of Trp86 χ_1 and Ile451 χ_2 ; they highly correlate to the larger

opening of the back door. The side chain of Trp86 sweeps through an angle of almost 90° and flattens out horizontally to the Tyr449 side chain when the back door opens (see Supporting Information).

Conformer Variations of the Omega Loop (Residues 69–96). As can be seen in Figure 6, the heavy atoms of the omega loop of the FAS-bound mAChE deviate significantly, up to approximately 6.0 Å from the crystal structure. In contrast, those of the apo-mAChE are more similar to the crystal structure. It has also been observed that this loop is highly flexible in the fluorescence anisotropy study by Shi et al.¹⁵ A large difference of the RMSD points to possible conformer variations of the loop. Hence, secondary structure analysis was carried using DSSP²⁸ to explore whether the sizable RMSD of the omega loop results from side chain fluctuations or secondary structure changes. Figure 7A shows that the omega loop exhibits considerable changes in its secondary structure. Particularly, the portion of the omega loop including residues 81–84, which do not contribute to the gorge rim and are not in direct contact with the FAS, is coiled up, forming a 3-10 or α helix segment when the back door is open to molecules of 1.4 Å or larger. The rapid conversion of this segment from the coil structure to the 3-10 helix or α helix of this segment is expected to affect the main gorge and alternative passages because the residues of this loop make up one side of the gorge. Evidently, the formation of the helical segment is coupled to the rotational isomerization of the side chain of the Trp86 as discussed above. Because Trp86 is an important component of one side of the gorge, rotation of its side chain coupled with the backbone displacement can lead to the opening of the back door.

It is of interest to correlate the motions of FAS to the changes in the secondary structure of the omega loop. The secondary structure analysis of FAS was, therefore, also performed, and the results are shown in Figure 7B. The five strands of the antiparallel beta sheets of FAS are very stable. However, the

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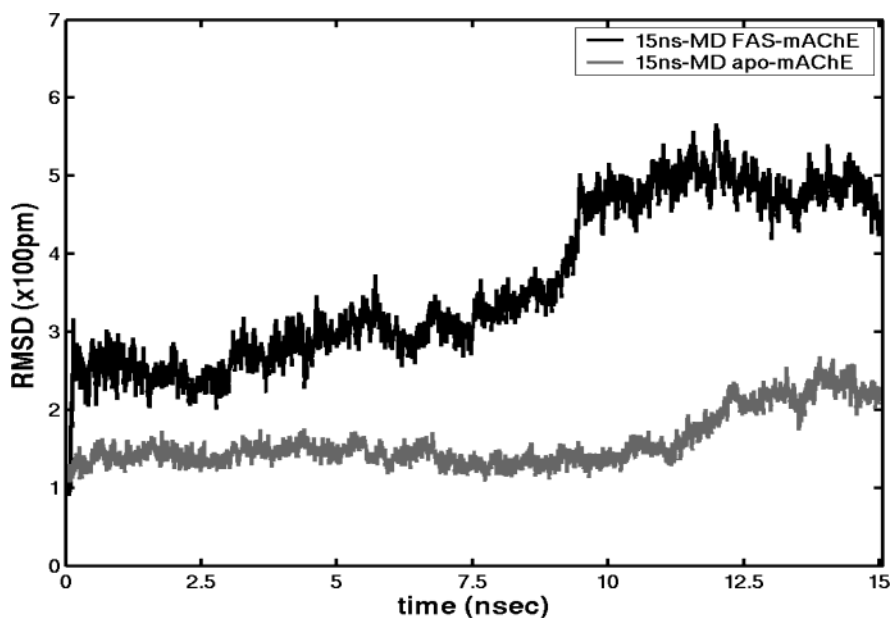


Figure 6. The comparison of the RMSD relative to the crystal structure of the heavy atoms of the omega loop residues 69–96 of mAChE: FAS (black line) and apo-AChE (gray line).

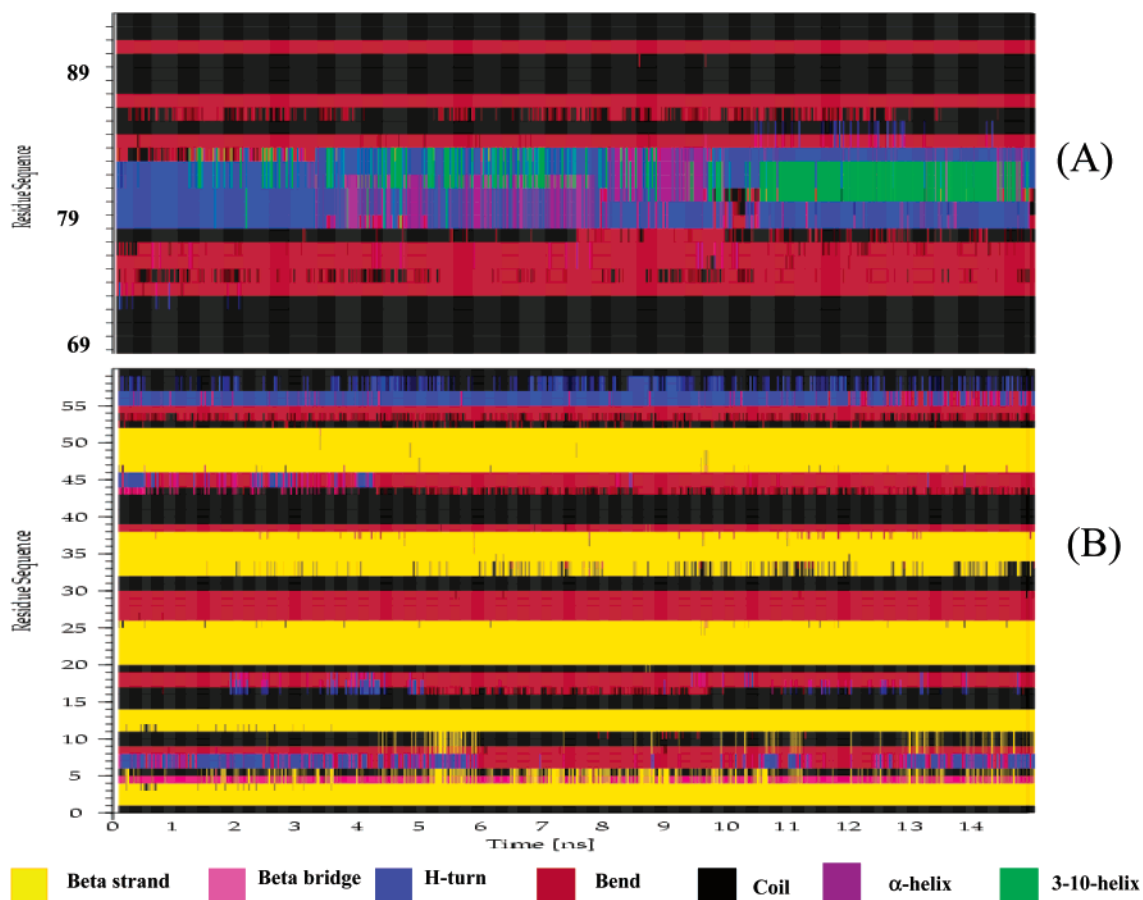


Figure 7. Secondary structure analysis: (A) Depiction of the secondary structure of the omega loop of mAChE through the 15 ns MD trajectory. (B) The secondary structure of FAS.

turns that connect these sheets together exhibit some fluctuations in the secondary structure. Of particular interest, residues 6–10 of the FAS loop I, which are mostly in the turn configuration, form an extended beta sheet as the outer portion of the omega loop is coiled up.

Motions of the Omega Loop. As can be seen in Figure 8, the long omega loop exhibits a significant displacement when the back door opens or closes. There are two main movements of this loop when the back door opens: the whole loop moves away from the main gorge axis, and the residues that belong to

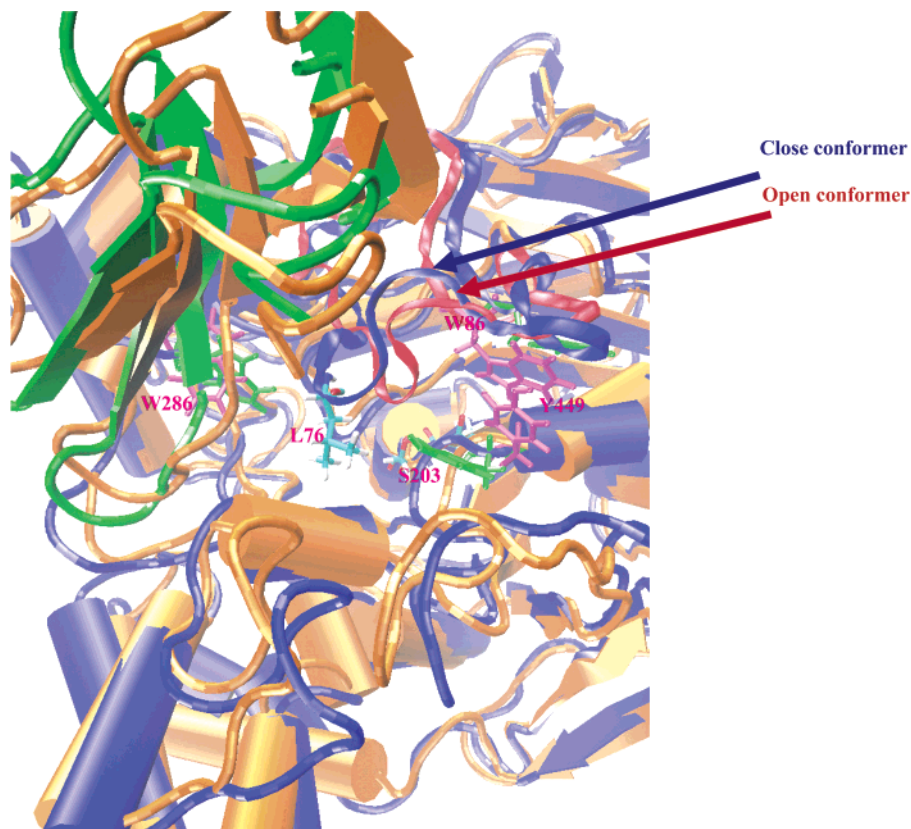


Figure 8. Secondary structure of the omega loop for the open back door (red) and closed back door (blue). AChE of the open snapshot is in orange, and the closed one is blue. The FAS structure is green in the conformer with the closed back door; it is orange when it facilitates the opening of the back door of the enzyme. The viewer looks down the back door passage to the active site, which is marked by Ser203. Tyr449 and Trp86 mark the back door passage, in green when the back door is closed and in purple when it is open.

the outer side of the loop coil up and form the helical segment (see Supporting Information). The later motion shifts the Trp86 toward a position where it interacts with Tyr449 by aromatic stacking to open the back door as shown in Figure 8. The flexibility of Trp86 couples with other flexible loops near active sites such as Gly448 and Tyr449 to open the back door passage.^{25,26}

To correlate the motions of loop I of FAS to the opening of the back door, the distances of all of the heavy atoms of the FAS loop I to those of the outer portion of the omega loop were computed and correlated to the radius of the back door. At a given width of the back door, these pairwise distances were calculated and averaged. The average distance between FAS:Arg11:NH1 and mAChE:Glu91:O_e displays the highest correlation. Figure 9 shows that, as the back door widens, the average distance of FAS:Arg11:NH1 to mAChE:Glu91:O_e gets shorter; leading to stronger interactions.

Fluorescence Anisotropy Decay. The segmental mobility of the mAChE omega loop has been probed by fluorescence anisotropy decay experiments, with and without bound-FAS.¹⁵ Corresponding time correlation functions have been calculated from the MD trajectory. The simulated anisotropy decays and the experimental measurement are compared in Figure 10. As expected, these are quantitative differences, but the mobility rankings are in agreement.

Motions of the Omega Loop Correlate with the Transient Opening of Alternative Channels to the Active Site. As discussed above, the motions of the omega loop seem to be linked to the transient existence of the alternative passages that

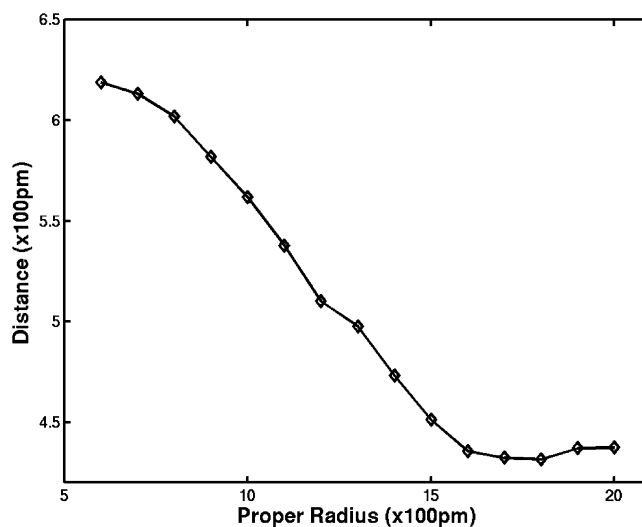


Figure 9. Interactions of loop I of FAS with the outer portion of the omega loop; the correlation of the average distance of the guanidino moiety of FAS:Arg7 to the carboxyl oxygen of mAChE:Glu92 with the opening of the back door channel.

might allow water, ions, or even substrates to gain access to or be released from the active site. This linkage can be gauged by the correlation parameter C_i :

$$C_i = \frac{\langle (R_i(t) - \langle R_i \rangle) (\xi_i(t) - \langle \xi \rangle) \rangle}{\sqrt{\langle (R_i(t) - \langle R_i \rangle)^2 \rangle \langle (\xi_i(t) - \langle \xi \rangle)^2 \rangle}}$$

where ξ is the proper radius which indicates the width of a

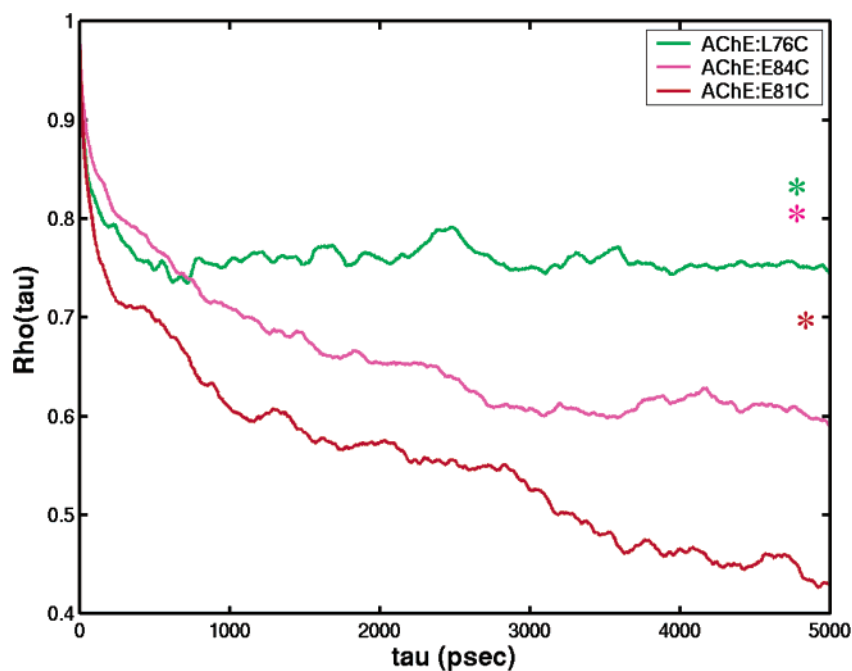


Figure 10. Comparison of the experimental and computed anisotropy decay for residues of the omega loop. The “*” symbols denote the estimated experimental values¹⁵ of the selected omega loop residues and are colored according to the data legend of the figure.

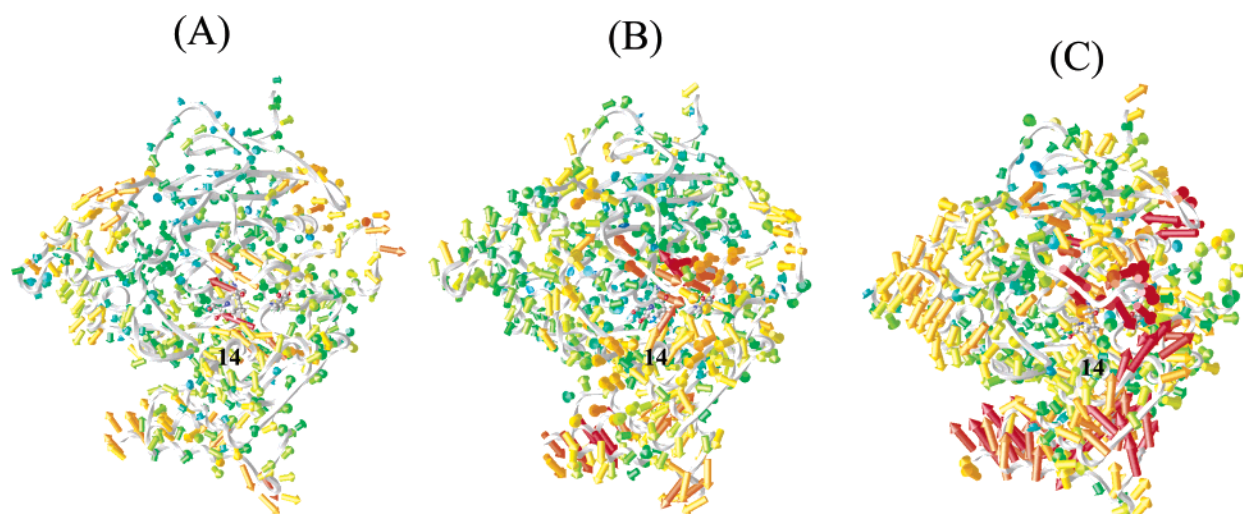


Figure 11. Porcupine plots showing the collective motions of the enzyme, and particularly of the omega loop, for three passages leading to the active site of the enzyme. (A) The main gorge. (B) The side door channel. (C) The back door passage. In each, the view is down into the gorge into the active site marked by mAChE:Ser203 in the middle of the enzyme, the N-terminus is on the top of the figure, and the C-terminus is on the lower left. The back door channel begins near mAChE:Trp 86 on the right. The side door passage is marked by mAChE:Leu76 between mAChE:Ser203 and mAChE:Trp86; numeral 14 is used to indicate the location of helix 14.

passage and R_i represents the Cartesian coordinates of each C_α atom of the enzyme. Three porcupine plots²⁶ of these correlation parameters, for the main gorge, the side door, and the back door, respectively, are shown in Figure 11. The color gradient from red (the most) to blue (the least) indicates the extent of correlation of each residue's displacement with the opening of each passage. The porcupine plot representation highlights the concerted motion of the enzyme for the three possible channels to its active site. As can be seen in this figure, the displacements of the omega loop are highly correlated with the opening of the given passages. Figure 11A illustrates a so-called “sardine can opening” of the omega loop as the main gorge enlarges. The loop is curled up, but at the same time helix 14 is moved inward which is opposite of the motions observed by Bui et

al.²⁹ when the main gorge enlarges to allow ligands to cross the bottleneck region in apo-mAChE. In the absence of FAS, the enzyme may favor the state with the smaller opening of the main gorge. Motions of the omega loop also contribute to the opening of the side door, as can be seen from Figure 11B. The opening of the back door is collectively coupled with many parts of the enzyme in addition to the omega loop.

Conformation of the Acylation Site. As shown in Figure 12, the oxyanion hole of the open back door conformer is almost unperturbed. The choline pocket, where the quaternary trimethylammonium group of acetylcholine binds, is in a configuration that favors the translocation of small molecules such as

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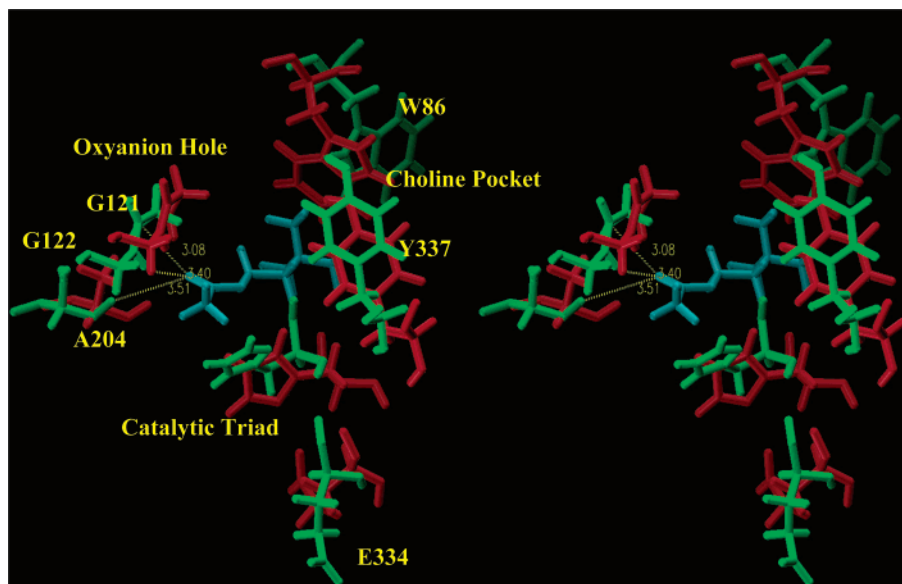


Figure 12. The acylation site of the mAChE: The average structures of the bound-mAChE (green) that have the back door opened by 2.0 Å or more are superimposed onto the mAChE structure with the corrected docking structure (red) of acetylcholine (cyan).³¹ This figure is generated using VMD,³³ and the crossed-eyes stereo convention is used.

the products of the hydrolysis. The observed flexibility of the omega loop supports the possibility that the back door of mAChE may have functional relevance. Other studies, such as estimations of the energetic cost of product release by Eneydy et al.,³⁰ have shown that a unidirectional flow from reactant to product through the short back door may be favored. In addition, the docking studies by Kua et al.³¹ showed that the product of the acylation step, choline, has its headgroup oriented toward the back door, leading to a possible exit of the molecule via the back door. The large ($\sim 90^\circ$) rotations of the side chains of the choline pocket residues support a functional role for this passage in the FAS–AChE complex. These results are consistent with the experimental fact that mAChE retains some catalytic activity in this complex. The rate constants of TFK^+ and TFK^\ominus dissociation from the FAS–AChE complex were increased by 30-fold and 3-fold, respectively,³² suggesting that FAS allosterically influences the access of the trifluoroacetophenone conjugates to the active site of mAChE.

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Conclusion

FAS alters the activity of mAChE by altering the dynamics and the structure of the enzyme, particularly of the large omega loop. This loop undergoes a flexible rearrangement during which some of its secondary structure rapidly interchanges from a coil to a 3–10 or α helix segment. Enhanced interactions well beyond the Trp:286 peripheral site are shown here, providing a structural basis for an inhibitory conformational effect on the acylation site. Displacements of the large omega loop appear to favor alternate routes of access to the enzyme's active site.

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Supporting Information Available: Dynamics of back door opening and the omega loop (video mpeg). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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