Open "Back Door" in a Molecular Dynamics Simulation of Acetylcholinesterase


The enzyme acetylcholinesterase (AChE) generates strong electrostatic fields that can attract the cationic substrate acetylcholine (ACh). The crystal structure of AChE (1) raises questions regarding substrate entry and product release. First, the active site gorge is very deep and appears too narrow to admit ACh (1, 2). However, the demonstration that quaternary amines enter the active site of crystallized AChE (3) proves that the protein is sufficiently flexible to admit substrate by some route. Second, the inward electrostatic field at the gorge, which is likely to accelerate penetration of the positively charged substrate (4, 5), would seem to impede the exit of the product choline from the mouth of the active site. A thin wall near the base of the active site, at residues Met and Trp, could offer an alternative route for the escape of products (4). This back door might also provide a vent for water molecules during the passage of substrate, products, or both through the narrow gorge. We have studied these issues by performing a molecular dynamics (MD) simulation of AChE and analyzing the resulting time series of protein conformations with regard to active site accessibility.

Unobserved atoms were added to the Torpedo californica AChE dimer (1), as described in (5), using the program Quanta (Molecular Simulations, Waltham, Massachusetts). Protonation states at neutral pH were assigned on the basis of atomic solvent accessibilities, of salt-bridging and hydrogen-bonding opportunities, and of the putative catalytic mechanism, which requires the active site His to be neutral (6). The model enzyme was immersed in water, and the classical laws of motion solved numerically with weak coupling to a heat bath at 300 K to generate 119 ps of analyzeable conformations (7).

We searched for conformations in which the active site had any aperture large enough to pass a water molecule, generating for each conformation a Lee and Richards (8) solvent-accessible dot surface with a 1.4 Å probe. The molecular
surface was separated into the outer surface and some number of cavity surfaces, based on distances between surface points (9). Of these surface subsets, the active site surface was defined as that contacted by Glu199 OE2, because this atom was consistently at the surface of the active site interior. Conformations with an open active site were those in which the numbers of connected points in both the active site and outer surfaces were identical.

Conformations with an open back door were sought as follows. For each conformation having an open active site, the active site entry was blocked by the deletion of all surface points within 8 Å of a point located approximately in the middle of residues Glu153, Asp280, Asp285, and Leu333 that defines the entrance to the gorge. If the active site surface was still continuous with the outer surface of the enzyme, an alternative aperture existed.

This surface analysis detected a transient opening, occurring in the first 20 ps of production MD and persisting for 0.3 ps. A channel (Fig. 1) formed in the thin active site wall at Trp64, the side chain of which probably contributes to binding of the quaternary ammonium of ACh (1, 3, 10). The channel began near Trp64 CH2 and CZ2, Gly441, and the ring of Tyr442 and curved around the edge of Trp64 to emerge at a surface dipole near Glu445. Its path led directly through a solvent-sized cavity that is observed in the crystal structure and which lies near Val129. The displacement of the Trp64 indole, relative to its location in the crystal structure, resembles that of a camera shutter because it is completely in-plane.

The channel can be opened by shifting only residues Trp64, Val129, and Gly441 from their crystal coordinates to their positions in the open conformation. The average atomic displacement for these residues, relative to the crystal conformation, is only 1.3 Å. The Trp64 residue is somewhat more mobile than average: The average root-mean-square (rms) fluctuation of its nonhydrogen atoms is 1.2 Å (main chain 0.8 Å, side chain 1.4 Å), compared with 0.72 Å for all nonhydrogen atoms within 25 Å of the dynamical center. The Val129 and Gly441 residues have unremarkable rms fluctuations of 0.70 Å.

Several points are significant to this opening mode. First, the time required for AChE to hydrolyze one substrate molecule is about 0.1 ms (11). Thus, although the observed opening event is brief, it occurs at all in a 119-ps simulation suggests that the back door can play a functional role on the longer time scale of catalysis. Second, the small size of the atomic displacements required to open the back door means that the open conformation should be easy to achieve; experiments have demonstrated that protein structures fluctuate substantially (12). Third, the presence in the crystal structure of a solvent-sized cavity in the thin wall of the active site where the channel forms suggests that the x-ray data may reflect similar opening events in a time-averaged fashion. Finally, no opening events were observed in other portions of the active site wall, although every stored conformation was analyzed. Thus, Trp64 defines the only permeable part of the active site wall in this simulation.

Because AChE appears to generate a functionally important electrostatic field (4, 5), we examined the effect of the open back door on this field (13). When the back door is open, field lines initiated at the active site Glu199, near Trp64, exit preferentially through this aperture (Fig. 2A). However, for the crystal structure most of these field lines emerge from the mouth of the active site gorge (Fig. 2B). When the electrostatic potential was evaluated at each point on a Lee and Richards—type surface (8), the surface point with the lowest electrostatic potential lay at the bottom of the active site, near Ile445. Therefore, ACh and choline are attracted to the base of the gorge, either through the front or the back door. This observation militates against the idea that the same field that guides ACh into and down the gorge urges choline out the back door. However, the field accelerates the departure of the other product, acetate ion. Also, a more widely opened back door might significantly weaken the active site fields by increasing exposure of the active site to the high dielectric solvent. This effect would facilitate the release of choline.

One way to test whether a functionally important back door does exist would be a kinetic study of a mutant enzyme whose back door is sealed. Of the three residues whose movement opens the channel, Trp64, Val129, and Gly441, it appears that only Val129 could be modified without the disruption of enzyme function by undesired mechanisms. Steric analysis of the crystal structure suggests that a Lys or Arg would fit well at position 129, with the positive group between Met109, Asp128, Glu445, and Leu455 (Fig. 1). In this position, the side chain would fill the surface dipole where the back door channel emerges. The positive group of the mutant side chain would be far (~11 Å) from the interior of the active site. To our knowledge, no special role has been suggested for Val129, and because it lies at the protein surface, it appears to be structurally unimportant. However, aligned cholinesterase amino acid sequences show little variation at this position: Eight have Val and two have Ile (14). Isoenzyme seems unlikely to block the back door because it possesses only one more methylene than Val. If the mutation of Val129 does slow the enzyme, it will be necessary to confirm the absence of structural distortion through crystallographic studies.

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Fig. 1. Active site gorge with open back door. Green dots, solvent-accessible surface of active-site gorge (T, top of gorge); white dots, channel to back door. Space-filling atoms are shown for Trp64 (W84), Val129 (V129), Gly441 (G441), and Glu445 (E445). Vectors indicate directions of displacement of three of these residues relative to their positions in the crystal structure. Stick diagrams indicate catalytic residues His443 and Ser200.

Fig. 2. Electric field lines for AChE with (A) open and (B) closed back door. The field lines (yellow) are traced from the region of negative potential near Glu199 OE2 deep in the active site. Blue atoms highlight the opening to the active site gorge. The back door is clearly marked by the vertical bundle of field lines in (A). Field lines were computed and displayed with GRASP (16). The orientation is changed relative to Fig. 1.

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Experiments have shown that thermal fluctuations make proteins somewhat porous, especially to small, nonpolar molecules (15). Our analysis supports the concept that the active site of AChE has a particularly porous wall at Trp34, which might be of functional importance. Kinetic energy gained by the catalytic residue His442 during hydrolysis may pass by way of the peptide linkage to Gly441, one of the charged residues. This energy might increase the probability of opening, causing an organized sequence of catalysis and channel opening.

REFERENCES AND NOTES

6. All Arg, Lys, Asp, Glu, NH2-terminal, and COOH-terminal groups were assigned "ionized" (except Glu442). The Glu442 residue was assigned "neutral" (Glu protonated) because it is fully deprotonated and appears to form only two to three hydrogen bonds. All Tyr and Cys side chains were set neutral. Histidine protonation sites were His3, His8, His26, ND1; His139, NE2; His181, ND1, NE2; His220, NE1; His224, ND1; His236, NE1; His298, ND1; His382, NE2; His440, ND1; His492, NE2; and His513, ND1, NE2. The net charge on monomer was +5. Both monomers were assigned identical protonation states. Polar hydrogens were added with the ARGOS package (17).
7. With only crystallographic solvent included, the energy was minimized with respect to hydrogen positions for 200 steepest descent steps. The energy was then minimized for 200 steepest descent steps with respect to the coordinates of residues 536 to 537 and 1072 to 1074 to form the COOH-terminal disulfide bond. A pre-equilibrated box of waters [SPC/E model (16)] was overlaid repeatedly on the system to fill in a 40-Å sphere centered on atom NE2 of the catalytic His442. The 142 crystallographic waters were retained. Waters closer than 2.5 Å to any crystallographic heavy atom were rejected. Atoms more than 35 Å from His440 NE2 were fixed in space for all subsequent calculations to create a constant-volume-dynamical system containing 2525 protein atoms and 3117 water molecules. Energy was minimized with respect to water coordinates for 200 steepest descent steps. Then MD on water only was performed for 20 ps, with velocity reassignment at 300 K every 0.2 ps and velocity rescaling (19) with time constant 0.2 ps. Energy was then minimized with respect to protein coordinates for 200 steepest descent steps. MD on the protein only was performed for three segments of 5 ps each, with velocity reassignment every 0.2 ps and rescaling with time constant 0.1 ps, at 100 K, 200 K, and 300 K, respectively. Before production calculations were made, MD was used to equilibrate the entire system for 20 ps at 300 K, with separate solute and solvent velocity rescaling with time constant 0.2 ps. During the first 20 ps of production, coordinates were saved every 20 steps. Subsequently, coordinates were stored every 5 steps. All interactions in a short-range (10 Å) pair list were updated each step, while all interactions in a long-range (12 Å) pair list were updated every five steps. Separate solute-solute, solute-solvent, and solute-solute pair lists were updated every 10, 15, and 20 steps, respectively. Pair lists were based on charge groups.

SHAKE (20, 21) was used to constrain bond lengths, permitting the use of a 2-śis time step. All calculations were made with the ARGOS program package (17), with GROMOS atomic parameters (22). System temperatures approximated 301 K with solute temperatures of about 298 K and solvent temperatures of about 302 K. Total system potential energy averaged ~220 \times 10^3 \text{kJ mol}^{-1}, with a drift of ~8.0 \text{kJ mol}^{-1} \text{ps}^{-1} during the final 100 ps. During the final 100 ps, the rms atomic position deviation from crystal coordinates of nonhydrogen atoms in the innermost 25 Å of the simulation sphere averaged 1.7 Å, with a residual upward slope of 0.001 Å ps^{-1}.


9. Surface generation started with 100 surface points per atom, representing possible locations of the center of a solvent probe sphere. For simplicity, hydrogen atoms were excluded, and all heavy atoms were assigned a radius of 1.8 Å. Two surface points were assigned to the same surface subset if they were closer than the greatest distance between any point and its nearest neighbor on the initial 100-point surface sphere of each atom.


13. We used the finite-difference Poisson-Boltzmann method (23), implemented in the program UHBD (24) to compute electrostatic fields in and around AChE. Finite-difference Poisson-Boltzmann calculations were made with protein and solvent dielectric constants of 2 and 80, respectively, physicist's ionic strength 0.15 M, and a 1 Å solvent layer. The dielectric boundary was defined as the Richards (25) probe-accessible surface. The only source charges were the formally charged groups of the molecular dynamics calculation. We used a 65 by 65 by 65 grid with 1.5 Å spacing.


16. GRASP, vers. 1.0; A. Nicholls and B. Honig, Columbia University, New York.


26. Atomic coordinates for the open back door conformation are available by anonymous Internet file transfer program (ftp) to mtsun.chem.uhn.edu. We thank B. Honig and A. Nicholls for making available the graphics program GRASP and R. Gillian and H. S. R. Gilson for helpful discussions. Supported by the Robert A. Welch Foundation, the National Institutes of Health, the National Science Foundation, the Grand Challenge Program of the National Science Foundation supercomputer centers, the Minerva Foundation, the Kimmelman Center, and U.S. Army Medical Research and Development Command Contract No. DAMD17-93-C-2070. M.K.G. is a Howard Hughes Physician Research Fellow and I.S. is Bernstein-Mason Professor of Neurochemistry. P.H.A. is supported by the Markey Charitable Trust.

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