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Efficient product clearance through exit channels in substrate hydrolysis by acetylcholinesterase

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Abstract

The channels connecting the active site of acetylcholinesterase (AChE) to the protein exterior were mapped by computational techniques in order to find potential exit routes for charged reaction products. 3.9% of the total volume of the AChE monomer is hollow space and over 50% of the void is located in the center; it is partitioned into three chambers, a deep entry channel, below it a wide channel located in a slightly positive region of AChE and ideally suitable for the exit of negatively charged fragments and a small chamber above Trp^{84} and Met^{83}. The latter serve as gates for the departure of the positively charged choline product of the hydrolysis of acetylcholine into the small cavity. An efficient product clearance is a prerequisite to a very low energy pathway for the irreversible hydrolysis of acetylcholine.

Key words: Acetylcholinesterase catalysis; Acetylcholinesterase active site; Acetylcholinesterase structure; Acetylcholinesterase mechanism

1. Introduction

Acetylcholinesterase (AChE, acetylcholine acetylhydrolase), hydrolyzes its natural substrate acetylcholine near the rate of encounter of substrate with enzyme [1–11] and 10^{17} faster than the neutral hydrolysis of acetylcholine [4–11]. A generalized scheme of the reaction is given below.

$$SerOH + (CH_3)_3N^+CH_2CH_2COOCH_3 \rightleftharpoons SerOCOCH_3 + (CH_3)_3N^+CH_2CH_2OH \text{ acylation}$$

$$SerOCOCH_3 + H_2O \rightleftharpoons SerOH + CH_3COO^- + H^+ \text{ deacylation}$$

Both phases involve a proton transfer from the nucleophile to the catalytic His followed by another proton transfer from $HisH^+$ to the leaving group [1–11].

Decades of intensive investigations of the dynamics of this enigmatic biocatalyst illuminated many molecular details of its mechanisms [1–10]. Nevertheless, a critical question of the catalytic mechanism remaining is how to find the escape route for products of the reaction of substrates and inhibitors. In order to answer this question, we have mapped, by a grid search algorithm, the channels connecting the active site to the protein exterior to find potential exit routes for charged reaction products [12]. We found that 3.9% of the total volume of the AChE monomer is hollow space and over 50% of the void is in the center: it is partitioned into three chambers, a deep entry channel, below it a wide channel located in a slightly positive region of AChE and ideally suitable for the exit of negatively charged fragments and a small chamber above Trp^{84} and Met^{83}. The latter may serve as gates [12,13] for the departure of the positively charged choline product of the hydrolysis of acetylcholine into the small cavity. This cavity has the highest concentration of negatively charged residues on AChE. We propose a mode of product clearance which is *consistent with irreversible hydrolysis* of the physiological substrate as needed in signal transmission in the cholinergic system [14].

The three-dimensional structure of an α-subunit of AChE from *Torpedo californica* electric organ has recently been determined at 2.8 Å resolution by X-ray crystallography of the homodimer [15]. Subsequent reports have identified in further detail the key catalytic residues at the active site, the anionic and the hydrophobic residues lining the entrance to substrates and inhibitors [16,17] and sequence conservation with other esterases and lipases [18]. The enzyme has been described as a strong dipole aligned along the gorge with the negative pole pointing away to attract positively charged reactants [17].

A recent molecular dynamics simulation on the 120 ps time scale of the solvated AChE from *Torpedo californica* confirmed the possibility of an existing 'backdoor' for the exit of choline [13]. Simulations of this kind would be especially informative with the inclusion of reactants and products, a difficult task to accomplish. While we have been working on this approach, here we would like to propose a working hypothesis for product clearence in the AChE-catalyzed reactions [12]. In this work, an unambiguous representation of cavities and channels has been implemented by program CADRAW, a grid search routine. Three adjacent cavities separated by thin walls seem to communicate via gating mechanisms for the effi-

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cient release of products which then enforce the unidirectionality of acetylcholine hydrolysis catalyzed by the enzyme.

2. Materials and methods

2.1. Generation of cavities and channels in an α-subunit of the homodimer of Torpido californica AChE

The X-ray coordinates for the enzyme were obtained from the Brookhaven Protein Data Bank [19]. The water molecules were deleted for the purpose of computing void volume and the protein structure was optimized with molecular mechanics program YETI (V5.3) [20,21] as described earlier [22]. The missing atoms from the side chains of 27 amino acid residues on the protein surface were also inserted and their positions were optimized. YETI was used to generate the hydrogen positions at heteroatoms. His residues were protonated on the N facing a potential hydrogen bond acceptor. His^{440} was protonated only on the Nδ to mimic the physiologic condition. Then the grid search for empty space within the enzyme was carried out at a grid size of 0.5 Å.

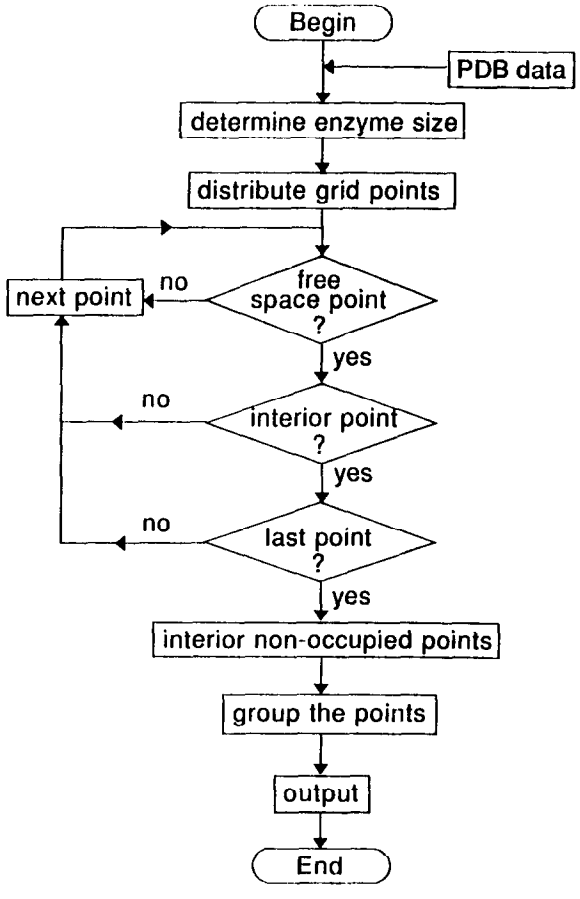
Program CADRAW, a grid search routine has been developed for surveying internal cavities and channels of macromolecules. Scheme 1 provides a flow-chart for the program [23]. Results of CADRAW can be input to molecular graphics program GEMM(V7.8) [24] for three dimensional visualization. The van der Waals radii used by Lee and Richards [25] were adopted in CADRAW. The conventional 1.4 Å was used for probe size [26,27]. The program was written in C and computations were carried out on a SGI Personal Iris 4D35TG Workstation. The program was tested on computing the volume of C_{240} with a diameter of 14 Å and a grid size of 0.5 Å [28].

3. Results and discussion

Table 1 lists the distribution of void volume of AChE. Fig. 1 shows the ribbon structure of the AChE monomer with the three cavities partitioned from one another by flexible loops. All figures are views down the active site gorge. Residues Asp^{72}, Glu^{73}, Glu^{199}, Glu^{285} and Glu^{327} surround the hydrophobic residues lining the entrance gorge of AChE, and exert an increasing negative electrostatic field in the direction of the active site. Thus the continuum of increasing negative electrostatic field promotes the influx of acetylcholine, unnatural substrates and inhibitors. The concentration of negative charge increases beyond the bottom of the gorge in the upper cavity, separated by a loop formed by residues 64–95, in which direction residues Glu^{273}, Asp^{276}, Glu^{278}, Glu^{247}, Glu^{199}, Glu^{443}, Glu^{445}, Glu^{327}, Asp^{326}, Asp^{392} and Asp^{393} are located. This most negative region of the enzyme seems ideal for cationic binding, however, is physically separated from the catalytic site. The three methyl groups of the choline fragment of acetylcholine were suggested [15–17] to interact with Trp^{84} and Met^{83} which may undergo a conformational change to release the positively charged choline product [12,13]. With an upward motion of the loop, the indole side chain of Trp^{84} under the thin wall could move to yield way to the choline fragment into the top cavity (Scheme 2). This dynamic process may be coupled with the water attack on the acyl enzyme, which may require a small conformational change of the catalytic His base [6]. The energy cost of this conformational change may be well invested if it assists implementation of a unidirectional flow from reactant to product toward the increasing local negative electrostatic gradient. The strongly negative environment seems also to promote dealkylation of a branched alkyl substituent in an organophosphorus adduct of AChE [22].

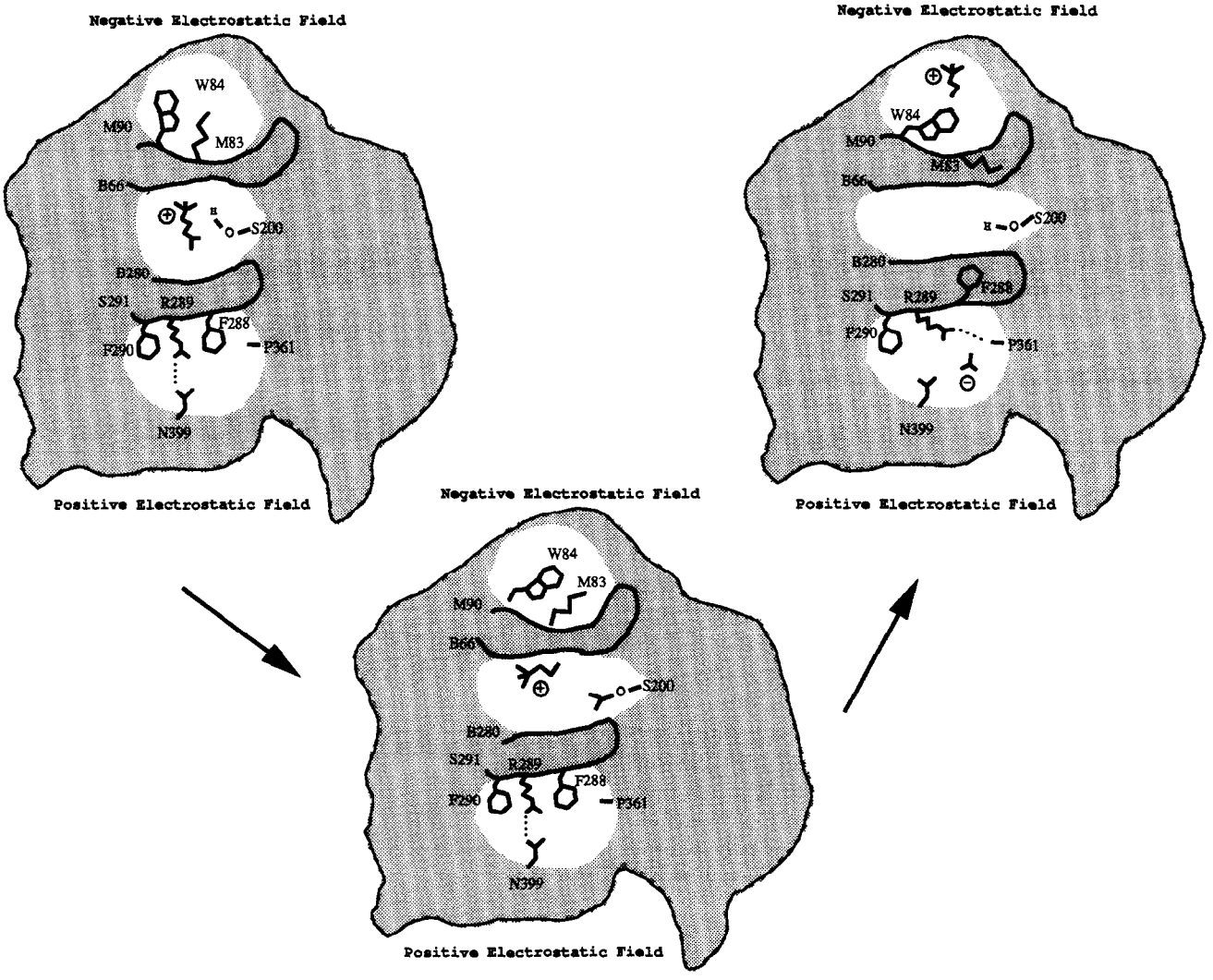
The other fragment of the reaction with acetylcholine is acetate ion, which bears a negative charge, will be *repelled by the negative electrostatic field*, and may use a different escape route. A chamber connected to the active site gorge, but *separated* by a thin wall to the right of and beneath another loop, formed by residues 279–292, is also discernable in Fig. 1. Arg^{289} in the loop is hydrogen-bonded to the carbonyl of Asn^{399} in the main chain, but approach of a negatively charged ion may disrupt the interaction and cause formation of a new hydrogen bond between Asn^{289} and the adjacent carbonyl of Pro^{361}. This rearrangement, an electrostaticaly-operated opening of the loop, can connect the two chambers and allow for passage of a negative leaving group.

The conformational change can also restore the native conformation of the upper loop region. The residues scattered along the walls of the lower chamber are Lys^{410}, Lys^{413}, Lys^{498}, Lys^{501}, Asn^{515}, Asn^{517}, Asn^{220}, Lys^{221},



Scheme 1. Flow-chart for the algorithm of CADRAW.

Fig. 1. Stereoscopic image of the AChE monomer looking down the active site gorge and two other cavities that are partitioned by flexible loops: the ribbon structure of the α-C backbone is in blue and the partitioning loops are in magenta (residues 66–90 top and 280–291 bottom). The upper cavity in yellow is lined with negatively charged residues, the central cavity in orange is the active site gorge and the lower cavity in green is mostly lined with positively charged residues.



Scheme 2. A cartoon of the mechanism of product release of the AChE-catalyzed hydrolysis of acetylcholine.

Table 1
Void and percent void volume in AChE

Region	V (Å^3)	%
Total void	3,737	3.90
Entrance channel	586	0.61
Exit channel for a positively charged product	217	0.23
Exit channel for a negatively charged product	1,196	1.24

Lys^{315}, Lys^{316} and Lys^{216}, which create a positive electrostatic field leading out of the active site to the backside of the AChE subunit. Here, bulk water can dissipate the ionic field and wash out the product.

This 'conducted tour' across the protein is enforced by the strong dipole of the protein. Specific orienting effects in the microenvironment of the active site further enforce a minimum energy reaction pathway for substrate(s), illustrated on Scheme 2, as well as covalently binding inhibitors.

3.1. Conclusion

Using the simple algorithm of CADRAW, two channels were identified that connect the active site gorge of AChE with the protein exterior: the cavities are separarted by apparently flexible loops that might serve as swinging gates operated by local changes in the electrostatic microenvirenment in the course of product release. Such interactions may be tested by replacing some of the constituent residues of the loops. Alternatively, computational experiments with the reaction products present at the active site of the enzyme might be performed. Molecular dynamics simulation of the interaction of charged reaction fragments with residues of the active site cavity is now in progress in this laboratory.

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[22] Qian, N. and Kovach, I.M. (1993) FEBS Lett. 336, 263–266. Optimization in YETI is carried out in an internal/cartesian coordinate space with a conjugate-gradient minimizer. All the bond lengths and bond angles and all the positions of main chain atoms are kept constant during calculation. The YETI force field consists of only non-bonded energies: electrostatic, hydrogen-bonding, van der Waals, metal-ligand and torsional energy terms. Electrostatic energies were calculated with the distance-dependent dielectric parameter set to $D(r) = 2.0r$. The cut-off criteria were as follows: 9.5/10.0 Å for electrostatic interactions, 6.5/7.0 Å for van der Waals interactions and 4.5/5.0 Å for hydrogen-bonding interactions. Convergence criteria were set to 0.025 kcal·mol^{-1}·deg^{-1} for torsional RMS first derivative. The energy convergence criterion was ± 0.05 kcal·mol^{-1}.
[23] The following steps describe the logic of the program: 1. Obtain the crystallographic coordinates for the macromolecule in PDB format. 2. Determine the length, width, and height of the macromolecule. 3. Divide the macromolecule into grids with dimensions per unit-grid-size. 4. Classify each grid-point into either occupied or non-occupied type: Occupied type is defined as a grid-point that is located inside the van der Waals sphere of **any** atom of the molecule and non-occupied type is defined as the grid-point which is located outside the van der Waals sphere of **every** atom of the molecule. Divide all grid-points of nonoccupied type into one of the four categories A B C and D : Category A is for a cavity point defined as a point surrounded by six occupied points along the X Y and Z axes; category B is a channel point defined as a point around which no occupied point can be found along one and only one of the six directions of the three axes, X, Y and Z; category C is for a valley point defined as a point around which no occupied point can be found along one of the three axes, X, Y and Z; and category D is a 'sky point' defined as a point around which occupied point can be found only in one of the six directions. 6. Compute and record the positions of all cavity and channel points. 7. Check the distance of each point from all other cavity or channel points. Identify the size of the vacant space. 8. Assign the points on the interface of the cavity or channel to occupied points. The continuum of these points circumscribe the cavity or space for

visual display. 9. Record the connectivity of all vacant points in a group. 10. Discard unoccupied points for cavities below the size of interest. 11 Compute the Cartesian coordinates of each recorded point and output the coordinates together with their connectivity with other points and their group number in NIH XC format. The output obtained in XC format serves as input into molecular modeling program GEMM. CADRAW is also supplied with a subroutine for visualization of a cavity or channel.

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[28] The three-dimensional structure of C_{240} was built in MacroModel (Still, W.C., Richards, N.G.J., Guida, W.C., Lipton, M., Liskam, R., Chang, G. and Hendrickson, T., MacroModel V4.0, Dept. Chemistry, Columbia University, New York, NY 10027, 1986.) 1814 grid points were generated with CADRAW in a spherical arrangement. This result is in agreement with the 223 $Å^3$ volume calculated with a diameter of 14 Å minus the probe volume. To test the capability of the program for generation of channels, 40 atoms from C_{240} were removed and the computation repeated.