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Key active site residues in the inhibition of acetylcholinesterases by soman

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Molecular modeling (GEMM 7.3) and molecular mechanics calculations (YETI V 5.3) using the X-ray coordinates for acetylcholinesterase (AChE) from *Torpedo californica* indicate electrostatic stabilization by the active site, Glu-199, of the developing positive charge on the incipient carbonium ion in the dealkylation in the adducts of AChE with P_SC_R and P_SC_S diastereomers of 2-(3,3-dimethylbutyl) methylphosphonofluoridate (soman). His-440 is indispensable as a general acid catalyst of C–O bond breaking in the dealkylation reaction and that of bond breaking to the Ser γ–O in reactivation. This demand for catalysis seems to be satisfied for the reactivation of enzyme from the P_SC_S diastereomer of soman, but not from the P(S)C(R) diastereomer.

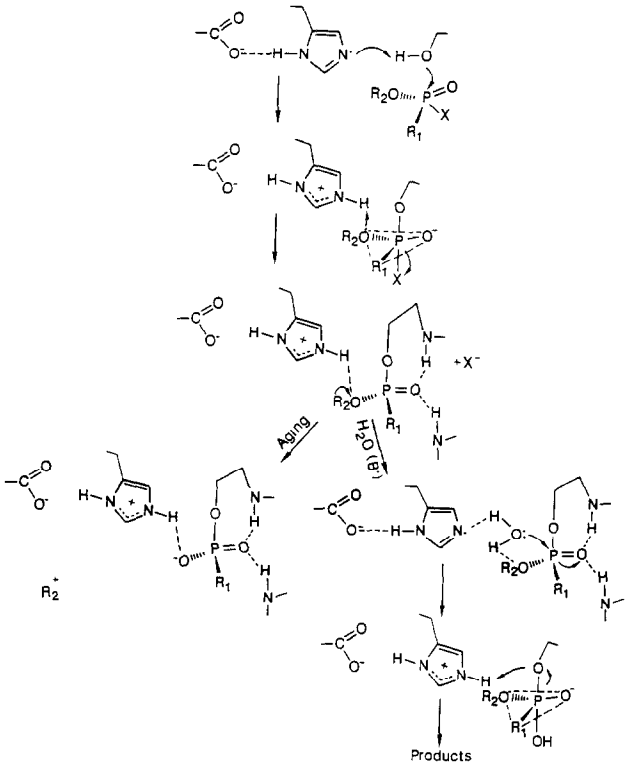
Acetylcholinesterase inhibition; Serine hydrolase inhibition; Irreversible enzyme inhibition; Enzyme inhibition by organophosphorus compound

1. INTRODUCTION

In the elucidation of the molecular mechanisms of inhibition by organophosphonates, the nature and stability of the phosphonylated serine hydrolases, particularly AChE, and the extent of enzymic participation in the spontaneous and nucleophilic dephosphonylation remained in the twilight.

We have previously reported [1–5] on the molecular origin of irreversible inhibition of serine hydrolases, as portrayed in Scheme 1, being the complete impairment of the acid base catalytic apparatus of the enzymes. This is because the proton transferring from the catalytic Ser residue to the catalytic His remains on the His as indicated by the increased pK of His in the adducts [6,7]. The evolutionary destination of the proton is to transfer to the leaving group in the natural substrate in the next step. In organophosphorus compounds, however, the leaving group is at a more distant location, not at 109° but at 180°, from the attacking group. Since protonated, the His cannot serve as a general base catalyst in the next cycle, in the hydrolysis of the phosphonylated enzyme [1]. The positive charge on His may also promote dealkylation of a branched alkyl substituent. Resistance to, even the most potent, nucleophilic reactivation has been attributed to the development of negative charge in the hemiester product of dealkylation. This event should raise the pK of the His even higher because of the stabilizing effect of the negative charge in the vicinity [1].

Now, with the availability of the three-dimensional structure of AChE from *Torpedo californica*, [8,9] results of mechanistic studies of dealkylation and nucleophilic reactivation of the adducts of AChE with enantiomerically pure phosphonates, such as soman (see Structure 1), can be interpreted in terms of specific interactions between active site residues and inhibitor. Molecular mechanics calculations designed for small molecule–macromolecule interactions are uniquely suited for evaluation of preferential orientations in geometry-optimized structures.



Scheme 1

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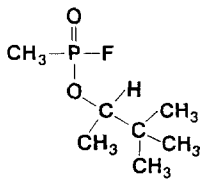

Structure 1

2. MATERIALS AND METHODS

The X-ray crystallographic coordinates for AChE from *Torpedo californica* [8] were obtained from the Brookhaven Protein Data Bank [10]. A missing five-amino-acid segment (485 to 489) of the AChE structure was reconstructed. The ϕ and ψ dihedral angles of the main chain of the segment were adopted from the results of a statistical search of the database in molecular modeling package GEMM (V7.8), [11] and the side chains were optimized with molecular mechanics program YETI (V6.3) [12]. 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. 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 $D(r) = 2.0r$ [13]. 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, to 0.025 kcal-mol^{-1} · deg^{-1} for rotational RMS first derivative and to 0.250 kcal · mol^{-1} · Å^{-1} for translational RMS first derivative. The energy convergence criterion was ± 0.05 kcal · mol^{-1}.

The structure of soman was built by using program MacroModel (V4.0) [14]. Its geometry was adjusted according to the crystallographic structures of several phosphonate analogues. Then, the structure of soman was subjected to energy minimization using MM2, a module of MacroModel. Finally, the MM2-minimized structure was further refined in MNDO, as implemented in MOPAC (V6.0) [15] to obtain partial atomic charges.

Covalent adducts of AChE with soman were generated from the refined structures, respectively. Refinement was performed first on the phosphonylated Ser-200, then gradually extended to an increasing number of residues around the active site.

3. RESULTS

Figs. 1 and 2 show some selected active site residues of the two refined structures of the adducts of AChE with P_SC_R-soman and P_SC_S-soman, respectively. In both adducts, the phosphonyl oxygen forms hydrogen bonds with Gly-118 and Gly-119, and the *t*-butyl group shows strong van der Waals attraction to Trp-84 and Phe-330. The chiral C center of the soman moiety is 3.3 Å away from one of the two oxygens of the side chain of Glu-199 in both adducts. Superimposition of the two refined structures (Fig. 3) shows that the two active sites are not substantially different except for His-440. In the adduct with C_S configuration, the active site of AChE can accommodate the soman moiety without noticeable conformational change. However, in the adduct with C_R configuration, the methyl group at the chiral carbon is in steric interference with His-440. As a consequence, the imidazole plane of His-440 is forced to rotate 60 degrees out of the optimum position.

4. DISCUSSION AND CONCLUSIONS

Figs. 1 and 2 show the stereoscopic image of the refined structures of adducts of AChE with P_SC_R-soman and P_SC_S-soman, respectively. The phosphonyl oxygen forms hydrogen bonds with G-118 and G-119, and the *t*-butyl group engages in hydrophobic interactions with W-84 and F-330. Glu-199 is ideally suited, at 3.3 Å from the pinacolyl moiety, for electrostatic stabilization of the positive charge on the developing carbonium ion in the transition state for dealkylation. The carbocation is known to undergo rapid rearrangement into predominantly alkene products and a minor amount of secondary alcohol [16].

Bell-shaped pH–rate profiles of earlier studies of the

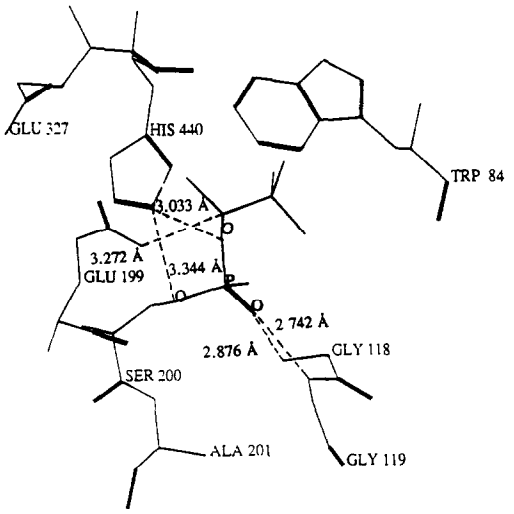

Fig. 1. Some selected residues in the active site of the P_SC_R-soman-AChE adduct showing the 'push–pull' mechanism of dealkylation.

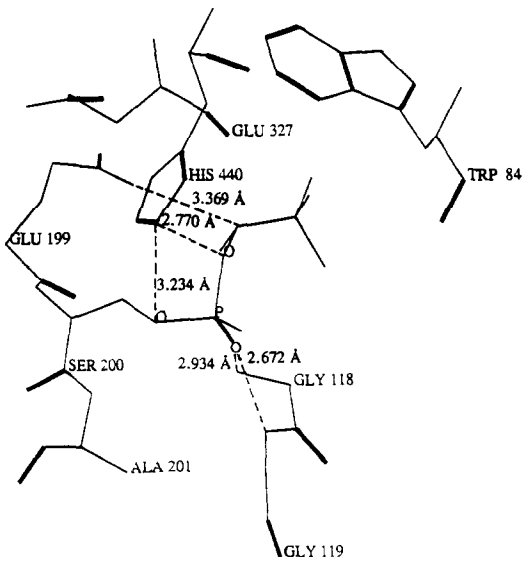

Fig. 2. Some selected residues in the active site of the P_SC_S-soman-AChE adduct showing the 'push–pull' mechanism of dealkylation.

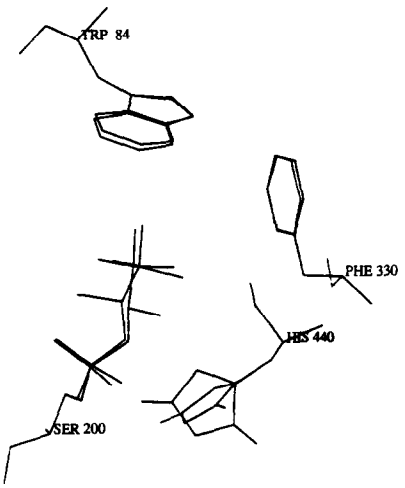

Fig. 3. Superimposed active sites of the adducts of AChE with P_SC_S-soman and P_SC_R-soman.

dealkylation of soman-inactivated-AChE indicate participation of a residue with pK 4.5 [17] and another residue with pK~6.0 [17,18]. The former is most likely to be E-199 and the latter is protonated His-440. These two residues seem to work in concert as in a 'push–pull' mechanism. Our studies of the pH dependence of the dealkylation reaction of both diastereomeric adducts of AChE with soman between pH 8 and 10 show the diastereomers dealkylate at similar rates in first-order dependence on hydronium ion concentration [19]. pH dependence of the dealkylation of cycloheptyl from the cycloheptyl methylphosphonyl adduct of AChE *Torpedo californica* [20] and from bovine erythrocytes [21] were similar. An inflection point corresponding to the residue with the lower pK was also very ionic strength sensitive [20], which is fully consistent with electrostatic stabilization of the developing positive charge.

Dealkylation of the soman-inactivated AChEs are associated with a solvent isotope effect of 1.6 [19]. A solvent isotope effect near 2 is expected for rate-determining protonation, whereas the smaller effect of 1.6 may indicate protonation by an acid catalyst prior to rate-determining C-O bond breaking.

The two active site conformations are not substantially different except for H-440 as discernable from Fig. 3. In the adduct with C_S configuration, the active site of AChE can accommodate the soman moiety while preserving the native conformation. In contrast, in the adduct with C_R configuration, the methyl group at the chiral C is in steric interference with H-440. As a consequence, the imidazole ring of H-440 is forced to rotate 60 degrees out of the optimum position and *out of alignment for an optimal, linear, proton transfer to the γ-O of S 200*. This impaired general acid catalysis of P-O bond cleavage to S 200 can reduce the efficiency of nucleophilic reactivation of AChE activity from the adduct with $P_S(-)C_R(-)$-soman [22] relative to the one with $P_S(-)C_S(+)$-soman. This observation explains that Electric eel AChE could be reactivated with 5 mM HI-6 to only about 15% from the adduct of AChE with the P(−)C(−) diastereomer of soman and up to 60% from the adduct with the P(−)C(+) diastereomer [19,23]. Reactivation of the latter is two and a half times faster than the former.

Glu-199, Asp-276 and the hydrophobic residues lining the entrance gorge of AChE, promote the influx of acetylcholine, unnatural substrates and inhibitors by exerting a negative electrostatic field [9]. Since the leaving group cleaved off from soman bears a negative charge, it should be repelled by the negative electrostatic field and needs a different escape route. We have recently mapped the exit channels for product release [24] and found a mobile loop, consisting of residues 279–292, separating the gorge from a positively charged bay region. The loop may move to give way to the departing F^- ion in the direction of the bay region.

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