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Docking Study of Enantiomeric Fonofos Oxon Bound to the Active Site of *Torpedo californica* Acetylcholinesterase

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Abstract—Molecular interaction between enantiomeric fonofos oxon (*O*-ethyl *S*-phenyl ethylphosphonothiolate) and acetylcholinesterase (AChE) of *Torpedo californica* was evaluated by using the Cerius2 program. It was suggested that the difference in the inhibitory activity of the two enantiomers of fonofos oxon on AChE is due to the steric hindrance in binding to the AChE active site.

Introduction

Acetylcholinesterase (AChE) catalyzes ester hydrolysis of the neurotransmitter acetylcholine, resulting in termination of neurotransmission.[1,2] The X-ray structure of the catalytic subunit has been solved for the *Torpedo californica* enzyme.[3] The active site is found at the bottom of a deep and narrow gorge and consists of at least six domains: (1) an esteratic subsite containing a catalytic triad:[3–6] the nucleophilic serine (Ser 200)[3,4] and the residues (His 440 and Glu 327)[3] responsible for the transition state stabilization, (2) the hydrophobic site[7–9] for the alkoxy group of the substrate including residues Trp 84, Phe 330, and Phe 331, (3) the oxyanion hole[3,10–12] consisting of residues Gly 118, Gly 119, and Ala 201, (4) an acyl pocket (Phe 288 and Phe 290)[7,13,14] that binds the acetyl group of the substrate, (5) an anionic subsite (Trp 84)[3,6,15–17] that accommodates the positive choline moiety of the substrate, and (6) a second peripheral anionic subsite (Trp 279)[6,12,16,18] which lines the gorge entrance.

Torpedo AChE employs a catalytic triad of Glu 237, His 440, and Ser 200, where the Ser 200 is the proximal nucleophile in the hydrolysis. AChE also catalyzes the hydrolysis of organophosphonates, ligands which react with the enzyme by rapidly phosphonylating the active site Ser 200, by acylation of the enzyme.[19] The reaction of AChE with organophosphonates displays marked stereospecificity and dependence on substituent size.[20] The absolute configuration of fononofos (*O*-ethyl *S*-phenyl ethylphosphonodithioate) and its active form, fonofos oxon (*O*-ethyl *S*-phenyl ethylphosphonothiolate) was established by X-ray diffraction analysis.[21] This is the first case of the resolution and determination of the absolute configuration of a thionate organophosphorus insecticide, to our best knowledge. The activity of chiral isomers of fonofos oxon was determined in the in vitro inhibition of electric eel, house fly-head and bovine erythrocyte AChE, horse serum cholinesterase, and variety of esterases.[22] Although the data for *Torpedo* AChE is not available, the results for electric eel were used in the molecular simulation. Thus, the availability of X-ray chrystallography of resolved enantiomerically pure fonofos oxon offers a unique means for analysis of configuration and spatial orientation in the active center in respect of the available three dimensional coordinates of *T. californica* AChE. Herein, we examine the role of these six domains of *T. californica* AChE indicating stereospecificity for enzyme phosphonylation by fonofos oxon and describe the positioning of tetrahedral ligands in the active center of the enzyme. The difference in inhibition between these enantiomers is investigated in terms of stabilization of fonofos oxon in an oxyanion hole and the Ser 200 toward the phosphorus atom.

Results and Discussion

When the structure of a more active enantiomer (*S*)-form is viewed with the leaving group (thiophenyl) toward the observer and P=O group vertical (P down),

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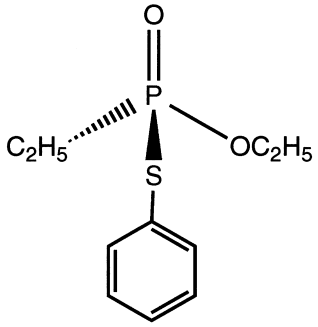
a smaller substituent (ethyl) is located at the left side and a larger one (ethoxy) toward the right (Fig. 1). This is the case for the most reported compounds.[23–28] When the inhibitor with a stable conformation interacts with the enzyme active center to form hydrogen bonds between the phosphoryl oxygen and the backbone amide nitrogens of Gly 118, Gly 119 and Ala 201 in the oxyanion hole, the most probable leaving group is placed in the direction of Trp 84. The leaving group points toward the gorge entrance, favoring a backside attack of the active Ser 200 on the phosphorus atom. The Ser 200 OH, which is activated by the charge relay system consisting of His 440 and Glu 327, attacks the phosphorus from the opposite side of the leaving group, i.e. aromatic ring, of (*S*)-form (Fig. 2). This places the leaving group approximately 180° from the side chain oxygen of Ser 200 and directed out of the gorge, facilitating concomitant apical displacement with apical attack. This orientation has already been evidenced for *O*-alkyl methylphosphonothiolates.[29,30]

Meanwhile, the Ser 200 OH attacks the phosphorus from the opposite side of the phosphoryl-*O* of (*R*)-form (Fig. 3). The smaller non-leaving group of the (*R*)-enantiomer, i.e. ethyl, suffers steric restraint with Ser 200 and does not fit into the acyl pocket. Steric limitation of the acyl pocket may prevent the accommodation of bulkier *O*-ethyl moiety in the (*R*)-form. Total energy value of (*S*)-form for binding with the activie site of AChE was 32,443.4 kcal, whereas that of (*R*)-form was 35,252.4 kcal. Thus, minimization of chiral fonofos oxon and AChE showed that the less active (*R*)-form had larger steric hindrance in its interaction with the active site of AChE than the more active (*S*)-form. Therefore, it is most likely that the more active (*S*)-form adapts the orientation depicted in Figure 4 when binding to the active site of the enzyme. This clearly shows that substrate selectivity and enantiomeric preference of AChE as well as rates of covalent reaction are all governed in a large part through steric limitations imposed with the narrow acyl pocket.



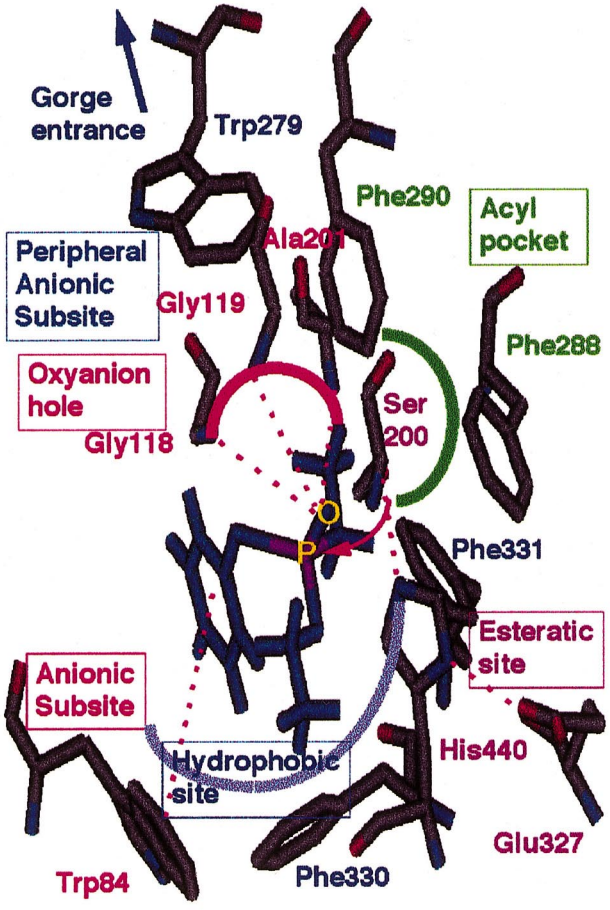


Figure 2. Superposition of (*S*)-fonofos oxon with six domains of AChE.

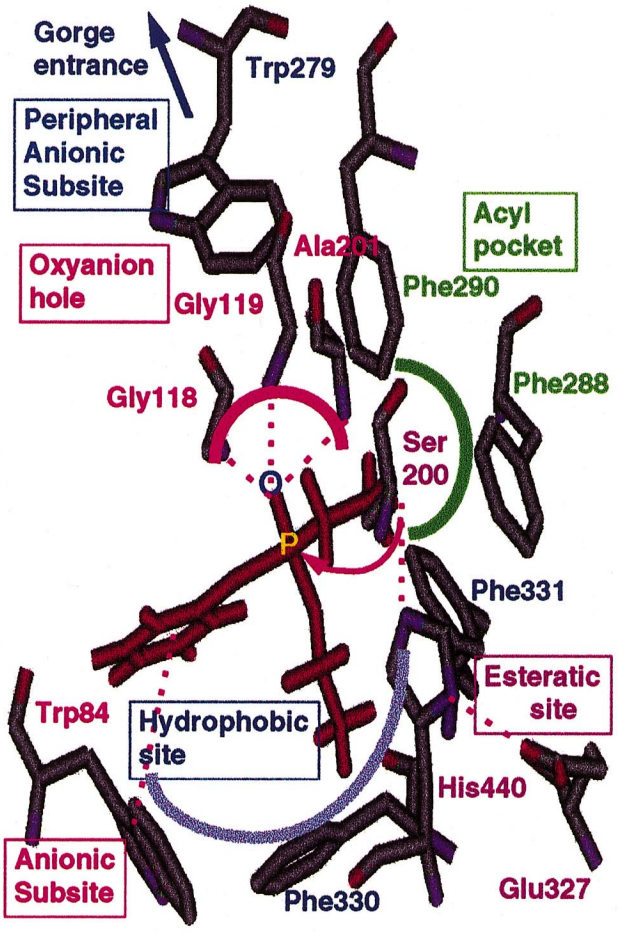


Figure 3. Superposition of (*R*)-fonofos oxon with six domains of AChE.

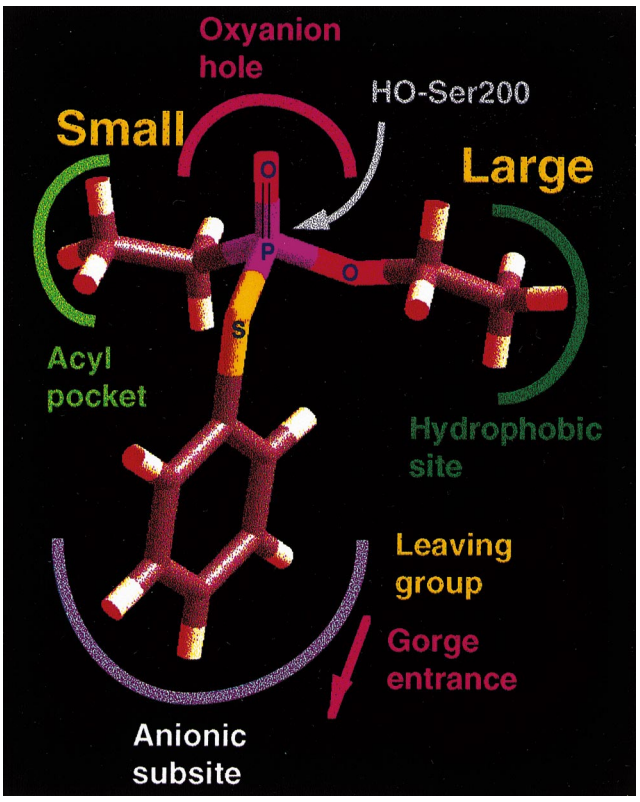


Figure 4. Model of interaction of (*S*)-fonofos oxon with AChE.

Since the pentacoordinate intermediate would have to undergo at least one Berry pseudorotation[31] (or turnstile rotation[32]) before the release of the leaving group, this process results in the retention of the phosphorus configuration. Whereas the process for (*R*)-form results in the inversion of the phosphorus configuration, corresponding to the model of Järv et al.[33] However, this is still to be clarified by X-ray crystallography of the active center of AChE phosphonylated with enantiomeric fonofos oxon.

Experimental Procedure

Enzyme and ligands

The coordinate for *T. californica* AChE was obtained from the Protein Data Bank at Brookhaven National Laboratory[3,34] and fonofos oxon from Allahyari et al.[21] (*S*)-Fonofos oxon was 9.3 times more active than the (*R*)-form in the inhibition of electric eel AChE.[22]

Molecular modeling

All molecular simulations were conducted with Molecular Simulations Incorporated's Cerius2 3.8 environment on a Silicon Graphics O2, runnning under the IRIX 6.5 operating system. The ligands were docked manually in the active site in a position that would minimize collisional interactions between molecules and in a position appropriate for an SN2 type reaction involving Ser 200. AChE-fonofos oxon complexes were minimized as follows in two steps using Universal 1.02 forcefield and steepest descents method. First, the energy expression (an equation describing the energy of the system as a function of its coordinates) was defined and evaluated for a given conformation. Energy expressions were defined that include external restraining terms to bias the minimization, in addition to the energy terms. Next, the conformation was adjusted to lower the value of the energy expression. A minimum was found after a maximum number of 500 iterations. The efficiency of the minimization is therefore judged by both the time needed to evaluate the energy expression and the number of structural adjustments (iterations) needed to converge to the minimum.

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References

1. Rosenberry, T. L. *Adv. Enzymol.* **1975**, *43*, 103.
2. Taylor, P. In *Pharmacological Basis of Therapeutics*; Macmillan: New York, 1985; pp 110–129.
3. Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. *Science* **1991**, *253*, 872.
4. Gibney, G.; Camp, S.; Dionne, M.; MacPhee-Quigley, K.; Taylor, P. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 7546.
5. Shafferman, A.; Kronman, C.; Flashner, Y.; Leitner, M.; Grosfeld, H.; Ordentlich, A.; Gozes, Y.; Cohen, S.; Ariel, N.; Barak, D.; Harel, M.; Silman, I.; Sussman, J. L.; Velan, B. *J. Biol. Chem.* **1992**, *267*, 17640.
6. Shafferman, A.; Velan, B.; Ordentlich, A.; Kronman, C.; Grosfeld, H.; Leitner, M.; Flashner, Y.; Cohen, S.; Barak, D.; Ariel, N. *EMBO J.* **1992**, *11*, 3561.
7. Ordentlich, A.; Barak, D.; Kronman, C.; Flashner, Y.; Leitner, M.; Segall, Y.; Ariel, N.; Cohen, S.; Velan, B.; Shafferman, A. *J. Biol. Chem.* **1993**, *268*, 17083.
8. Ordentlich, A.; Barak, D.; Kronman, C.; Ariel, N.; Segall, Y.; Velan, B.; Shafferman, A. *J. Biol. Chem.* **1995**, *270*, 2082.
9. Ordentlich, A.; Barak, D.; Kronman, C.; Segall, Y.; Velan, B.; Shafferman, A. *J. Biol. Chem.* **1996**, *271*, 11953.
10. Barak, D.; Ariel, N.; Velan, B.; Shafferman, A. In *Multidisciplinary Approaches to Cholinesterase Functions*; Shafferman, A., Velan, B., Eds.; Plenum Press: New York, 1992; pp 195–199.
11. Cygler, M.; Grochulski, P.; Kazlauskas, R. J.; Schrag, J. D.; Bouthillier, F.; Rubin, B.; Serreqi, A. N.; Gupta, A. K. *J. Am. Chem. Soc.* **1994**, *116*, 3180.
12. Harel, M.; Sussman, J. L.; Krejci, E.; Bon, S.; Chanal, P.; Massoulié, J.; Silman, I. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 10827.
13. Vellom, D. C.; Radic, Z.; Li, Y.; Pickering, S. N.; Camp, A.; Tayor, P. *Biochemistry* **1993**, *32*, 12.
14. Tayor, P.; Radic, Z. *Annu. Rev. Pharmacol. Toxicol.* **1994**, *34*, 281.
15. Weise, C.; Kreienkamp, H.-J.; Raba, R.; Pedak, A.; Aaviksaar, A.; Hucho, F. *EMBO J.* **1990**, *9*, 3885.
16. Harel, M.; Schalk, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Hirth, C.; Axelsen, P.; Silman, I.; Sussman, J. L. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 9031.
17. Sussman, J. L.; Harel, M.; Silman, I. In *Multidisciplinary Approaches to Cholinesterase Functions*; Shafferman, A., Velan, B., Eds.; Plenum Press: New York, 1992; pp 95–107.

18. Schalk, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Hirth, C. In *Multidisciplinary Approaches to Cholinesterase Functions*; Shafferman, A., Velan, B., Eds.; Plenum Press: New York, 1992; pp 121–130.
19. Aldridge, W. N.; Reiner, E. In *Enzyme Inhibitors as Substrates*, Neuberger, A.; Tatum, E. L., Eds.; North-Holland Publishing Amsterdam and American Elsevier Publishing: New York, 1972.
20. Berman, H. A.; Decker, M. M. *J. Biol. Chem.* **1989**, *264*, 3951.
21. Allahyari, R.; Lee, P. W.; Lin, G. Y.; Wing, R. D.; Fukuto, T. R. *J. Agric. Food Chem.* **1977**, *25*, 471.
22. Lee, P. W.; Allahyari, R.; Fukuto, T. R. *Pestic. Biochem. Physiol* **1978**, *8*, 146.
23. Wu, S.-Y.; Hirashima, A.; Takeya, R.; Eto, M. *Agric. Biol. Chem.* **1989**, *53*, 165.
24. Sasaki, M. In *Bioorganic Chemistry of Pesticides*; Eto, M. Ed.; Soft Science: Tokyo, 1985; pp 210–222.
25. Eto, M. In *Chemistry of Plant Protection*, Springer-Verlag: Berlin, 1990; Vol. 6; pp 60–107.
26. de Jong, L. P. A.; Benshop, H. P. In *Stereoselectivity of Pesticides, Biological and Chemical Problems;* Ariëns, E. J., van Resen, J. J. S., Welling, W., Eds.; Elsevier: Amsterdam, 1988; pp 109–149.
27. Wustner, D. A.; Fukuto, T. R. *Pestic. Biochem. Physiol* **1974**, *4*, 365.
28. Wustner, D. A.; Fukuto, T. R. *J. Agric. Food Chem.* **1973**, *21*, 756.
29. Hosea, N. A.; Berman, H. A.; Taylor, P. *Biochemistry* **1995**, *34*, 11528.
30. Albaret, C.; Lacoutière, S.; Ashman, W. P.; Froment, D.; Fortier, P.-L. *Protein* **1997**, *28*, 543.
31. Berry, R. S. *J. Chem. Phys* **1960**, *32*, 933.
32. Ugi, I.; Marquarding, D.; Klusacek, H.; Gillespie, P.; Ramirez, F. *Acct. Chem. Res.* **1971**, *4*, 288.
33. Järv, J.; Aaviksaar, A.; Godovikov, N.; Lobanov, D. *Biochem. J.* **1977**, *167*, 823.
34. Berstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, Jr., E. F.; Brice, M. D.; Rogers, J. R.; Kennard, O.; Shimanouchi, T.; Tatsumi, M. *J. Mol. Biol.* **1977**, *112*, 535.