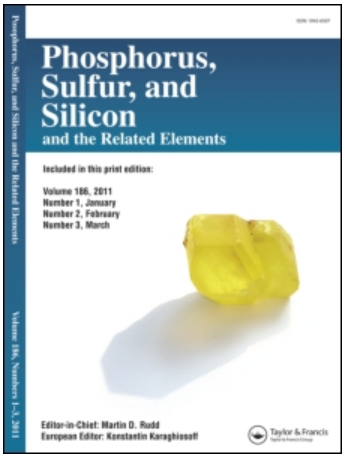

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INTERACTIONS IN TETRAVALENT AND PENTAVALENT PHOSPHONATE ESTERS OF SER AT THE ACTIVE SITE OF SERINE ENZYMES

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Abstract The stabilizing interactions in pentavalent and tetravalent phosphonate esters of the active-site Ser in selected serine hydrolase enzymes can be used to explain how these man-made molecules recruit enzyme catalytic power up to 70% of that of the natural substrate. The removal of the leaving group of a substrate or inhibitor is very efficient by acetylcholinesterase (AChE) because it is aided by hydrophobic forces and the repulsive interaction with the negatively charged Glu199 at the active site of AChE. The interactions between protein and small molecular fragments were evaluated with molecular mechanics and dynamics. The conclusions should be informative to the design of haptens for antibodies and efforts to drug design and detoxification after enzyme inhibition.

Key Words serine hydrolase inhibition, acetylcholinesterase inhibition, chymotrypsin inhibition, trypsin inhibition, molecular mechanics, molecular dynamics.

INTRODUCTION

Phosphonylation of serine hydrolase enzymes probably takes place via an in-line attack of the Ser nucleophile at the central P with the leaving group at 180 ° from the entering group and involves the formation of a pentacoordinate transient along the reaction path [1-2]. The life-time of the transient depends on the electronic and steric nature of all the substituents not only the leaving group [2-3]. Reactions of the enzymes with organophosphorus compounds that have good leaving groups most likely occur with a nearly concerted departure of the leaving group to Ser attack and the acceleration by the enzymes relative to the uncatalyzed aqueous hydrolysis is astonishing (10^6-10^{11}) [4]. Most recently, we studied the propensity of the catalytic machinery of serine hydrolases to promote leaving-group departure in phosphonates. We generated high-quality geometric and charge parameters by an *ab initio* 6-31$^+$G* basis set (GAMESS) [5] for dimethoxy methylphosphonofluoridate, a trigonal bipyramid. Using the parameters we generated pentavalent adducts of the active-site Ser modified with 2-(3,3- dimethyl-butyl) methylphosphonofluoridate (soman). Soman is one of the most efficient inhibitors of the enzymes [6] and it has a small leaving group, F^-, an advantage in computations. These fragments were then incorporated into the active site of AChE, trypsin and chymotrypsin to evaluate the specific nonbonding interactions that contribute to

molecular recognition of phosphonate esters and the elements that may preferentially promote leaving-group departure. We provide here an analysis of the results that should be informative to the design of haptens for antibodies and efforts to drug design and detoxification after enzyme inhibition.

METHODS

The molecular mechanics-optimized (YETI V5.3) [7] structures reported earlier for the fully solvated AChE, trypsin and chymotrypsin were used in these calculations [2]. Both nitrogens Nε and Nδ were protonated on the catalytic His to represent the protonation state of phosphorylated adducts according to earlier measurements on trypsin and chymotrysin [8]. Each diastereomer of the serine ester of soman was then incorporated into the structure of the enzyme to be studied and the entire structure was again energy-minimized in YETI. Molecular dynamics simulations were carried out with program CHARMM (Vc22g5) [9] on energy-minimized native AChE and trypsin and covalently modified AChE all solvated with one water shell. All atoms were represented explicitly. The TIP3P model, as implemented in CHARMM was employed for the simulation of solvate water molecules. The parameters for the amino acid residues were from the standard parameter file of the CHARMM program. No H-bonding term was used in the empirical potential energy function. The stochastic boundary calculation was carried out with a < 18 Å radius reaction zone centered on Ser200 Oγ, a 2 Å shell buffer zone, and the region beyond the 20 Å radius was kept constant. Langevin dynamics were used in the buffer zone. Numerical integration by the leap frog integrator with a 1 fs step size was used for the molecular dynamics calculations. A switching function was used on the force for the long range non-bonding energy terms with the cutoff value of 12 Å.

RESULTS AND DISCUSSION

The geometric parameters for critical interactions are in Table 1. Figure 1 shows the equilibrium structures of the active site of AChE, trypsin and chymotrypsin with the pentavalent intermediate formed immediately after attack of the catalytic Ser on soman.

TABLE I

Distances (Å) between the F atom and stabilizing residues in the pentavalent adducts of serine hydrolases with diastereomers of soman; residue numbering AChE/trypsin

Interaction[a]	AChE		Trypsin		Chymotrypsin	
	P_SC_S	P_RC_S	P_SC_S	P_RC_S	P_SC_S	P_RC_S
Gly119/193N-H---FP	2.73	3.09	3.74	3.74	3.02	3.60
Glu199O1---FP	6.14	5.94				
Glu199O2---FP	6.58	6.42				
WAT536O---FP	4.49	4.45	2.60	2.68	3.30	4.24

The P_SC_S diastereomers is formed in an in-line attack by Ser on the faster-reacting soman diastereomers.

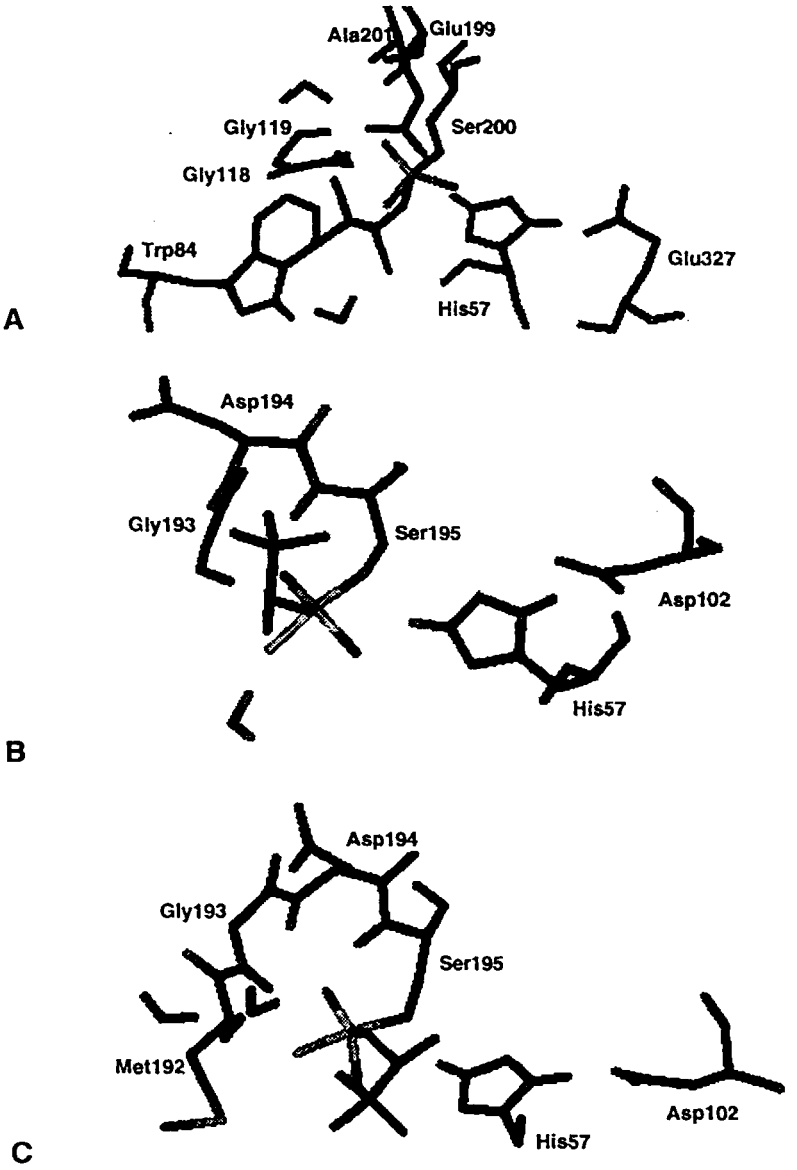

Figure 1. Active-site interactions in the pentavalent P_SC_S dieastereomers of soman-inhibited A. AChE, B trypsin and C chymotrypsin. Protein-protein interactions were within 5 kcal/mol for each adduct of an enzyme and protein-water interactions were within 100 kcal/mol corresponding to no more than a difference of 2-8 water molecules.

A salient feature of the results is the much more crowded active site of AChE than those of the serine proteases. Although the total sum of stabilizing energies are greater for the AChE structures, favorable electrostatic and H-bonding interactions are often counter-balanced by severe van der Waals repulsions reflected in a net repulsive interaction within the phosphonate fragment. Repulsion energies are also observable in the Glu-His diad. Molecular dynamics simulation of the structure on the 200 ps time scale ameliorated the repulsion within the protein but compressed the pentavalent structure. The interactions in the oxyanion hole were diminished during the simulation and those with the F^- were enhanced. The observation is consistent with propositions for the origins of enzyme catalytic power in general and serine hydrolase catalysis in particular. Earlier mechanistic investigations indicate that the distortions, at least partly, could precede bond formation with the active-site Ser [1a,d,f]. Inactivation of AChE by soman was characterized by a partly rate-determining conformational adjustment that probably serves to optimize the fit between active site and inhibitor. Apparently, the adjustment needs to be gradual and is induced as the inhibitor tumbles down the active-site gorge and approaches the bottom.

A key focus of the calculations was the general question of leaving group departure from the pentavalent transient. The F atom was placed in the apical position as expected by the Westheimer rules [10]. Elimination of F^- from these structures is very efficient, which requires the presence of stabilizing forces. Since the protein was adequately solvated, the promotion of F^- departure comes partly from solvation and partly from the strong positive electrostatic field in the oxyanion hole. In addition, an electrostatic push from Glu199 of AChE may play a role in stabilizing the departing F^-. This would also be consistent with the great efficiency of AChE inactivation.

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