Catalytic Recruitment by Phosphonyl Derivatives as Inactivators of Acetylcholinesterase and Substrates for Imidazole-Catalyzed Hydrolysis: β-Deuterium Isotope Effects

Andrew J. Bennet,† Ildiko M. Kovach,* and Jeffrey A. Bibbs

Contribution from the Center for Biomedical Research, University of Kansas, 2099 Constant Avenue, Lawrence, Kansas 66046. *Received February 28, 1989*

Abstract: β-Deuterium secondary isotope effects for the phosphonylation of the active site serine of acetylcholinesterase (AChE) by phosphonyl derivatives at 25 °C and pH 7.70 in 0.05 M phosphate buffer were as follows: 3,3-dimethyl-2-butyl methylphosphonofluoridate (soman), 0.90 ± 0.03; 2-propyl methylphosphonofluoridate (sarin), 0.91 ± 0.04; 4-nitrophenyl 2-propyl methylphosphonate (IMN), 0.93 ± 0.05. β-Deuterium isotope effects for the imidazole-catalyzed hydrolysis of the same three compounds and of bis(4-nitrophenyl) methylphosphonate (NMN) were similar: soman, 0.96 ± 0.03; sarin, 0.96 ± 0.02; IMN, 0.96 ± 0.02 (73.0 ± 0.01 °C); NMN, 0.94 ± 0.02. The results indicate an increase in the force constants for the CL (L = H, D) bonds adjacent to phosphorus at the transition state for phosphonylation. This trend is pronounced in the AChE reaction, conceivably due to a more compressed structure at the transition state for the AChE reaction in comparison to the imidazole-catalyzed hydrolysis of the phosphonyl derivatives.

We have recently published observations on the recruitment of the catalytic power of serine hydrolase enzymes by reactive phosphonyl derivatives.[1-3] The compounds in our investigations are nearly irreversible inhibitors of serine hydrolases, because they activate the catalytic apparatus at the active site of the enzymes by strong (and quite specific) interactions.[3] In doing so, they elicit general base catalyzed phosphonylation of the active site serine. Acetylcholinesterases (AChE), the physiologically most consequential target enzymes for phosphonylation,[2] showed more complex mechanisms of phosphonylation than did serine proteases.[1] For example, solvent isotope effects for phosphonylation of AChE are only 1.3-1.6, rather than 2-4 as expected for general-base catalysis. This suggests that the reaction proceeds through a partially rate-limiting proton-transfer process with a contribution of solvent isotope insensitive events.[2a] One of these is the bond-breaking process when 4-nitrophenyl 2-propyl methylphosphonate (IMN) is the inhibitor. This conclusion is inferred from a 6 ± 3% ^{18}O leaving group effect on the inactivation of AChE by IMN.[2b] We further wished to investigate the features of activation of the catalytic potential of serine hydrolases by probing the extent of structural changes and distortions experienced at the phosphorus center in the transition state of enzymic and comparable nonenzymic phosphonylation reactions.[4]

In the recent past β-deuterium isotope effect measurements have been developed as diagnostic tests of the extent of rehybridization in acyl-transfer reactions.[5] We now have extended this concept to phosphoryl-transfer reactions, on the basis of the premise that some similarities in the overall course of structural rearrangement at carbonyl and phosphoryl may exist. The origin of all secondary isotope effects lies in the alteration of the vibrational force field of the atoms involved with only a minor contribution from changes in mass and moments of inertia in the course of the reaction.[6] Changes in the vibrational force constants themselves stem from hyperconjugative, inductive, and steric effects.[7] In the case of β-secondary deuterium isotope effects in substitution at carbonyl, the changes in hyperconjugation appear to be predominant over the other two components. Systems in which a combination of inductive and steric effects is revealed in the deuterium isotope effects have also been reported. For instance, inductive effects combined with steric effects, due to the hydration of the positive charge, in the ionization equilibrium for methyl- and dimethylammonium ion [CL_3 and $(CL_3)_2$, L = H, D] have been inferred from β-deuterium isotope effects of 3-4% per D.[8] A predominance of the contribution of steric effects has been suggested for the methylation of *N,N*-dimethylaniline and dimethylphenylphosphine [$(CL_3)_2$, L = H, D] by methyl tosylate.[9] This study gave inverse isotope effects of 2% per D for the amines and 1% per D for the phosphines respectively in the direction of positive charge development at the transition state. The authors proposed that the larger inverse effect for the quaternization of amines than for the quarternization of phosphines is a sign of greater steric crowding at the shorter C-N bond in the transition state.

It is difficult to predict the occurrence, origin, and range of magnitude of force constant changes in phosphonyl-transfer reactions in which nucleophilic attack at phosphorus is occurring. This is particularly true because of the unknown behavior of the d orbitals as acceptors of electrons in different structural situations. This behavior would determine the hyperconjugative contribution to the isotope effect. The simplest view would be that hyperconjugation would decrease with nucleophilic attack, as in carbonyl compounds. This would produce an inverse isotope effect ($k_D > k_H$). Since here the positive charge on phosphorus is vanishing at the transition state upon nucleophilic attack, the inductive

† Present address: Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada.

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Table I. Rate Constants and β-Deuterium Secondary Isotope Effects for Methyl(CL_3)phosphonates (L = H, D) as Inhibitors of AChE and Substrates for Imidazole-Catalyzed Hydrolysis

	substrate or inhibitor, temp, concn	$k(CH_3) \pm SD$, $M^{-1} s^{-1}$	$k(CD_3) \pm SD$, $M^{-1} s^{-1}$	$k(CH_3)/k(CD_3) \pm SD$
AChE[a]	soman, 25.0 ± 0.1 °C, 30–100 nM	$(1.56 \pm 0.03) \times 10^{6}$ [b]	$(1.74 \pm 0.02) \times 10^{6}$ [b]	0.90 ± 0.02
	sarin, 25.0 ± 0.1 °C, 150–400 nM	$(4.61 \pm 0.18) \times 10^{5}$ [b]	$(5.03 \pm 0.16) \times 10^{5}$ [b]	0.91 ± 0.04
	IMN, 25.0 ± 0.1 °C, 8–16 μM	$(7.11 \pm 0.35) \times 10^{3}$ [b]	$(7.65 \pm 0.22) \times 10^{3}$ [b]	0.93 ± 0.05
imidazole	soman, 25.0 ± 0.1 °C, 20–100 μM[c]	$(3.30 \pm 0.08) \times 10^{-3}$	$(3.44 \pm 0.07) \times 10^{-3}$	0.96 ± 0.03
	sarin, 25.0 ± 0.1 °C, 20–100 μM[c]	$(5.47 \pm 0.05) \times 10^{-3}$	$(5.67 \pm 0.07) \times 10^{-3}$	0.96 ± 0.02
	IMN, 73.0 ± 0.1 °C, 50–80 μM[d]	$(4.72 \pm 0.07) \times 10^{-4}$	$(4.91 \pm 0.09) \times 10^{-4}$	0.96 ± 0.02
	NMN, 25.0 ± 0.1 °C, 20–80 μM[e]	$(3.11 \pm 0.04) \times 10^{-3}$	$(3.30 \pm 0.04) \times 10^{-3}$	0.94 ± 0.02

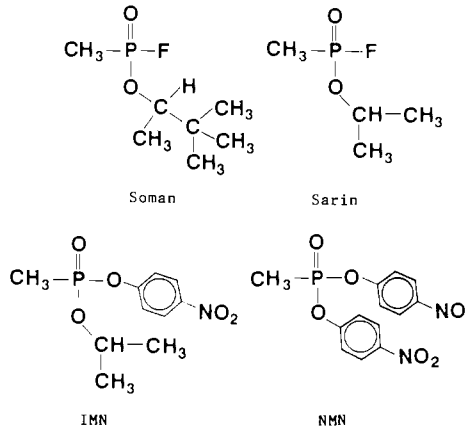
[a] Sigma V-S, 10 units/L. All solutions contained 0.0434 M K_2HPO_4 and 0.0066 M KH_2PO_4 and 5% v/v CH_3OH to yield pH 7.70. [b] The measured value of the rate constant is sensitive to conditions such as ionic strength and the presence of cosolvents. [c] Imidazole/imidazole nitrate = 10.0, total concentration 0.11–0.7 M, pH 8.2, ionic strength 1.0 M with KNO_3, 0.001 M EDTA. [d] Imidazole/imidazole hydrochloride = 10.0, total concentration 0.11–0.7 M, pH 8.2, ionic strength 1.0 M with KCl. [e] Imidazole/imidazole hydrochloride = 1.0, total concentration 0.10–1.0 M, pH 7.2, ionic strength 0.50 M with KCl, 1% v/v CH_3CN.

contribution should generate a normal ($k_H > k_D$) isotope effect. The greater crowding in the transition state should produce an inverse steric contribution ($k_D > k_H$). On the analogy of the examples above,[8,9] theoretical calculations,[6,7] and the experience with carbonyl compounds,[5] the magnitude of inductive and steric effects should be smaller than 1–2% per D. The major effect, if this is so, would be hyperconjugative.

In this study, we have measured β-deuterium isotope effects for phosphonylation reactions of the active site serine of AChE and for hydrolysis catalyzed by imidazole base.

Results

AChE Inhibition. Second-order rate constants for inhibition of AChE [k_i/K_i or $k(CL_3)$, where L = H, D] at 25.0 ± 0.1 °C were determined for 3,3-dimethyl-2-butyl methylphosphonofluoridate (soman), 2-propyl methylphosphonofluoridate (sarin), and 4-nitrophenyl 2-propyl methylphosphonate (IMN), and are listed in Table I. These bimolecular rate constants for AChE



inactivation were obtained by the competitive method described in detail elsewhere.[10] In this procedure, the first-order decline in AChE activity is monitored by a proportional decline in turnover of a good substrate, phenyl or naphthyl acetate. The inverse of a series of first-order rate constants k_{obs} at a fixed substrate concentration [S] was plotted against the inverse of a series of inhibitor concentrations [I] according to eq 1. The intercept at

$$1/k_{obs} = 1/k_i + [K_i/(k_i[I])][1 + [S]/K_m] \quad (1)$$

$1/[I] = 0$ was not well-defined in our experiments to yield a value of k_i with acceptable precision because the concentration of the inhibitors had to be kept below K_i under our conditions. The value of K_m was 2.28 ± 0.1 mM for phenyl acetate and 1.30 ± 0.1 mM for naphthyl acetate.[11] The experiments were carried out at 8 mM phenyl acetate and 2 mM naphthyl acetate. Direct measurements were essential because inhibitor depletion or leaving-group production took place in stoichiometric equivalence to the quantity of AChE inactivated; isotopic competitive techniques using high-precision isotope-ratio mass spectrometry was impossible. The β-secondary deuterium isotope effects [$k(CH_3)/k(CD_3)$] were calculated and are also listed in Table I.

Nonenzymic Hydrolysis. Second-order rate constants for the imidazole-catalyzed hydrolysis [k_{Im} or $k(CL_3)$, where L = H, D] of soman, sarin, and bis(4-nitrophenyl) methylphosphonate (NMN) were measured at 25.0 ± 0.1 °C. IMN was less reactive under these conditions so the hydrolysis was observed at 73.1 ± 0.1 °C. The β-secondary isotope effects [$k(CH_3)/k(CD_3)$] were calculated, and the values along with those for the rate constants are listed in Table I.

Discussion

Catalytic Recruitment in the Inactivation of AChE. Second-order rate constants for the inactivation of AChE by soman, sarin, and IMN involves rate accelerations of 10^{11}–10^{12} relative to their neutral hydrolysis rates.[2,3] Since the mechanism of inactivation involves general-base catalysis by the imidazole of the histidine residue of the active site of AChE, it seems appropriate to express the enzymic acceleration as the ratio of the enzymic rate constant to the rate constant for nonenzymic, imidazole-catalyzed hydrolysis of the phosphonyl derivatives. The acceleration factors are around 10^7 (IMN), 10^8 (sarin), and 10^9 (soman).

β-Deuterium Isotope Effects. All β-deuterium isotope effects are inverse and 0.90–0.96 per CD_3 within experimental error for the seven reactions in Table I. Except for the imidazole-catalyzed reaction of IMN, these reactions were all measured at 25 °C. The temperature dependence of β-deuterium isotope effects in acyl-transfer reactions has been studied in this laboratory lately,[12] and it was found that for the equilibrium hydration of dichloroacetone the effect is 0.94 ± 0.009 per D within 15–45 °C, and for the basic hydrolysis of ethyl acetate it is 0.97–0.98 per D between 5 and 50 °C. Similar temperature dependence is anticipated for the β-deuterium isotope effects in the phosphonylation reactions. Therefore, the force constants for the CL (L = H, D) bonds in the transition state of the phosphonyl-transfer reactions of this study are larger than those for the reactant state.

The effects are less inverse, 0.94–0.96 per CD_3, for the imidazole-catalyzed hydrolysis of four compounds than for the inactivation of AChE by the phosphonyl derivatives.[13] For two of the inhibitors, soman and NMN, the solvent isotope effects are also substantially larger (2.6–2.8)[4] for the imidazole-catalyzed hydrolysis than for the AChE-catalyzed hydrolysis (~1.3).[2] The earlier solvent isotope effect data in accord with the β-deuterium isotope effects of this study indicate an earlier transition state with more advanced proton transfer and probably less heavy-atom rearrangement in the transition state for the imidazole-catalyzed hydrolysis than for the AChE-catalyzed hydrolysis of these phosphonate esters. It appears that a somewhat less increase in the CH(D) force constants occurs at the transition states for the

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nonenzymic reactions than at the transition states for AChE inactivation. One interpretation of this observation would be that the enzyme stimulates its own phosphonylation by factors of 10^7–10^9 in a manner that involves compressing the transition state structure about the transferring phosphorus. Since AChE evolved to catalyze transacylation rather than transphosphonylation, the accommodation of greater bond lengths and different bond angles at P, from carbonyl compounds, may present a particularly tight fit at the active site of AChE.[3] Possibly, compression creates a greater similarity between the transphosphonylation transition state and the "natural" transacylation transition state. The small solvent isotope effect, 1.28 ± 0.1, and large ^{18}O leaving group effect, 1.06 ± 0.03, reported[2b] for the inactivation of AChE with IMN also support the proposition of advanced bond making and breaking at the transition state for AChE inactivation by phosphonate esters.

β-Deuterium Isotope Effect as a Probe of Transition Structure in Phosphonyl Transfer. The origin of inverse β-deuterium isotope effects is an increase in the force constants, i.e., a tightening of the potential for the stretching and/or bending motions of the β-hydrogens.[5] As described in the introduction, the physical reason for such an observation most probably lies in the loss of hyperconjugation of electron density from a CL bond into an electron-deficient, likely to be, d orbital on the adjacent P. The consequence of the loss of this stabilizing interaction is a transition state tighter than the ground state. Hyperconjugation of alkyl groups into electron-deficient orbitals on P has been proposed[14] to explain ^{31}P and ^{19}F chemical shifts in the NMR spectra of $RFP(O)X$ and R_FF_2PX. The shift depends on the number of H or F atoms attached to α-carbon of the alkyl (R) or perfluoroalkyl (R_F) group. This study indicated that the importance of hyperconjugation diminishes as the coordination number on phosphorus increases. The NMR work then lends support to the possibility of the origin of the β-deuterium isotope effect being the loss of hyperconjugation during rehybridization at P from sp^3 to sp^3 d.[15] Back-donation of electron density from an alkoxy oxygen into an empty d orbital has been invoked for the explanation of the greater stability of tetravalent phosphates than phosphonates.[16] Hyperconjugation from CL bonds into d orbitals at P indeed should be less important than that from the lone-pair electrons on alkoxy oxygens, but apparently is measurable by special techniques such as NMR and β-deuterium isotope effects.

The hyperconjugative component of β-deuterium isotope effects is expected to be geometry dependent. The maximum loss of hyperconjugation when the transition state is reached is from the CL bond exactly aligned with the electron-deficient orbital in the ground state and vanishes on the approach of the orthogonal position. How exact the alignment between the CL bond and any of the d orbitals in the methylphosphonates of this study can be is difficult to answer without some theoretical calculations. The maximum β-deuterium isotope effect with freely rotating CL_3 groups could be calculated. In the lack of this information one can only use the β-deuterium isotope effect as a qualitative test of buildup or decrease in charge on a vicinal P and as a probe of transition state conformation.

The other important contribution to inverse β-deuterium isotope effects can conceivably come from enhanced steric interactions at the transition state. The exact magnitude of such contribution is not known for phosphonate derivatives, but it has been shown[9] to be small in other systems. A third contribution to the β-deuterium isotope effect could come from inductive effects, which are expected to be in the opposite direction and thus would cancel out some of the inverse effects. These effects, however, have been found to be very minor for many systems[7,8] including phosphines.[9] The origin of the inductive effect is customarily attributed to anharmonicity,[7b,c] i.e., the greater proximity of the center of electron density of the CD bond to the C atom than that of the CH bond. Recently, however, Williams[6] could reproduce secondary isotope effects on solvolysis reactions by calculation of the inductive effect without invoking anharmonicity. Whatever the origin of the inductive effect might be, its contribution to the β-deuterium isotope effect may be minor.

Conclusion. β-Deuterium isotope effects in phosphonyl transfer for seven different reactions were significantly inverse, 0.90–0.96 per CD_3, on changing tetrahedral geometry at P in the ground state to presumably pentavalent P at the transition state. Since these were the first measurements of β-deuterium isotope effects on phosphonyl transfer, it is not yet possible to tell what the range is for these isotope effects. It is nevertheless clear that the effects are large enough to be useful probes of changes in geometry during phosphonyl transfer. AChE-catalyzed phosphonyl transfers show more inverse β-deuterium isotope effects than imidazole-catalyzed analogues, which is indicative of tighter enzymic than nonenzymic transition states. Tight enzymic transition state structures would be consistent with advanced bond making and breaking, which was the conclusion of previous studies involving other isotopic probes of transition states. Ongoing investigations include the use of β-deuterium isotope effects in other phases of the inhibition process of serine hydrolases by phosphonate esters, and future studies will be extended to the requisite theoretical calculations.

Experimental Section

Materials. Inorganic salts and buffer components were reagent-grade chemicals, which were used as purchased or were dried, recrystallized, or distilled as necessary. Water was distilled, from a copper-bottom still, passed through a Barnstead mixed-bed ion-exchange column, boiled for 20 min, and cooled suddenly. Phenyl acetate (Eastman) was purified by column chromatography and vacuum distillation.

Inhibitors. Isotopomers of soman and sarin labeled in the β-position (CL_3, L = H, D) in 0.01 M aqueous solutions were provided by the Army Medical Institute for Chemical Defense. These solutions were further diluted for kinetic work to 5–25 nM for soman and 50–100 nM for sarin with 10^{-4} M HCl. The concentration of these solutions was determined directly by a chymotryptic method.[17]

Di-2-propyl Methyl(CD_3)phosphonate.[18] ICD_3 (Aldrich, 5 g, 34.5 mM, 98% deuterium content) was added to triisopropyl phosphite (Aldrich, distilled from Na, 9 mL, 35 mmol). The mixture was gradually warmed over a period of 2 h to 100 °C, while the isopropyl iodide side product was refluxing. The mixture was maintained at reflux for 1 h. The bulk of the isopropyl iodide was removed at atmospheric pressure. The product was then distilled at 0.95 mmHg and 48–50 °C to give 6.01 g: yield 95%.

2-Propyl Methyl(CD_3)phosphonochloridate.[19] Diisopropyl methyl(CD_3)phosphonate (3.41 g, 18.6 mmol) was added to dry diethyl ether (distilled from $LiAlH_4$, 20 mL), and the solution was cooled in an ice bath for 10 min. Oxalyl chloride (Aldrich, 1.85 g, 21.2 mmol) in dry diethyl ether (5 mL) was then added dropwise under a dry nitrogen atmosphere with constant stirring. Thereafter, the solution was warmed to room temperature, and stirring was continued for 36 h. Fractional distillation at 15–25 mmHg, 82–84 °C, gave 2.33 g of the chloridate: yield 78.4%.

2-Propyl 4-Nitrophenyl Methyl(CL_3)phosphonate (L = H, D; IMN). The coupling of 4-nitrophenolate with both isotopomers of 2-propyl methyl(CL_3)phosphonochloridate (L = H, D; H form from Alfa Products) in 86.3% yield was carried out as reported earlier.[1,4] The compounds were further purified by HPLC. The aqueous methanolic solutions of the eluate were used in kinetic experiments; therefore, their analytical concentrations were determined spectroscopically after their hydrolysis in strong base to 4-nitrophenol. L = H: NMR (60 MHz; CCl_4, Me_4Si) δ 1.33 [dd, 6 H, $CH(CH_3)_2$], 1.51 [d, 3 H, CH_3, J(P–H) = 27 Hz), 4.4–5.0 [m, 1 H, $CH(Me)_2$], 7.28 [d, 2 H, Ar(2,6), $J_{2,3}$ = 9 Hz], 8.10 [d, 2 H, Ar(3,5)]; MS (70 eV), *m/e* 259 (8.6), 244 (17.7), 218 (27.6), 217 (25.0), 201 (19.8), 139 (60.3), 109 (100.0). L = D: MS (70 eV), *m/e* 262 (78.7), 247 (24.5), 221 (43.6), 220 (34.0), 204 (18.1), 139 (100.0), 109 (45.7).

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Methyl(CD_3)phosphonodichloridate.[20] Diisopropyl methyl(CD_3)-phosphonate (2.82 g, 15.4 mmol) was heated at 145 °C with 2.5 equiv of concentrated HCl to produce the methyl(CD_3)phosphonic acid (1.50 g, 99% yield). The dried acid was then chlorinated with thionyl chloride at 105 °C until the production of HCl gas ceased. The product was separated by fractional distillation under vacuum (20 mmHg, 85–90 °C).

Bis(4-nitrophenyl) Methyl(CL_3)phosphonate (L = H, D; NMN). Methyl(CL_3)phosphonodichloridate (L = H, D; H form from Aldrich) was combined with 2 equiv of the potassium phenolate salt at room temperature to afford the isotopomers of NMN in 95% yield as reported earlier.[1,4] L = H: MS (70 eV), *m/e* 338 (36.0), 337 (35.2), 260 (31.4), 200 (59.7), 154 (43.0), 122 (50.6), 75 (100.0). L = D: MS (70 eV), *m/e* 341(30.0), 340(30.0), 260(29.0), 203(50.0), 157(45.0), 122(62.0), 75(100.0).

Kinetics. Rate measurements for chromophoric species involved the automated acquisition of 500–1000 data points with a Perkin-Elmer Lambda-7 spectrophotometer interfaced to a Zenith Z-100 microcomputer. The temperature was controlled with a Lauda K4/DR circulating water bath furnished with a thermistor probe attached to a digital readout. Measurements of the pH of kinetic solutions before and after reaction were performed with a Radiometer RHM 84 pH meter. Nonenzymic hydrolyses of phosphonofluoridates were carried out with a Radiometer PHM 84 Research pH meter that had been interfaced into a Zenith-158 PC. Radiometer F-1052F fluoride electrode and Radiometer K801 AgCl reference electrodes were used. The temperature was controlled at 25.0 ± 0.1 °C with a Neto 01-T-623 temperature controller that provided circulation of water into the jacket of a cell holder, and it was monitored continually inside the reaction vessel with a thermometer. All fluoride determinations were carried out in polyethylene vials. MV readings were converted to concentrations with the Nernst equation, which were then analyzed by a nonlinear least-squares program. All reactions were followed and calculated for four half-lives.

AChE Inactivation. AChE from *Electrophorus electricus* was obtained with an activity of 1000 μmol units/mg (Sigma type V-S, lyophilized powder). Solutions of AChE were prepared in 0.1 M phosphate buffer at pH 6.9 and 46 ng/mL by dilution of the Sigma preparation and were frozen until use. Reactions were conducted at enzyme concentrations of 0.2–0.5 unit/mL of AChE on the basis of the Ellman assay. The enzyme was introduced into the reaction mixture in 50 μL of a stock solution. The pH was maintained at 7.60 (0.0066 M KH_2PO_4, 0.0434 M K_2HPO_4) in distilled water. Phenyl acetate stock solutions were hydrolyzed to phenol, and the liberated phenoxide ion was measured at 286.9 nm (ϵ 2544 M^{-1} cm^{-1}) to verify actual substrate concentrations. Nonenzymic hydrolysis of phenyl acetate was completely negligible under our conditions.

In a typical kinetic experiment the appropriate volume of buffer was equilibrated within 0.05 °C of the working temperature (as monitored with a thermistor probe) in a quartz cuvette in the cell compartment of the instrument. The inhibitor was injected in a 50–100-μL volume from cold stock solutions, and phenyl acetate was delivered in a 50-μL volume from 0.16 M methanolic stock solutions (8 mM in reaction mixture). The absorbance was monitored at 275 nm after each injection to assure that phenyl acetate was intact before the enzymic reaction began. The reaction was initiated with the injection of the enzyme in a 50-μL volume to bring the total to 1 mL. Some experiments were carried out in an identical manner with 1 mM naphthyl acetate at 233 nm for control.

The irreversible inhibition of AChE in the presence of substrate was studied under pseudo-first-order conditions with a >1000-fold excess of inhibitor over enzyme concentration to obtain a signal equal to 10% decomposition of the substrate. First-order rate constants were calculated by a least-squares fit of absorbance time coordinates. Substrate-independent, second-order inhibition constants were calculated by a linear least-squares fit of the inverse observed rate constants to substrate concentration.

Imidazole-Catalyzed Hydrolysis of Phosphonyl Derivatives. The reactions of phosphonofluoridates were carried out at pH 8.2 in imidazole buffers that contained a ratio of 10:1 free base to the HNO_3 salt. The reactions of NMN were carried out at pH 7.2 in buffers of imidazole to imidazole hydrochloride of 1:1. The imidazole base (Aldrich) was twice recrystallized from benzene, and the HNO_3 was titrated against NaOH standards (Fisher). In the reactions of IMN and NMN, dry HCl gas was used to generate the imidazole hydrochloride. Serial dilutions of the buffer with 1 M total imidazole concentration were made with 1 M KNO_3 solutions and were 1 mM EDTA for the phosphonofluoridates and with 1 M KCl solutions for the 4-nitrophenolates.

Reactions of the phosphonofluoridates were followed by electrometric monitoring of the fluoride release. The fluoride electrode was calibrated with solutions of the serial dilution of a 0.1 M fluoride standard (Radiometer) into the appropriate imidazole buffers and 1 M KNO_3.

Reactions of the 4-nitrophenyl esters were followed by monitoring the release of 4-nitrophenol at 400 nm. The temperature was at 73.0 ± 0.1 °C for the imidazole-catalyzed hydrolysis of IMN. Least-squares slopes from rate vs concentration of imidazole-free base gave second-order rate constants.

Acknowledgment. Many helpful consultations with Professor R. L. Schowen are gratefully acknowledged. This research was supported by Contract DAMD-17-83-C-3199 from the U.S. Army Medical Research and Development Command. The provision of space and facilities by the Center for Biomedical Research is also acknowledged.

Supplementary Material Available: Double-reciprocal plots of average observed rate constants versus concentration of soman, sarin, and IMN for the inactivation of AChE and plots of first-order rate constants as a function of imidazole concentration for the hydrolysis of soman, sarin, IMN, and NMN (7 pages). Ordering information is given on any current masthead page.

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