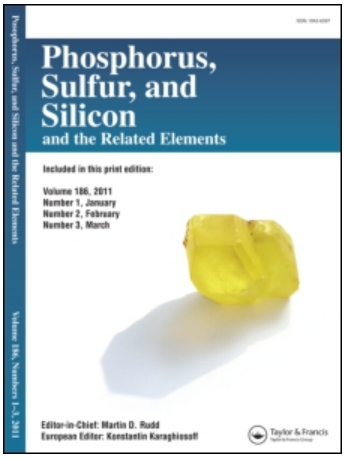

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Solution Dynamics of Phosphonate Ester Hydrolysis

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SOLUTION DYNAMICS OF PHOSPHONATE ESTER HYDROLYSIS

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Abstract The character of buffer-catalyzed hydrolysis of four phosphonates, bis-(4-nitrophenyl) methylphosphonate (NMN), 4-nitrophenyl 2-propyl methylphosphonate (IMN), 2-(3,3-dimethyl)butyl methylphosphonofluoridate (soman), and 4-nitrophenyl 4-methylphenacyl methylphosphonate (MPMN), was investigated. The latter hydrolyzes by an intramolecular nucleophilic mechanism. The transition state for each reaction is discussed.

INTRODUCTION

Imidazole-catalyzed hydrolysis of fluorophosphonates and of phosphonate esters of 4-nitrophenol is of particular interest,[1] because it mimics some features of the inhibition of serine hydrolase enzymes by organophosphorus compounds. Recently, we have reported results of solvent isotope effects and proton inventories for the inactivation of serine hydrolase enzymes.[2-5] We have concluded from these studies that a single proton participates in the reaction coordinate motion in the reaction of serine proteases. This one-proton general base catalysis is consistent with the removal of the proton from the active-site serine hydroxyl by the imidazole of the catalytic histidine.

RESULTS

Rates of reactions of the 4-nitrophenyl phosponate esters were measured by spectroscopic monitoring of the release of 4-nitrophenol. Repetitive scans (240-440 nm) of the the reactions showed clear isosbestic points. The first-order rate constants (k_{obs}) were calculated from automated data acquisition of thousand data points at controlled temperatures. Fluoride ion release from soman was monitored with a Radiometer PHM84 Research pH meter that had been interfaced into a Zenith-158 computer and furnished with a F-1052 fluoride electrode and a K801 AgCl reference electrode. The data were then fit to the following relationship, $k_{obs} = k_{HOH} + k_B[\text{Buffer}]$, where [Buffer] means total concentration of acidic and basic components of the buffer. The activation parameters and their errors were obtained by non-linear least-squares fit of the temperature dependence of the rate constants. Rate measurements for solvent isotope effects and partial solvent isotope effects were carried out in isotopic waters buffered with identical ratios of buffer acid to conjugate base.

TABLE 1. Activation Parameters and Kinetic Isotope Effects for Buffer-Catalyzed Hydrolysis of Phosphonyl Derivatives.

Substrate, Concentration Range Buffer, Temperature Range (°C)	$\Delta H^{\ddagger} \pm SD$ kJ/mol	$\Delta S^{\ddagger} \pm SD$ J/mol K	$k_{HOH}/k_{DOD} \pm SD$	$k_{3H}/k_{3D} \pm SD$[h]
NMN, $CH_3PO(OpNP)_2$[m], 2-8 x 10^{-5} M				
Imidazole[a], 25.0-55.0 ± 0.1	43.7 ± 1.8	-146 ± 5	2.69 ± 0.09[h]	0.94 ± 0.02[l]
Hydroxide[b], 25.0-45.0 ± 0.1	69.1 ± 3.8	27 ± 12	0.51 ± 0.02[h]	0.91 ± 0.04
Water[a,b], 25.0-45.0 ± 0.1	27.6 ± 9.8	-270 ± 31	2.91 ± 1.24[h]	
Phosphate[c]			1.54 ± 0.26[h]	
IMN, $CH_3PO(OiPr)(OpNP)$[m], 1-7 x 10^{-5} M				
Imidazole[d], 45.0-80.0 ± 0.1	56.0 ± 1.5	-150 ± 4	1.40 ± 0.07[i]	0.96 ± 0.02[l]
Hydroxide[e], 25.0-46.0 ± 0.1	33.6 ± 1.6	-143 ± 5	0.94 ± 0.02[h]	0.936 ± 0.005
Water[e]			1.70 ± 0.72[h]	
Water + Hydroxide[d], 45.0-80.0 ± 0.1	60.0 ± 4.0	-170 ± 13	1.20 ± 0.18[i]	
Phosphate[f], 55.0-75.0 ± 0.1	71.8 ± 3.0	-93 ± 9	1.11[j]; 1.15[k]	
Soman, $CH_3PO(OPin)F$[m], 10-12 x 10^{-5} M				
Imidazole[g]			2.79 ± 0.03	0.96 ± 0.02[l]
MPMN, $CH_3PO(OMPh)(OpNP)$[m], 2 x 10^{-5} M				
TRIS[n],			1.70 ± 0.10[h]	
Phosphate[n], 10.0-35.0 ± 0.1	49.0 ± 2.0	-118.0 ± 6.9	2.10 ± 0.10	
Hydroxide[n], 10.0-35.0 ± 0.1	76.0 ± 4.0	77.0 ± 12.0	2.10 ± 0.10	
Hydroxide[o],			0.97 ± 0.02[h]	

[a]Imidazole/imidazole•HCl = 1.0, total concentration 0.1-1.0 M, pH 7.2 at 25.0 ± 0.1°C, μ = 0.5 M (KCl), 1% v/v CH_3CN, [b]0.025 M Sodium borate-HCl, hydroxide concentration 0.001-1 x 10^{-5} M, 0.2 M KCl, 1% v/v CH_3CN, [c]K_2HPO_4/KH_2PO_4 = 1.25, 0.05-0.20 M, pH 6.8 at 25.0 ± 0.1 °C, μ = 0.6 M (KCl), [d]Imidazole/imidazole•HCl = 9.0, total concentration 0.2-1.0 M, pH 8.0 at 25.0 ± 0.1 °C, μ = 1.0 M (KCl), [e]0.001-0.48 M NaOH, μ = 1.0 M (KCl), [f]K_2HPO_4/KH_2PO_4 = 1.0, 0.05-0.20 M, pH 8.0 at 25.0 ± 0.1 °C, μ = 1.0 M (KCl), [g]Imidazole/imidazole•HNO_3 = 9.0, total concentration 0.4-0.8 M, pH 8.2 at 25.0 ± 0.1 °C, μ = 1.0 M (KNO_3), 10^{-3} M EDTA, [h] 25.00 ± 0.05 °C, [i]73.0 ±0.2 °C [j] 54.7 ± 0.2 °C, [k] 70.0 ± 0.2 °C [l] reference 3, [m] OpNP = 4-nitrophenyl; OiPr = isopropyl; OPin = pinacolyl; OMPh = 4-methylphenacyl, [n] 0.05-0.30 M, μ = 1.0 M (KCl), [o] 0.005-0.5 M, μ = 1.0 M (KCl).

Table 1 shows activation parameters, solvent isotope effects and secondary β-deuterium isotope effects (β-DIE, k_{3H}/k_{3D}) for the reactions studied. Partial solvent isotope effects for the imidazole-catalyzed reactions of NMN and soman depended linearly on the atom fraction of deuterium. β-DIE were measured for the reactions of hydroxide ion with NMN and IMN. These, along with previous measurements[5] for the imidazole-catalyzed reactions of the compounds, are listed in the last column.

The log rate - pH profile for the MPMN showed linear dependence with a slope of one above pH 9.0 and decreasing dependence on [HO^-] below that.

DISCUSSION AND CONCLUSIONS

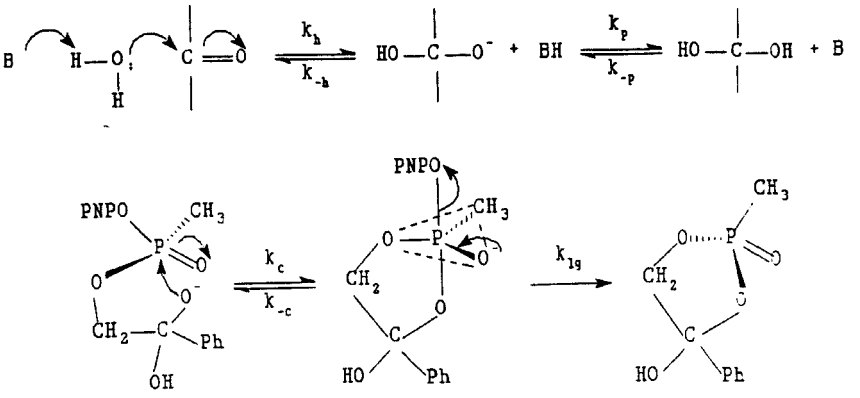
We find that three prototypical phosphonate inhibitors of serine hydrolases show a range of transition state structures in reactions with four different nucleophiles. Imidazole acts as a general base catalyst in the hydrolysis of NMN and soman: the transition states for these reactive phosphonates, at least until high concentrations of imidazole, have a significant component from a single-proton transferring from water to imidazole. The water-catalyzed hydrolysis of NMN is also similar. The imidazole -catalyzed reaction of IMN involves a small solvent isotope effect, probably, because of a direct nucleophilic attack on IMN by imidazole is a viable competing mechanism with imidazole base-catalysis of water attack. This difference in the mode of reaction between the good electrophiles, NMN and soman, and IMN is likely to be a consequence of a much smaller change in the charge at phosphorus between reactant and transition states for IMN. The reactant state of IMN is likely to be less positive at P than for NMN and soman, and our isotope effect data supports the contention that negative charge does not accumulate significantly at P in the transition state either. Transition states for inactivation of serine proteases have similar characteristics to the imidazole-catalyzed hydrolysis of these compounds.[2-5]

Phosphate dianion and hydroxide ion react with NMN and IMN nucleophilically. The hydroxide reactions involve a fairly advanced transition state for NMN and an earlier transition sate for IMN.

Secondary β-DIE on hydroxide ion attack at P of NMN and IMN were both inverse and distinctly lower than the values for the imidazole reaction with the same compounds. Most likely, in phosphonate esters, as in acyl esters, the major contribution to the changes in the vibrational force constants (and inverse β-DIE) are generated through rehybridization on going from reactants to transition states. The more inverse the effect is, the greater the departure from the sp^3d configuration in the reactant state is in the activated complex. Formation of a full-fledged pentavalent intermediate certainly would mean greater changes in geometry along the reaction coordinate than in a concerted reaction. Overall, results of this probe fully support the solvent isotope effects and activation enthalpies, which all point toward a late transition state for NMN and an earlier one for IMN with hydroxide ion. The β-DIE also indicate greater geometric changes for reactions of NMN than for IMN, which is consistent with greater charge separation in the reactions of NMN than those of IMN.

MPMN hydrolyzes orders of magnitude faster than analogs without the β-keto substituent and the facilitation is at least 10 times greater than what has been observed with carboxylic esters containing a β-keto group.[1] The pentavalent structure

in the transition state might involve less strain then the quasi-tetravalent carbonyl transition state. The data support the mechanism involving β-keto group participation via cyclic oxyphosphorane formation[1,6] in the displacement of 4-nitrophenol as follows;



$$k_{obs} = \frac{k_{lg}k_c k_h[B]}{k_{lg}k_c + k_{lg}k_{-h} + k_{-h}k_{-c}}$$

Our results are consistent with an, at least partially, rate determining proton transfer in the hydration step with solvent isotope effects over 2 at pHs 7.45 and 9.25, perhaps concurrent with partially rate-determining formation of the oxyphosphorane intermediate. The entropy of activation for the reaction of hydroxide ion with MPMN is very large positive and consistent with rate-determining intramolecular attack. The ionic strength dependence is consistent with a greater charge delocalization at the transition state than in the ground state in which the base catalyst is fully ionized. The pH - rate profile and inverse solvent isotope effects at higher pH values indicate the nucleophilic involvement of hydroxide ion at pH values greater than 10.

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