J|A|C|S
COMMUNICATIONS

Published on Web 06/25/2004

Lithium-Catalyzed Hydroxide Attack at the Carbon Atom of Methyl Phosphate

Richard Wolfenden* and Frances Zhao

Department of Biochemistry & Biophysics, University of North Carolina, Chapel Hill, North Carolina 27599

Received May 5, 2004; E-mail: water@med.unc.edu

The spontaneous hydrolysis of phosphate ester monoanions is relatively easy,[1] but the reaction of water with simple phosphate ester dianion appears to be the slowest biomimetic reaction whose spontaneous rate has been measured in water,[2] with an estimated half-time of $\sim 10^{12}$ years at room temperature in the absence of a catalyst.[3] So unreactive is the methyl phosphate dianion that the monoanion remains a major target of water attack even in 1 M KOH, where it constitutes approximately one part in 10^8 of the total methyl phosphate that is present. Because water attack on phosphate ester dianions is so sluggish, enzymes that catalyze water attack at the phosphorus atom of dianionic forms of their substrates (fructose 1,6-bisphosphatase, protein phosphatase-1, and inositol 1-phosphatase, for example) exceed other known enzymes in the rate enhancements that they produce. Here, we report an alternative mode of cleavage of methyl phosphate that involves hydroxide attack at the carbon atom of methyl phosphate and proceeds at a rate proportional to the square of the concentration of lithium hydroxide.

Using PTFE-lined reaction vessels to examine the hydrolysis of methyl phosphate in strongly alkaline solutions at elevated temperatures as described earlier,[3] we observed no significant variation in rate as the concentrations of KOH or RbOH were raised to their solubility limits at room temperature. Pronounced increases in rate were observed in the presence of NaOH or LiOH (Figure 1).

The activity of water is much reduced at high concentrations of NaOH,[4] and the hydrolysis of some phosphate ester dianions has been shown to proceed much more rapidly in aqueous solutions that contain aprotic solvents.[5,6] Thus, the enhanced rates observed in concentrated sodium hydroxide might be considered to arise, at least in part, as an indirect effect of changes in the activity of solvent water. However, water activity has been shown to be very much less depressed in LiOH than in equivalent molalities of NaOH at elevated temperatures.[7] We confirmed that result by direct manometric measurements of the vapor pressure of water, observing a water activity coefficient of 0.87 over a saturated solution of LiOH at 25 °C. Thus, a reduction in water activity seemed unlikely to explain the rate-enhancing effect of LiOH.

The hydrolysis of methyl phosphate was found to proceed at a rate that was roughly proportional to the *square* of the concentration of LiOH (Figure 2). To test the possibility that two lithium ions might be involved in this reaction, we examined the effects of varying the component ions of lithium hydroxide independently by varying the concentrations of LiCl and KOH, neither of which catalyzes methyl phosphate hydrolysis by itself. The rate of hydrolysis was found to vary with the first power of the concentration of lithium at constant hydroxide ion concentration and, with the first power of the concentration of hydroxide ion, at constant concentrations of lithium or sodium (data not shown). Thus, hydrolysis in concentrated LiOH appears to proceed by hydroxide attack on the monolithium complex of the methyl phosphate dianion. The more rapid hydrolysis of the lithium than the sodium salt (Figure 1) may arise from a difference between the dissociation constants

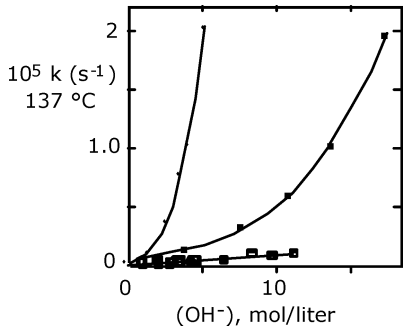


Figure 1. Apparent first-order rate constants (s^{-1}) for hydrolysis of methyl phosphate at 137 °C, plotted as a function of increasing concentrations of LiOH (▲), NaOH (■), KOH (□), or RbOH (○) up to their limits of solubility.

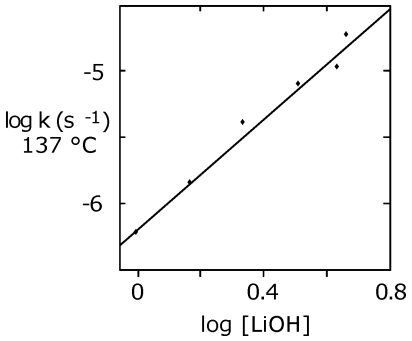


Figure 2. Apparent first-order rate constants (s^{-1}) for hydrolysis of methyl phosphate, at 137 °C, plotted as a logarithmic function of increasing concentrations of LiOH. The slope of the regression line is 2.1.

of the complexes formed by these cations with the methyl phosphate dianion in water, from a difference between the inherent rates of reaction of their complexes with the hydroxide ion, or from both.

Plotted as a function of reciprocal temperature (Kelvin), the logarithm of the apparent rate constant for hydrolysis in the presence of 4.4 M LiOH yielded a linear Arrhenius plot (Figure 3) that could be extrapolated to yield an apparent first-order rate constant (3×10^{-9} s^{-1}) at 25 °C. Adopting the rate expression in eq 1, that apparent first-order rate constant is equivalent to an actual third-order rate constant (k_3) of 1.5×10^{-10} M^{-2} s^{-1}, at 25 °C. The catalytic effect of lithium hydroxide shows no evidence of saturation even at its solubility limit (Figures 1 and 2), and thus the reactivity of the lithium complex can be estimated only as a lower limit. Even at that lower limit, at which the presumed complex is incompletely formed, the apparent first-order rate for hydroxide attack at the carbon atom of lithiated methyl phosphate (Scheme 1c) is 3×10^{-9} s^{-1} at 25 °C, exceeding the first-order rate constants for P−O cleavage of either the monoanion or the dianion of methyl phosphate (Table 1).

$$-\mathrm{d[MeP]/dt} = k_3[\mathrm{MeP}][\mathrm{Li}^+][\mathrm{OH}^-] \qquad (1)$$

10.1021/ja0473586 CCC: $27.50

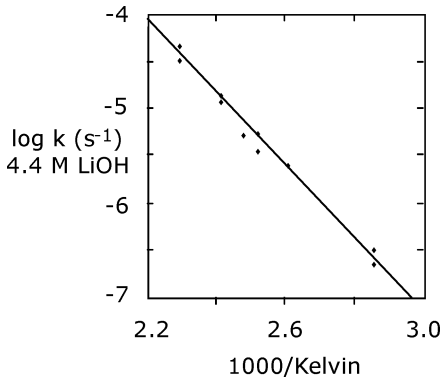
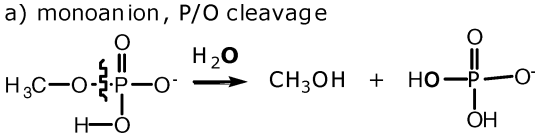


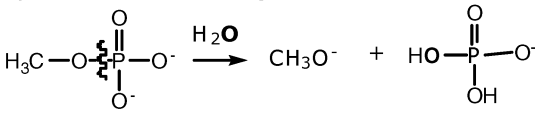
Figure 3. Arrhenius plot of the common logarithm of the apparent first-order rate constant (s^{-1}) for hydrolysis of methyl phosphate, plotted as function of the reciprocal of temperature (Kelvin).

Scheme 1. Methyl Phosphate Reactions

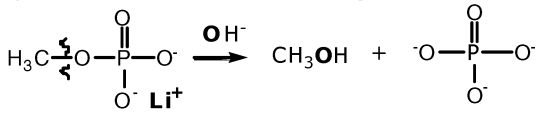
a) monoanion, P/O cleavage



b) dianion, P/O cleavage



c) lithiated dianion, C/O cleavage



After hydrolysis had been carried to completion in 4.4 M LiOH containing 90% $H_2{}^{18}O$, at 130 °C, the methanol product was found to be 88% enriched in ^{18}O, indicating that hydrolysis proceeds by cleavage between the oxygen and carbon atom of the monolithium complex (Scheme 1c). As expected for nucleophilic displacement at carbon, no reaction (<1%) could be detected for isopropyl or neopentyl phosphate under the same conditions, and ethyl phosphate was found to be ~20-fold less reactive than methyl phosphate.[8]

Many enzymes act on phosphoric ester substrates by facilitating oxygen attack at the phosphorus atom, rather than at the carbon atom. One of the few exceptions is glycogen phosphorylase (E. C. 2.4.1.1) reacting with glucose 1-phosphate to add single glucose residues at the 4-OH group at the terminus of glycogen, in the direction of reaction that is favored thermodynamically.[9] No metal ion is involved in the action of glycogen phosphorylase, and no covalent intermediate has been detected thus far.[10] It would be of interest to learn the extent to which the anomeric structure of glucose 1-phosphate enhances its intrinsic susceptibility toward attack by oxygen nucleophiles at C-1, in view of the extremely sluggish rate of spontaneous P/O cleavage of methyl phosphate dianion[1] and the fact that C/O cleavage of methyl phosphate must be slower still. Somewhat more closely resembling the present reaction, in that the carbon atom under attack is not anomeric, is the reaction catalyzed by methionine adenosyltransferase (E. C. 2.5.1.6), in which the sulfur atom of methionine reacts with the C-5′ atom of ATP to form *S*-adenosylmethionine. These reactions, like the decarboxylation of OMP, appear to be among the more formidable challenges that have been overcome during the evolution of modern enzymes.

Table 1. Rates of Methyl Phosphate Reactions in Scheme 1

	k_{25} (s^{-1})	k_{100} (s^{-1})	$\Delta H^{\ddagger}$ (kcal/mol)	$T\Delta S^{\ddagger}_{25}$ (kcal/mol)
(a)[a]	4×10^{-10}	8×10^{-6}	+30	−0.2
(b)[b]	2×10^{-20}	1.4×10^{-13}	+47	+2.1
(c)[c]	$>3 \times 10^{-9}$	$>8 \times 10^{-7}$	+17	<−12
(c)[d]	1.5×10^{-10}	4.4×10^{-8}	+17	−14

[a] References 1 and 2. [b] These values were estimated by extrapolation from the behavior of aryl phosphates (ref 2) and from the behavior of methyl phosphate in 1 M KOH as described in ref 3. [c] Nominal values of apparent first-order rate constants observed in 4.4 M LiOH (this work). The dissociation constant of the Li+ complex of the methyl phosphate dianion is unknown, but exceeds 4.4 M (see text). [d] Third-order rate constants (M^{-2} s^{-1}) calculated from values of apparent rate constants in footnote c, using eq 1.

Acknowledgment. We thank J. P. Guthrie, A. J. Kirby, and N. H. Williams for helpful discussions. This work was supported by Grant #GM-18325 from the National Institute of General Medical Sciences.

References

(1) Bunton, C. A.; Llewellyn, D. R.; Oldham, K. G.; Vernon, C. A. *J. Chem. Soc.* **1958**, *1958*, 3574.
(2) Kirby, A. J.; Varvoglis, A. G. *J. Am. Chem. Soc.* **1967**, *89*, 415.
(3) Lad, C.; Williams, N. H.; Wolfenden, R. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5607.
(4) Robinson. R. A.; Stokes, R. H. *Electrolyte Solutions*, 2nd ed.; Butterworths: London, 1959; pp 510−511.
(5) Abell, K. W. Y.; Kirby, A. J. *Tetrahedron Lett.* **1986**, *27*, 1085.
(6) Grzyska, P. K.; Czyryca, P. G.; Golightly, J.; Small, K.; Larsen, P.; Hoff, R. H.; Hengge, A. C. *J. Org. Chem.* **2002**, *67*, 1214.
(7) Stephan, E. F.; Miller, P. D. *J. Chem. Eng. Data* **1962**, *7*, 501.
(8) It would be of interest to compare the leaving group activity of the presumed lithium phosphate dianion with the leaving group activities of other anions, with respect to their ease of displacement from methane by the hydroxide ion. But since the catalytic effect of lithium hydroxide shows no sign of saturation even at its solubility limit (Figures 1 and 2), that reactivity lex can be estimated only as a lower limit.
(9) K_{eq} for glycogen phosphorylation is approximately unity (Hestrin, S. *J. Biol. Chem.* **1949**, *179*, 943), but under the conditions that typically prevail in mammalian tissue, the concentration of inorganic phosphate so greatly exceeds the concentration of glucose 1-phosphate that this reaction proceeds entirely in the direction of phosphorolysis.
(10) Pyridoxal phosphate is an essential cofactor for glycogen phosphorylase. Its position in the crystal structure suggests that it is (just) close enough to the reacting substrates to furnish a phosphoryl group that acts as a general base: (a) Sygusch, J.; Madsen, N. B.; Kasvinski, P. J.; Fletterick, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 4757. (b) Weber, I. T.; Johnson, L. N.; Wilson, K. S.; Yeates, D. G.; Wild, D. L.; Jenckins, J. A. *Nature (London)* **1978**, *274*, 433. Other evidence suggests that this may be an oversimplification however: (c) Withers, S. G.; Shechosky, S.; Madsen, N. B. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 322.

JA0473586