Determination of the pK_a of the 2′-Hydroxyl Group of a Phosphorylated Ribose: Implications for the Mechanism of Hammerhead Ribozyme Catalysis

Paul D. Lyne[†,‡] and Martin Karplus*[,†]

Department of Chemistry and Chemical Biology
Harvard University, 12 Oxford Street
Cambridge, Massachusetts 02138
Physical and Theoretical Chemistry Laboratory
Department of Chemistry, Oxford University
South Parks Road, Oxford OX1 3QZ, England

Received June 1, 1999
Revised Manuscript Received September 8, 1999

Hammerhead ribozymes[1] are small catalytic domains found in some viral satellite RNAs and are involved in RNA self-cleavage.[2−4] The reaction catalyzed by hammerhead ribozymes is a phosphate ester hydrolysis that is thought to proceed via activation of a specific 2′-OH group by proton abstraction. The resultant 2′-alkoxide acts as a nucleophile, attacking the electropositive phosphorus in an intramolecular fashion and displacing the 5′-oxygen of the cleaved nucleotide (Figure 1). Hammerhead ribozymes are metalloenzymes[5,6] with an absolute requirement for divalent metal ions for catalysis of the cleavage reaction.[7] Magnesium ions are the natural cofactors for the hammerhead ribozyme, but other divalent cations can replace magnesium. They include Mn^{2+}, Cd^{2+}, and Co^{2+}, which actually catalyze the reaction at higher rates than Mg^{2+} under the same conditions, and Ca^{2+}, which catalyzes the reaction at a slower rate; a recent study reports that very high concentrations of Li^{+} ions can replace the Mg^{2+} ions.[8] Cleavage rates are correlated with the pK_a of the solvated metal ion, with the rate increasing as the pK_a of the solvated ion decreases. This has traditionally been interpreted[9] as indicating that a metal-coordinated hydroxide acts as the general base in the reaction. An alternative interpretation of the data[10] suggests that the mechanism involves two metal ions but that a metal hydroxide ion is not the base in the reaction. The exact number of metal ions involved in the reaction and the nature of their interaction with the hammerhead ribozyme remains an area of discussion.[11] A knowledge of the pK_a values of ionizable groups at the active site of the ribozyme may aid in indicating possible mechanistic pathways and in inferring the nature of the chemically active species.[12,13]

Since deprotonation of the 2′-hydroxyl group is involved in generating the attacking nucleophile in the hammerhead ribozyme reaction, it is important to know the pK_a of this group. The experimental determination of pK_a values in complex systems, particularly the direct measurement of titrating groups of catalytically important residues in enzymes, is not a straightforward task. Kinetic assignments of pK_a values can be complicated by uncertainties in interpreting the pH dependence of the measured parameters.[14] Moreover, different values are often obtained with different techniques.[15] An estimate[16,17] of the pK_a of the 2′-OH group of a nucleotide is based on a value of 12.5 for the nucleoside 2′-anhydro-1-α-D-xylofuranosyluracil. However, the presence of the phosphate group could modify the pK_a value. Usher et al. have reported[18] a value of 13.9 for the pK_a of the 2′-OH group in *cis*-tetrahydrofuran-4-ol 3-phenyl phosphate, while Guthrie estimated[19] the pK_a of the 2′-OH group of nucleotides to be approximately 16 based on thermodynamic arguments. Kinetic pK_a values of 13 (at 333 K) have also been reported for dinucleotides.[20]

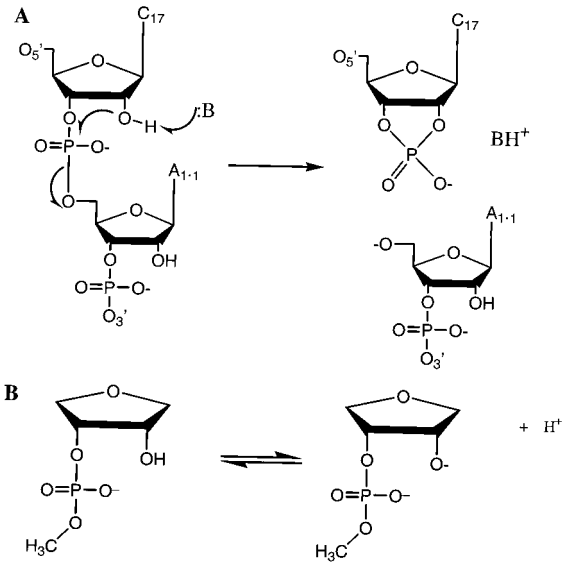

Figure 1. (A) Schematic of the proposed mechanism for the first step of phosphate ester hydrolysis in the hammerhead ribozyme (divalent metals not shown). (B) Model phosphorylated ribose used in this study.

Since the value for the pK_a of the 2′-OH group of a nucleotide is not well established, a theoretical estimate of the pK_a of the 2′-OH at the active site of hammerhead ribozymes is presented here based on quantum chemistry calculations of a phosphorylated ribose (Figure 1). The pK_a of a given acid HA in aqueous solution is related to the standard Gibbs free energy change of the ionization reaction by the thermodynamic relationship

$$\mathrm{p}K_a = -\log K_a = \frac{\Delta G_{aq}^{\,0}}{2.303RT} = \frac{1}{2.303RT} \times (\Delta G_g^{\,0} + \Delta G_{solv}^{\,0}(H^+) + \Delta G_{solv}^{\,0}(A^-) - \Delta G_{solv}^{\,0}(HA)) \quad (1)$$

corresponding to the thermodynamic cycle in eq 1.[21] In eq 1, $\Delta G_g^{\,0}$ and $\Delta G_{aq}^{\,0}$ are the standard free energies of ionization in the gas and in solution, respectively, and the $\Delta G_{solv}^{\,0}$ values are the free energies of solvation. All gas-phase free energies were calculated using the Gaussian 94 program.[22] Solvation energies were calcu-

† Harvard University.
‡ Oxford University.

(1) Birikh, K. R.; Heaton, P. A.; Eckstein, F. *Eur. J. Biochem.* **1997**, *245*, 1−16.
(2) Buzayan, J. M.; Gerlach, W. L.; Bruening, G. *Nature* **1986**, *323*, 349−353.
(3) Hutchins, C. J.; Rathjen, P. D.; Forster, A. C.; Symons, R. H. *Nucleic Acids Res.* **1986**, *14*, 3627−3640.
(4) Prody, G. A.; Bakos, J. T.; Buzayan, J. M.; Schneider, I. R.; Bruening, G. *Science* **1986**, *231*, 1577−1580.
(5) Pyle, A. M. *Science* **1993**, *261*, 709−714.
(6) Wilcox, D. E. *Chem. Rev.* **1996**, *96*, 2435−2458.
(7) Dahm, S. C.; Uhlenbeck, O. C. *Biochemistry* **1991**, *30*, 9464−9469.
(8) Murray, J. B.; Seyhan, A. A.; Walter, N. G.; Burke, J. M.; Scott, W. G. *Chem. Biol.* **1998**, *5*, 587−595.
(9) Dahm, S. C.; Derrick, W. B.; Uhlenbeck, O. C. *Biochemistry* **1993**, *32*, 13040−13045.
(10) Pontius, B. W.; Lott, W. B.; von Hippel, P. H. *Proc. Natl. Acad. Sci., U.S.A.* **1997**, *94*, 2290−2294.
(11) Zhou, D.-M.; Taira, K. *Chem. Rev.* **1998**, *98*, 991−1026.
(12) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; Dover: New York, 1987.
(13) Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; Freeman: New York, 1985.
(14) Knowles, J. R. *CRC Crit. Rev. Biochem.* **1975**, *3*, 165.
(15) Marcus, Y. *Ion Solvation*; Wiley: New York, 1985.
(16) Birnbaum, G. I.; Giziewicz, J.; Huber, C. P.; Shugar, D. *J. Am. Chem. Soc.* **1976**, *98*, 4640−4644.
(17) Izatt, R. M.; Hassen, L. D.; Rytting, J. H.; Christensen, J. J. *J. Am. Chem. Soc.* **1965**, *87*, 2760−2761.
(18) Usher, D. A.; Richardson, D. I., Jr.; Oakenfull, D. G. *J. Am. Chem. Soc.* **1970**, *92*, 4699−4712.
(19) Guthrie, J. P. *J. Am. Chem. Soc.* **1977**, *99*, 3991.
(20) Jarvinen, P.; Oivanen, M.; Lonnberg, J. *J. Org. Chem.* **1991**, *56*, 5396.
(21) Kollman, P. *Chem. Rev.* **1993**, *93*, 2395.
(22) Gaussian 94 (revision D.1) Gaussian Inc.: Pittsburgh, 1995.

10.1021/ja991820i CCC: $19.00
Published on Web 12/18/1999

Table 1. Solvation Free Energy Changes Involved in the Ionization of MeOH and H_3PO_4[a]

	ΔG_{aq}			
	MeOH	MeO^-	H_3PO_4	$H_2PO_4^-$
experiment[36,37]	−5.08	−95.00	−26.0	−76.00
B3LYP/6-31+G**	−5.50	−93.03	−20.8	−79.83

[a] All values in kcal/mol.

Table 2. Electronic and Solvation Energies for the Ribose (RH) and Phosphorylated Ribose (pRH) and Their Conjugate Bases (R^- and pR^-) Calculated at the B3LYP/6-31+G** Level[a]

	E_{Elec}	ZPE	$G°$(g)	ΔG_s	$G°$(s)	pK_a
RH	−382.9107	0.1259	−382.8161	−0.0213	−382.8374	13.1
R^-	−382.3388	0.1107	−382.2584	−0.1205	−382.3789	
pRH	−989.4385	0.16718	−989.3113	−0.1089	−989.4202	14.9
pR^-	−988.7286	0.15209	−988.6156	−0.3423	−988.9579	

[a] Units are hartrees.

lated using the Poisson−Boltzmann method as implemented in the Jaguar program.[23,24] The value of the solvation free energy of the proton was set equal to −262.23 kcal/mol.

Since pK_a calculations depend strongly on solvation free energies, accurate values for them are required. To address this issue, certain of the parameters used in the standard Jaguar model[23] were optimized for systems related to the one of interest here. In particular, it is known that anions often pose problems for the determination of accurate solvation energies with continuum methods.[25,26] The values of solvation energies obtained by using a continuum dielectric method depend on the van der Waals radii employed in the calculation. Several studies have used adjusted van der Waals radii to obtain agreement with experiment.[26−28] To determine the optimal set of radii for the molecules being studied here, in particular the radii for the 2′-alkoxide oxygen and the *pro*-R and *pro*-S oxygens of the phosphate group, the solvation free energies of methanol and H_3PO_4 and their conjugate bases were determined and compared with experimental data. The calculated thermodynamic values are given in Table 1. It was found that values of 1.42 and 1.26 Å for the phosphate oxygens and methoxide oxygen, respectively, gave good agreement with the available experimental data; the default radii in Jaguar for these atoms are somewhat larger (1.60 and 1.45 Å, respectively). The adjusted radii were used for the solvation calculations on the model nucleotides.

As a test of the method we have calculated the pK_a of the 2′-hydroxyl of ribose. The relevant gas phase and solvation energies are presented in Table 2. The pK_a of a ribose using the optimized radius for the alkoxide oxygen of the conjugate base is calculated to be 13.14, which is rather close to the experimental value of 12.5; the value of the pK_a calculated with the default Jaguar radii is 18.45, showing the importance of consistent van der Waals radii for continuum electrostatic calculations.[26−28] The various model calculations suggest that the basis set, the form of the nonlocal functional, and the optimized radii for the Poisson−Boltzmann calculations are appropriate for determining the pK_a of the phosphorylated ribose. The gas-phase electronic energies and corresponding thermochemical data for the phosphorylated ribose and its conjugate base are shown in Table 2. The pK_a calculated for the phosphorylated ribose (14.9) is considerably higher than the only available experimental pK_a (12.5) of a nucleoside (see above).[16,17,29] This is not surprising since the conjugate base of that nucleoside is expected to be stabilized by a hydrogen bond between the adjacent 3′-OH group and the deprotonated 2′-O^-. The pK_a of a nucleoside that has no such stabilization of the conjugate base should have a higher pK_a. Additionally, the molecule studied here has a negatively charged phosphate group in close proximity to the 2′-OH group which should make it thermodynamically less favorable for the 2′-OH group to ionize than for the same group in the unphosphorylated molecule. Thus, the upward shift by nearly 2 pK_a units is reasonable.

The large value for the pK_a of the 2′-OH group found here (pK_a = 14.9) has implications for the mechanism of hammerhead ribozyme catalysis and more specifically for the role played by metal ions in the reaction. It has been established, based on atomic substitution of the *pro*-R and *pro*-S oxygens by sulfurs (the thio-effect[30]) and by spectroscopic studies,[31] that a metal ion coordinates directly with the *pro*-R oxygen of the phosphate at the active site.[31] Kinetic studies of hammerhead cleavage have found that there is an inverse relationship between the pK_a values of solvated metal ions and the rate of the cleavage reaction.[9] This has been interpreted to indicate that a metal hydroxide ion acts as the general base in the reaction since the concentration of metal hydroxide ions increases as the pK_a of the hydrated metal ion decreases. However, increasing the acidity of the hydrated metal ion lowers the basicity of the metal hydroxide. This is expected to lower its ability to deprotonate the 2′-OH group at the active site of the hammerhead. The pK_a calculated here for a 2′-OH group in a nucleotide model is several units higher than the pK_a of several metal ions (e.g., Pb^{2+}(7.7), Zn^{2+}(9.0), and Mg^{2+}(11.4)[32]) that readily catalyze the reaction. It therefore seems unlikely that metal hydroxide ions act as the general base in the reaction.[10] Since a metal ion is known to bind directly to the adjacent *pro*-R oxygen of the phosphate, it is conceivable that it promotes the reaction by stabilizing the transition state. It is also possible that it could bridge the 2′-oxygen and *pro*-R oxygen, which is consistent with the. two metal ion model proposed for a number of phosphoryl transfer reactions[10,33,34] If so, it is expected that the hydroxide bond at 2′-OH would be polarized and that the pK_a of the group would be reduced. The magnitude of the polarization of the bond would be directly related to the identity of the metal ion.

Since other ribozymes[5] and ribonucleases[35] employ mechanisms that involve deprotonation of a 2′-OH at the active site, the result presented here has implications for phosphate hydrolysis reactions in many biological systems.

Acknowledgment. This work was supported in part by the Human Frontiers in Science Program (P.D.L.), the Wellcome Trust (P.D.L.), and National Institute of Health (M.K.). Calculations were performed at the Pittsburgh Supercomputing Center, the Goddard Space Flight Center (NASA), and the Oxford University Supercomputer Centre.

Supporting Information Available: Details are given of the methods used for the calculation of the results in the text and tables (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA991820I

(23) *Jaguar*, 3.5 ed.; Schrodinger, Inc.: Portland, OR, 1998.
(24) Tannor, D. J.; Marten, B.; Murphy, R.; Friesner, R. A.; Sitkoff, D.; Nicholls, A.; Rignalda, M.; Goddard, W. A., III; Honig, B. *J. Am. Chem. Soc.* **1994**, *116*, 11875−11882.
(25) Honig, B.; Sharp, K.; Yang, A.-S. *J. Phys. Chem.* **1993**, *97*, 1101.
(26) Stefanovich, E.; Truong, T. *Chem. Phys. Lett.* **1995**, *244*, 65−74.
(27) Lim, C.; Bashford, D.; Karplus, M. *J. Phys. Chem.* **1991**, *95*, 5610−5620.
(28) Sitkoff, D.; Sharp, K. A.; Honig, B. *J. Phys. Chem.* **1994**, *98*, 1978−1988.
(29) Saenger, W. *Principles of nucleic acid structure*; Springer-Verlag: New York, 1984.
(30) Scott, E. C.; Uhlenbeck, O. C. *Nucleic Acids Res.* **1999**, *27*, 479−484.
(31) Cunningham, L. A.; Li, J.; Lu, Y. *J. Am. Chem. Soc.* **1998**, *120*, 4518−4519.
(32) Pan, T.; Long, D. M.; Uhlenbeck, O. C. *Divalent Metal Ions in RNA Folding and Catalysis*; Pan, T., Long, D. M., Uhlenbeck, O. C., Eds.; Cold Spring Harbor Press: Plainview, NY, 1993; pp 271−302.
(33) Steitz, T. A.; Steitz, J. A. *Proc. Natl. Acad. Sci.* **1993**, *90*, 6498−6502.
(34) Sawata, S.; Komiyama, M.; Taira, K. *J. Am. Chem. Soc.* **1995**, *117*, 2357−2358.
(35) Fersht, A. *Structure and Mechanism in Protein Science*; W. H. Freeman and Company: New York, 1999.
(36) Cabani, S.; Gianni, P.; Mollica, V.; Lepori, L. *J. Solution Chem.* **1981**, *10*, 563−595.
(37) George, P.; Witonsky, R. J.; Trachtman, M.; Wu, C.; Dorwart, W.; Richman, L.; Richman, W.; Shurayh, F.; Lentz, B. *Biochim. Biophys. Acta* **1981**, *223*, 1−15.