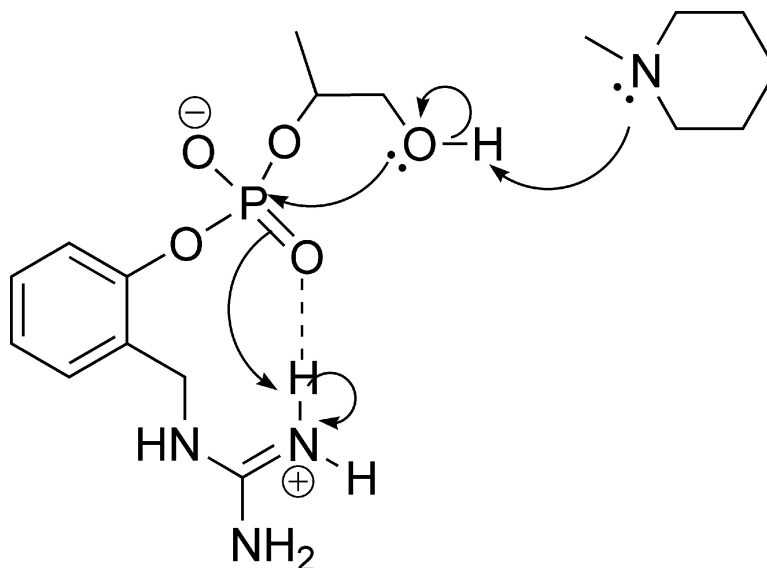## Communication

Guanidinium Groups Act as General-Acid Catalysts in Phosphoryl Transfer Reactions: A Two-Proton Inventory on a Model System

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Guanidinium Groups Act as General-Acid Catalysts in Phosphoryl Transfer Reactions: A Two-Proton Inventory on a Model System

Anna M. Piątek, Mark Gray, and Eric V. Anslyn*

Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas 78712

Received May 26, 2004; E-mail: anslyn@ccwf.cc.utexas.edu

Phosphoryl transfers from phosphodiesters to water and alcohols have been discussed in terms of two mechanisms.[1] The "classical" mechanism involves general-base/general-acid catalysis that deprotonates the attacking nucleophile and protonates the leaving group respectively, proceeding through a dianionic phosphorane transition state or intermediate.[2] The alternative is the so-called "triester" mechanism,[3] which involves a general-acid catalyst which protonates a phosphodiester peripheral oxygen simultaneous with nucleophilic attack to give a monoanionic phosphorane intermediate. These two mechanisms occur in parallel in buffer-catalyzed RNA cleavage/transesterification.[4] In RNase A, the classical mechanism receives the most support,[5] where His-12 is the general-base, His-119 is the general acid, and Lys-41 either acts as a general acid or electrostatic catalyst to stabilize the phosphorane-like transition state. In contrast, a triester mechanism for RNase T1 receives the most support.[6] In this enzyme Glu-58 and His-92 play the roles of the general-base and general-acid catalysts respectively, while Arg-77 acts as an analogue to Lys-41 in RNase A.

If the pK_{a2} value of the conjugate acid of an anionic phosphorane is higher than the pK_a value of any of the acid catalysts nature commonly employs (imidazolium, ammonium, or guanidinium in His, Lys, or Arg respectively) and that acid is hydrogen bonded to a peripheral oxygen of the substrate in the ground state, a proton transfer will necessarily occur during the creation of that phosphorane (see below). This results in the triester mechanism.[7] Previous estimates for pK_a values of phosphoranes derived from phosphodiesters are in the range 6.5−11 for pK_{a1} and 11.3−15 for pK_{a2}.[1a] Recently Dejeagere and Karplus calculated a pK_{a1} of 7.9 and a pK_{a2} of 14.3,[8a] whereas Kirby estimated pK_{a1} values of 9.8 and 14.2 for equatorial and apical OH groups, respectively, and pK_{a2} values would be estimated to be 4 or more units higher.[8b] These calculated and estimated pK_{a2} values indicate that even a guanidinium group from an arginine, which is the least acidic member of nature's general-acid catalysts (pK_a around 13), will protonate a proximal developing dianionic phosphorane.[9] Yet, this prediction has not been experimentally tested.

proton will transfer if pK_a of conjugate acid is above pK_a of HB⁺
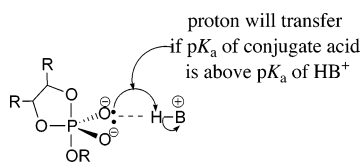
To probe the roles of general-acid catalysts in phosphoryl-transfer reactions, we herein report the design, synthesis (Supporting Information), and proton inventory study of a phosphodiester with an intramolecularly coordinated guanidinium group (Figure 1). The guanidinium group was placed ortho to the phosphoester to allow facile coordination to a peripheral oxygen of the ester, but in a manner where coordination to the leaving group phenoxide oxygen is nonoptimal. To confirm this coordination mode, modeling of **1**
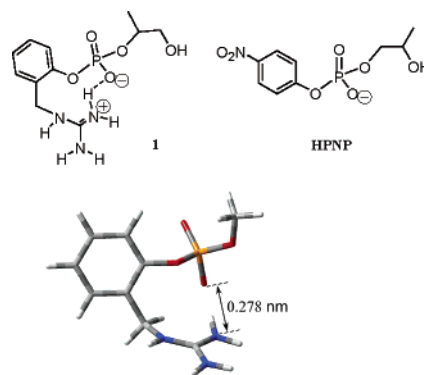

Figure 1.

was undertaken at the HF/6-31G* level, with a water solvent continuum (1-hydroxy-2-propyl replaced by methyl).[10] Several geometries involving a guanidinium hydrogen bond to the leaving-group phenol oxygen did not minimize with the hydrogen bond intact but instead led to separated noninteracting guanidinium and phosphodiester groups. However, a geometry involving a guanidinium to phosphoester peripheral oxygen coordination minimized to give a structure with a standard hydrogen-bond length (Figure 1).

To study the possibility of a proton transfer between the guanidinium group and a phosphodiester peripheral oxygen during transesterification/cleavage of **1**, we employed the proton inventory method.[11] This technique allows one to determine the number of protons undergoing bonding changes in a rate-determining step. We also explored the proton inventory of 2-hydroxypropyl p-nitrophenyl phosphate (HPNP) as a control, which is a substrate commonly used as an RNA mimic in hydrolysis studies.[12] HPNP was originally reported in 1965,[13] and the same experimental conditions used for hydrolysis in this early study were used in the proton inventories of **1** and HPNP (8.3×10^2 μM substrate, 99.2 mM N-methylpiperidine; pH = 10.4). The rate of hydrolysis of **1** in various mixtures of H₂O and D₂O were measured via UV/vis spectroscopy at 280 nm, and a first-order kinetics analysis was applied to at least the first three half-lives of the reaction.[14] The results are plotted in Figure 2, which shows that the rate constant versus D₂O fraction (n) is "bowl-shaped". The points were fit to the Gross−Butler equation ($k_n/k_0 = (1 - n + n\phi)^2$) giving two proton isotope effects: 2.22 and 2.44. As further evidence that a two-proton inventory is supported, Figure 2A gives a plot of the square root of the relative rate constants versus D₂O fraction. This plot is linear, as predicted for a two-proton inventory.

In contrast, HPNP does not contain a guanidinium group, and this substrate is well accepted to undergo cleavage/transesterification by simple general-base catalysis.[15] The experiment was performed with six points ranging between pure H₂O and pure D₂O (substrate at 203.5 μM, and the reaction was monitored at 400 nm). The data are plotted in Figure 2B, which shows a straight line, supporting one proton moving in the rate-determining step. This contrast with
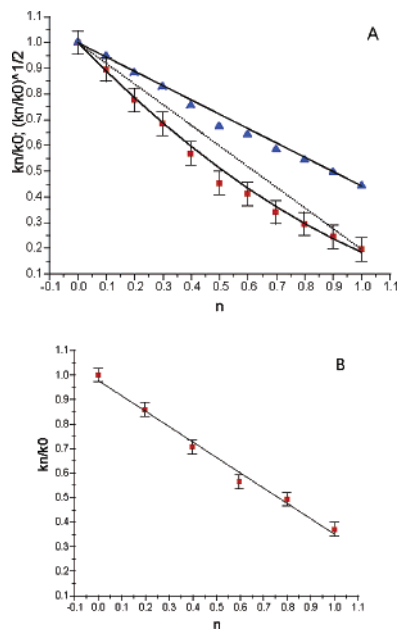
Figure 2. (A) Proton inventory for the hydrolysis of substrate **1** in water as a function of the mole fraction of D_2O (50 °C, pH = 10.4). The lower curve (—) is "bowl-shaped" fit of experimental points (■) to the Gross–Butler equation (two-proton inventory). The dashed line (···) is the theoretical curve for one-proton inventory connecting the H_2O and D_2O points. The upper curve is a straight line fit of the experimental points (▲) in a plot of $(k_n/k_0)^{1/2}$. (B) Proton inventory for the hydrolysis of HPNP in water as a function of the mole fraction of D_2O (50 °C, pH = 9.7).
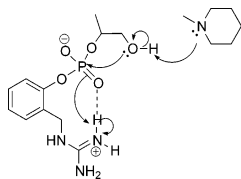

Figure 3. Proposed two proton-transfer mechanism.

structure **1** leads us to propose that two protons are moving in the rate-determining step of cleavage/transesterification of **1**, as shown in Figure 3.

The data given above were used to calculate a rate enhancement imparted by the guanidinium group in **1**. The k_{obs} for cleavage/transesterification of **1** was 16 times larger than that for HPNP, although HPNP possesses the better leaving group. To explore this effect, we measured the OH pK_a of *o*-guanidinylmethylphenol (pK_a = 9.1). Using a β_{LG} value of −0.62,[13,16] it was calculated that the cleavage/transesterification of **1** should be 26 times slower than HPNP based upon a pK_a of 7.15 for *p*-nitrophenol. This translates to a 42-fold rate enhancement imparted by the intramolecular guanidinium general-acid catalysis in **1**.

In summary, we find that a single guanidinium group in an exposed aqueous environment can impart a 40-fold advantage to phosphodiester cleavage/transesterification. In addition, a two-proton inventory for **1** relative to a one proton inventory for HPNP shows that a guanidinium group coordinated to a phosphodiester will act as a general-acid catalyst during cleavage/transesterification, not just as an electrostatic catalyst. Because a two-proton inventory

was found for a guanidinium group, it stands to reason that the more acidic ammonium group from lysine and imidazolium group from histidine will also act in a similar manner if they are coordinated to a phosphoester peripheral oxygen. The Brønsted catalysis law predicts that these groups should impart even greater rate enhancements, and we are currently examining this prediction using analogues of **1**.

Supporting Information Available: Experimental and analytical data of synthesis of compound **1**; the description of theoretical method that was used. This material is available free of charge via the Internet at http://pubs.acs.org.

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