Electrostatic Catalysis of the Hydrolysis of a Phosphate Diester in Water

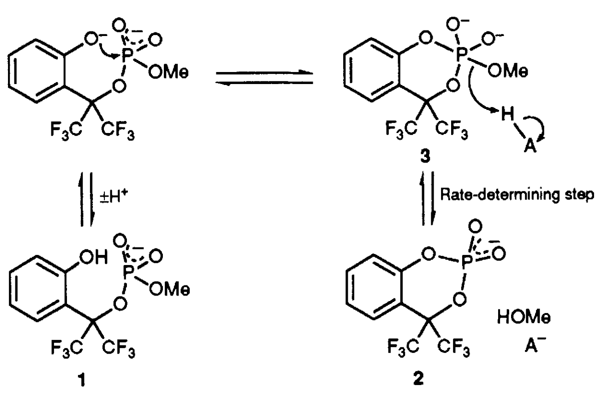
Kevin N. Dalby, Florian Hollfelder and Anthony J. Kirby*
University Chemical Laboratory, Cambridge CB2 1EW

The efficiency of general acid catalysis by protonated amines of the hydrolysis of a phosphate diester with a neighbouring nucleophilic OH group depends less on the pK_a of the acid than on the proximity of a second positive charge.

As part of a detailed examination of catalysis in systems where the hydrolysis of a phosphate diester is catalysed by a neighbouring hydroxy group, we report in this and the preceding communication[1] results for systems designed to react *via* pathways in which a single step is rate determining. Here we report catalysis by general acids of the hydrolysis of the diester **1**. In this system we are able to observe catalysis of the key step in which a poor leaving group departs from the phosphorus centre of a phosphate diester, without complications from other reactions. We find a remarkable enhancement of catalytic efficiency in the presence of a neighbouring positive charge.

Compound **1** exhibits highly efficient intramolecular catalysis of hydrolysis over the entire pH range. The reaction is completely specific at all pHs, and involves cyclisation to **2**. This is the only product detectable by ^{31}P NMR after ten half-lives except in KOH solutions, where **2** is hydrolysed further to a mixture of the two monoesters. The pH-independent reaction of the dianion at pH 10 and above has a half-life at 50 °C of less than 2 min. The results reported here refer specifically to the dianion reaction, which dominates the pH–rate profile for hydrolysis above pH 7.[2]

The displacement from phosphorus of the methoxide anion, six powers of ten more basic, by the phenolate oxygen of the substrate (pK_a 9.55), is remarkable, and would be difficult to explain if the reaction were concerted. This point will be discussed in detail in our full paper;[3] we discuss the mechanism here in terms of a pentacovalent addition intermediate **3**, the breakdown of which is expected to be rate determining (Scheme 1, HA = H_2O). Since water is not a stronger acid than methanol its role will be limited to hydrogen-bonding solvation, but the loss of a poor leaving group like methoxide is expected to be general acid catalysed, and we observe strong catalysis by general acids of the hydrolysis of both the monanion and the dianion.



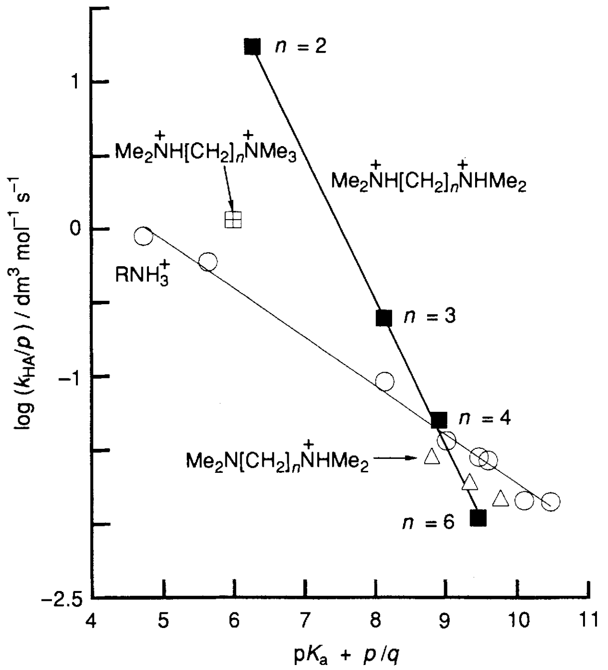
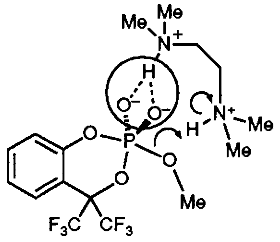


Fig. 1 Brønsted plot for general acid catalysis of the hydrolysis of the phosphate diester **1**. Open circles represent points for primary alkylammonium ions; triangles for trialkylammonium ions. The filled squares are for the series of dications, $Me_2NH^+[CH_2]_nNH^+Me_2$, with n = 2, 3, 4 and 6 as indicated. The pK_a values are also statistically corrected.[4]

A Brønsted plot (not shown) displaying all the data shows—unusually for general species catalysis—extensive scatter, indicating that the effectiveness of a general acid depends not only on its pK_a, but on other factors also. However, data for individual classes of general acid show the expected regularities. Thus points for eight primary alkylammonium cations, spanning a range of almost 6 pK_a units, are well correlated by a straight line of slope (Brønsted α) of −0.33 ± 0.01 (Fig. 1).

The tetramethylethylenediammonium dication is 100 times more effective than expected from its pK_a. We find a striking dependence of the effect on the distance between the second positive charge and the general acid centre (Fig. 1). For the series of dications $Me_2NH^+[CH_2]_nNH^+Me_2$, with n = 2, 3, 4 and 6, the rate enhancement decreases with increasing n, to such an extent that the apparent Brønsted α for the series is 0.99; that is to say, the effect on α of increasing the proximity of the positive charge is twice as large as the effect of increasing the strength of the general acid. The monocations derived from these bases define the behaviour of simple tertiary ammonium cations; they are correlated by a line of slope similar to that drawn (Fig. 1) for primary alkylammonium cations. The positive deviation from this line for the tetramethylethylenediammonium dication corresponds to a rate factor of almost 200 (half of which is statistical).



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The mechanism of this effect is of great interest. It may be presumed to stabilise the high concentration of negative charge on the pentacovalent transition state, and intermediate if one is involved, either electrostatically (through space) or by hydrogen bonding. Our evidence suggests that both factors may be involved; when the second proton of the tetramethylethylenediammonium dication is replaced by a methyl group, much but not all of the rate enhancement disappears (Fig. 1). Thus the rate constant for catalysis by the pentamethylethylenediammonium cation is 28 times smaller than that for the tetramethylethylenediammonium dication, but still some six-fold greater than expected for a tertiary ammonium cation of the same pK_a. The simplest explanation of these results is that the two effects—both normally weak in water—reinforce each other, possibly as in **4**; here (circled) intramolecular hydrogen bonding is strengthened by ion-pair association, which in turn is favoured by the close approach of the opposite charges made possible by the formation of the bifurcate hydrogen bond (impossible for the $^+NMe_3$ group). Such effects will be much stronger in less polar media, and no doubt in enzyme active sites also.[5]

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4 W. P. Jencks, *Catalysis in Chemistry and Enzymology*, McGraw-Hill, New York, 1969, pp. 173–175.
5 Enzymes hydrolysing phosphate diesters, such as ribonuclease A and staphylococcal nuclease, typically have lysine or arginine residues in the active site, in positions corresponding to the region circled in **4**. (See, for example, A. R. Fersht, *Enzyme Structure and Mechanism*, Freeman, New York, 2nd edn., 1985, pp. 428ff.)