Intramolecular Attack by the Hydroxy Group on Phosphate Diesters is Both General Acid and General Base Catalysed

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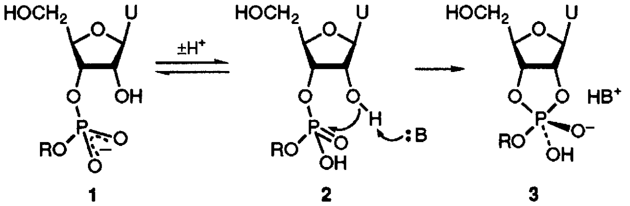
Buffers catalysing the hydrolysis of a phosphate diester with a neighbouring nucleophilic OH group act as general acids, general bases, or both, depending on their pK_a, though the rate determining step remains the same.

Of the phosphate esters which play a role in living systems, diesters are the most important—and the least reactive. Even RNA, which is readily hydrolysed in alkali *via* nucleophilic catalysis by the neighbouring 2′-OH group, is cleaved only very slowly at pH 7 in the absence of a relevant enzyme. So good quality data on catalysis of the reaction are difficult to obtain. The recent work of Breslow *et al.*[1] on the imidazole-catalysed hydrolysis of UpU **1** (R = uridylyl-5′) has raised a number of specific questions about the mechanism of catalysis which cannot be answered unambiguously by the data available. In particular it is suggested that the reaction proceeds by way of a pentacovalent intermediate **3** which is formed by initial general base catalysed attack of the neighbouring OH group on the protonated, neutral form **2** of the diester:



This would be observed as general acid catalysis of hydrolysis, since the substrate is present as the anion near pH 7, but the data for catalysis by imidazole in this system are complicated, at least in part because the breakdown to products of the intermediate **3** is also subject to buffer catalysis.

We have begun 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 following communication[2] observations of general importance, obtained in systems designed to react *via* pathways in which a single step is rate determining. Here we report results for catalysis by general acids and bases of the hydrolysis of the diester **4**, for which the breakdown of a pentacovalent intermediate should be rapid, and its formation thus securely rate determining.

The pH–rate profile for hydrolysis of **4** is shown in Fig. 1. This is closely similar to the comparable plot for the corresponding phenyl ester obtained by Usher and his coworkers,[3] with the exception of the 'step' near pH 4, which indicates that the spontaneous hydrolysis of the CO_2H form shown is faster† than that of the dianion. The reaction is

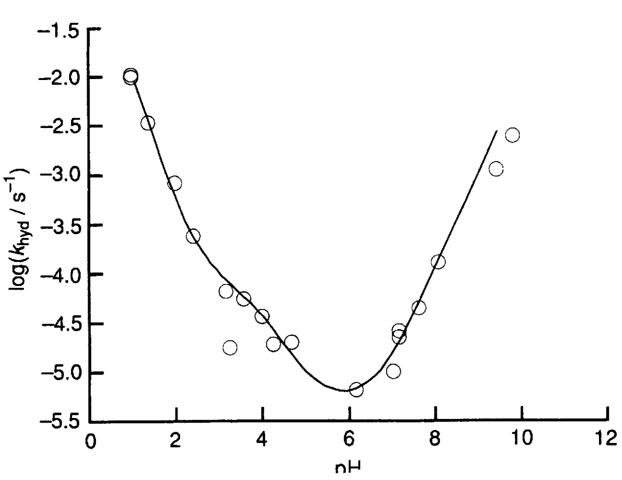


Fig. 1 pH–rate (s^{-1}) profile for the hydrolysis of **4** at 50 °C and ionic strength 10 mol dm^{-3} (KCl)

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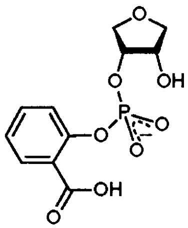
† By no more than would be expected from the electronic effect of the CO_2H group.



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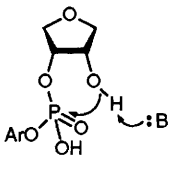

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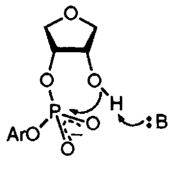

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catalysed by the buffers used to maintain pH, and the points shown were obtained by extrapolation to zero buffer concentration.

We observe general acid catalysis for formic and acetic acid, dihydrogen phosphate and TRIS‡ buffers. (No buffer catalysis was detectable for carbonate–hydrogen bicarbonate.) The data are accurately correlated by the Brønsted equation (statistically corrected,[4] $r = 0.999$), with a coefficient, $\alpha = -0.38$. Formic, acetic and dihydrogen phosphate act exclusively as general acids, but TRIS shows strong general base catalysis also (k_B 1.7×10^{-4} compared with k_{BH^+} 2.2×10^{-5} dm^3 mol^{-1} s^{-1}). The simplest, and probably correct, interpretation of these results is that catalysis by the weaker general bases, at lower pH, involves the protonated, neutral form of the diester (rate determining step as in **5**). TRIS, the strongest general base used, necessarily acts at higher pH, where less protonated form is present. In this case the predominant mechanism is general base catalysis **6** of the reaction of the substrate (di)anion.

It is of particular interest that the changeover occurs in the region of pH 7, indicating that a catalyst with a pK_a near 7 can act (kinetically) as both general acid and general base. This result also allows an estimate of the rate advantage conferred by protonation of the diester anion, as 10^{5-6} (the pK_a of the diester acid **5** is expected to be about 1).§

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‡ Tris(hydroxymethyl)aminomethane.

§ Similar comparisons for buffer catalysed reactions of the related substrate described in the following paper[2] give a range for this factor between 10^4 and 5.5×10^6. More extensive data for the relative reactivity of phosphate di- and tri-esters show that this can vary over a wide range, depending on the leaving group and the basicity of, and charge[5] on, the nucleophile.