Catalytic Hydrolysis of 2',3'-Cyclic Adenosine Monophosphate by Aqua(2,2':6',2"-terpyridine)copper(II): Breakdown of the Analogy Between Activated Phosphodiesters and RNA

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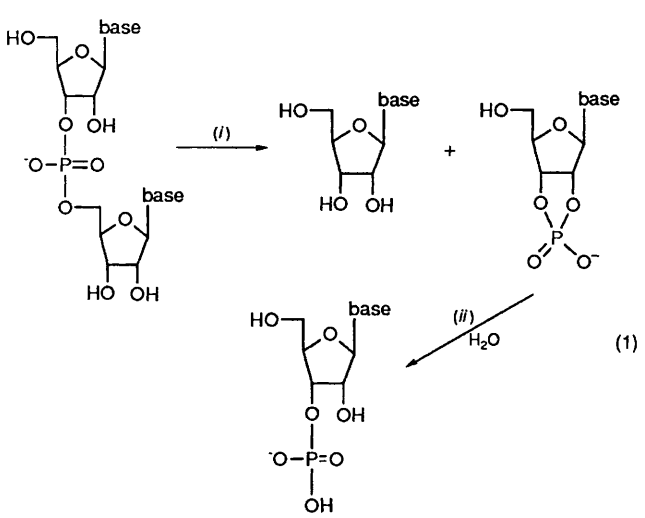
Aqua(2,2':6',2"-terpyridine)copper(II) catalyses the hydrolysis of 2',3'-cyclic adenosine monophosphate, but does not hydrolyse the 'activated' substrate bis(*p*-nitrophenyl) phosphate indicating that the latter is not a reliable model for biological phosphoric ester hydrolysis.

We report a breakdown of the analogy between activated phosphates commonly used to model RNA and RNA itself: 2',3'-cyclic adenosine monophosphate (cAMP) is hydrolysed by aqua(2,2':6',2"-terpyridine)copper(II) **1**, while 'activated' *p*-nitrophenyl phosphodiesters are not. The reported[1] failure of **1** to promote hydrolysis of activated phosphodiesters led to the recent conclusion that this complex must be incapable of hydrolysing unactivated phosphodiesters such as those present in RNA.[2] We have confirmed that **1** does not hydrolyse activated model phosphodiesters, but demonstrate here that the biologically important substrate 2',3'-cAMP is hydrolysed by **1**.

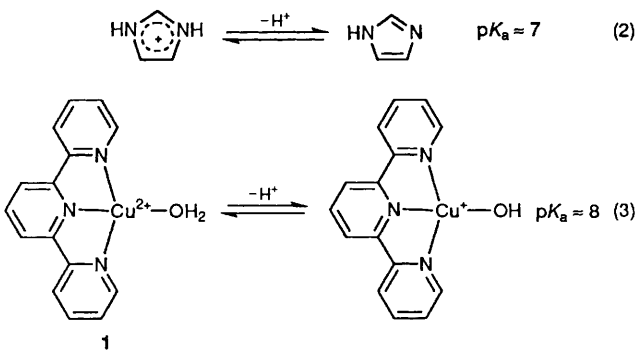
Metal-catalysed transesterification and hydrolysis of RNA are fundamental to the chemistry of living cells. For example, the maturation (*via* cleavage and hydrolysis) of transfer[3] and ribosomal[4] RNA is carried out by ribozymes in processes that require metal cofactors. Hydrolytic cleavage of RNA is usually viewed as a two-step process [equation (1)], as shown below for a dinucleoside phosphate. The first step is a transesterification that employs the 2'-OH of RNA as an intramololecular nucleophile, while the second step is hydrolysis. Both steps are



(1)

catalysed by imidazole–imidazolium (Him–H_2im^+) *via* general base–general acid mechanisms.[5] A parallel between imidazolium–imidazole[6] and M–OH_2/M–OH[7] complexes is illustrated below [equations (2) and (3)].

We recently reported the first examples of well-defined metal



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complexes (including **1**) that can promote step (*i*) of RNA hydrolysis, and we wished to determine if the second step is also promoted by metal complexes.[8] In contrast to imidazole, both the acid and conjugate base forms of **1** are cationic. Therefore, weakly acidic complexes such as **1** have the advantage over imidazolium that they are cationic in the form of their conjugate base, and both their acid and base forms can deliver themselves to RNA substrates by electrostatic or Lewis-acid interactions.

Although acid- and base-catalysed hydrolysis of 2',3'-cyclic nucleotide monophosphates has been studied over a wide pH range,[9] the chemical role that metals play in this reaction remains largely unknown. The commonly cited[8b-d,10] mechanism of metal-promoted phosphate ester hydrolysis derives from studies of 'activated' substrates such as the *p*-nitrophenyl phosphates.[11] The leaving group for these reactions is the stabilized anion *p*-nitrophenolate, and the established mechanism involves direct co-ordination between metal and phosphate and nucleophilic attack by free or co-ordinated hydroxide.

The importance of these steps has not been established for biologically relevant phosphodiesters. In fact, a different set of mechanistic themes is being developed for RNA hydrolysis and transesterification. For example, the self-cleavage of yeast phenylalanine transfer RNA by Pb^{2+} does not occur at the site where the lead ion binds, but rather at a remote site.[12] Furthermore, Cowan and Jou[13] have ruled out direct metal–phosphate binding during catalysis by Ribonuclease H, an enzyme that employs a metal cofactor to hydrolyse the RNA strand of DNA–RNA hybrids. Thus, it remains to be determined if direct metal–phosphate binding is important to activate RNA phosphodiester groups toward nucleophilic

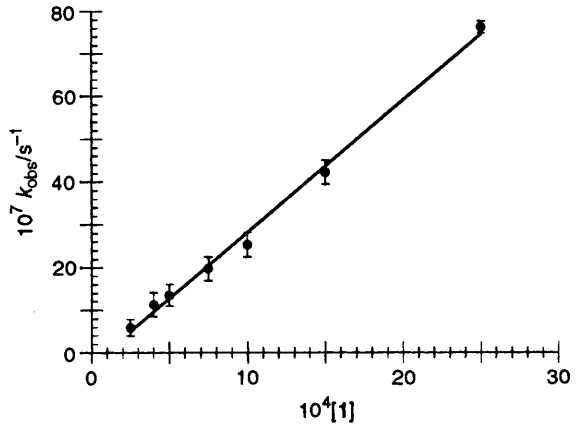
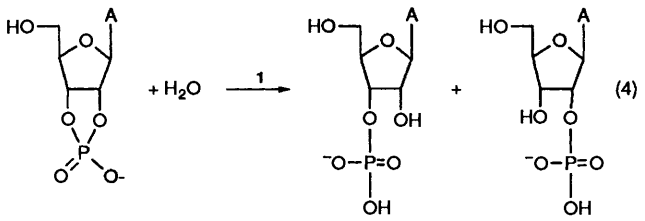


Fig. 1 Dependence of k_{obs} on [**1**]. Conditions: $[cAMP]_{initial}$ = 100 μmol dm^{-3}, [**1**] = 250–2500 μmol dm^{-3}, 5 mmol dm^{-3} hepes buffer, 50 mmol dm^{-3} $NaClO_4$, 4 mmol dm^{-3} toluene-*p*-sulfonic acid (internal standard), T = 37 °C. At pH 7.4, the second-order rate constant is 0.002 64 ± 0.000 06 dm^3 mol^{-1} s^{-1} (r = 0.997)

attack, and whether a metal-bound hydroxide acts as a nucleophile in these reactions. Additionally, protonation of the phosphate by $M–OH_2$ may activate the negatively-charged phosphate toward nucleophilic attack by stabilizing the transition states. To probe these questions, we are studying the kinetics and mechanism of metal-promoted RNA hydrolysis and cleavage.



The hydrolysis of cAMP by **1** [see equation (4)] at 37 °C and pH 7.4 was monitored by analysing aliquots *via* reverse-phase high performance liquid chromatography (HPLC), which separates the starting material from the products 2′-AMP and 3′-AMP.[2] The initial concentration of cAMP was 100 μmol dm^{-3}, and quantification was accomplished by comparing integrated peak intensities (detected at 260 nm) relative to the intensity of the internal standard toluene-*p*-sulfonic acid (4 mmol dm^{-3}). Reaction mixtures were buffered with 0.005 mol dm^{-3} hepes [*N*-(2-hydroxyethyl)piperazine-*N*-ethanesulfonic acid], and ionic strength was kept constant at a value of 56 ± 1 mmol dm^{-3} by the addition of 50 mmol dm^{-3} $NaClO_4$. The hydrolysis of cAMP is accelerated relative to background in the presence of complex **1**. All k_{obs} values were determined at least in triplicate and errors are reported as standard deviations. The products of the reaction are 2′-AMP and 3′-AMP, in a ratio of 1.5:1, as determined using calibration curves.

Fig. 1 illustrates the dependence of k_{obs} on [**1**] at pH 7.4 for the range 250 ⩽ [**1**] ⩽ 2500 μmol dm^{-3}. Based on least-squares analysis, we conclude that a second order reaction occurs with k_2 = 0.002 64 ± 0.000 06 dm^3 mol^{-1} s^{-1}. To place this rate constant in perspective, the second-order rate constant for imidazole-catalysed hydrolysis of cytidine 2′,3′-cyclic phosphate is 800-fold smaller.[14] Catalytic turnover (1.3 turnovers) was observed with [cAMP] = 2.5 mmol dm^{-3} and [**1**] = 1 mmol dm^{-3}, although some product inhibition appeared to occur. The pH dependence of the reaction is consistent with Brønsted–Lowry base catalysis by $[Cu(terpy)(OH)]^+$ (terpy = 2,2′:6′,2″-terpyridine). The additional role of **1** as a Lewis or Brønsted–Lowry acid catalyst is under investigation.

We have confirmed that under our experimental conditions (pH 7.4, [substrate] = 100 μmol dm^{-3}, [**1**] = 1000 μmol dm^{-3}), bis(*p*-nitrophenyl) phosphate is inert to hydrolysis by **1** (no acceleration over background was observed after 5 d at 37 °C). However, we have shown that the RNA cyclic phosphate ester is hydrolysed by complex **1**. We conclude that there is *no direct parallel* between the hydrolysis of activated phosphate esters (*i.e. p*-nitrophenyl phosphates) and unactivated phosphate esters such as those found in RNA. The reasons for this discrepancy may include the possibility that different reaction mechanisms operate for hydrolysis at pH 7 of activated esters (whose stabilized leaving groups need not be protonated) and unactivated esters (whose leaving groups must be protonated). Several mechanisms are consistent with the observed behaviour of complex **1**, including: (i) sequential Brønsted–Lowry acid/Brønsted–Lowry base catalysis analogous to the cleavage of RNA by imidazolium–imidazole buffers. This analogy may be particularly apt because the pK_a of $[Cu(terpy)(H_2O)]^{2+}$ is 8.08,[7] relatively close to that of imidazolium,[6] and (ii) Lewis acid–Brønsted–Lowry base catalysis by **1**, or a variant in which Cu–OH acts as a nucleophile instead of a base.

In conclusion, we have shown that **1** does promote the hydrolysis of RNA, in contrast to its inability to hydrolyse 'activated, model' phosphodiesters. The reaction is first order with respect to **1**, which parallels the reported behaviour of imidazole.[5] We suggest that **1** and its analogues are inorganic analogues of imidazole, with the additional property that **1** can deliver itself to RNA by means of electrostatic attraction or direct co-ordination. This difference may account for the 800-fold enhanced cleavage of 2′,3′-cyclic nucleotide monophosphates by the terpyridyl complex **1** *vs.* imidazole.[12]

Acknowledgements

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