Unprecedentedly Fast Hydrolysis of the RNA Dinucleoside Monophosphates ApA and UpU by Rare Earth Metal Ions

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Rare earth metal(III) ions rapidly hydrolyse adenylyl(3′-5′)adenosine and uridyl(3′-5′)uridine at pH 8, 30 °C; the half-life of the former is 10 min with 0.01 mol dm^{-3} Tm^{III}.

Catalytic hydrolysis of RNA has attracted much interest, mainly because of potential applications to artificial ribonucleases.[1–5] Hydrolysis of uridyl(3′-5′)uridine (UpU) by Eu^{III} has been reported (half-life 10 h at 80 °C).[5] However, no catalyst has ever hydrolysed RNA with sufficient efficiency under physiological conditions. We report here remarkably fast hydrolyses of adenylyl(3′-5′)adenosine (ApA) and UpU by rare earth metal(III) ions.

Hydrolysis of ApA and UpU (10^{-4} mol dm^{-3}) was carried out at pH 8 (Tris buffer, 0.05 mol dm^{-3}) and 30 °C (unless otherwise noted) under nitrogen, and was followed by periodic HPLC analysis (ODS column with water–aceto-

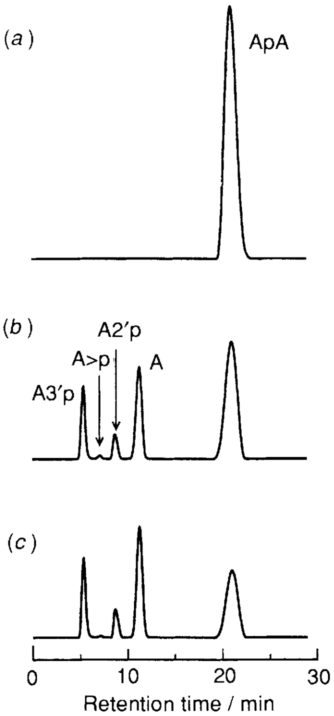

Fig. 1 Reversed phase HPLC patterns for the hydrolysis of ApA catalysed by $\mathrm{Tm^{III}}$ (0.01 mol $\mathrm{dm^{-3}}$) at pH 8, 30 °C: (*a*) $t = 0$ min, (*b*) $t = 10$ min, (*c*) $t = 20$ min

nitrile, 94 : 6 for ApA and 97 : 3 for UpU). The change in pH before and after the hydrolysis was at most 0.1 pH unit. Special care was taken to avoid contamination by ribonuclease. The absence of the enzyme was confirmed by repeated control experiments as well as formation of adenosine 2′-phosphate (see below).

At 30 °C and pH 8, $\mathrm{Tm^{III}}$ promptly hydrolyses ApA to adenosine (A) and its 2′- and 3′-phosphates (A2′p and A3′p), as depicted in Fig. 1. The pseudo-first-order rate constant with 0.01 mol $\mathrm{dm^{-3}}$ $\mathrm{Tm^{III}}$ is 6.8×10^{-2} $\mathrm{min^{-1}}$ (half-life 10 min). In the absence of $\mathrm{Tm^{III}}$, no hydrolysis takes place (half-life is estimated to be 130 years).[3] The acceleration by $\mathrm{Tm^{III}}$ is nearly 10^7 fold. The hydrolytic rate is, to the best of our knowledge, the largest value yet reported. UpU is also hydrolysed efficiently by $\mathrm{Tm^{III}}$ (rate constant 1.4×10^{-2} $\mathrm{min^{-1}}$ with 0.01 mol $\mathrm{dm^{-3}}$ $\mathrm{Tm^{III}}$).

Formation of small but definite amount of adenosine 2′,3′-cyclic phosphate (A>p) as the hydrolysis intermediate is detectable [see Fig. 1 (*b*) and (*c*)]. The A2′p and A3′p, formed from A>p, are further hydrolysed to A by the metal ion. The absence of any other products confirms that the cleavage proceeds totally *via* the hydrolytic pathway. Contamination by ribonuclease is ruled out by the formation of A2′p (the enzyme, if present, should yield A3′p with 100% selectivity).

Other rare earth metal(III) ions are also effective at pH 8, 30 °C; their catalytic activity is in the following order: Tm, Lu > Y > Nd, Eu, Sm > Ce, Sc, Gd, Tb > Pr, Dy > Ho, Er > Yb > La. Even $\mathrm{La^{III}}$, the poorest in this series, accelerates the hydrolysis by 10^5 fold at 0.01 mol $\mathrm{dm^{-3}}$. In contrast, catalysis by $\mathrm{Ca^{II}}$, $\mathrm{Mg^{II}}$, $\mathrm{Zn^{II}}$, $\mathrm{Al^{III}}$ and $\mathrm{Fe^{III}}$ was virtually absent.

The rate of cleavage by rare earth metal ions increases monotonically with increasing pH; the cleavage by $\mathrm{Tm^{III}}$ at pH 8 and 8.5 is faster than that at pH 7 by 43 and 190 fold, respectively. Thus the previously reported small acceleration by 0.01 mol $\mathrm{dm^{-3}}$ $\mathrm{Eu^{III}}$ (1460 fold at pH 7) for the hydrolysis of UpU[5] is reasonable. Probably metal–hydroxo complexes, formed at pH > 6.5,[6] are responsible for the catalysis, as in the hydrolysis of the activated phosphate esters ATP[7] and 2,4-dinitrophenyl diethyl phosphate.[8] The hydroxide ion functions as a general base catalyst, activating the 2′-hydroxy residue of the ribose for the intramolecular attack on the phosphorus atom.

In conclusion, unprecedentedly fast RNA hydrolysis has been achieved by rare earth metal(III) ions. The metal ions are promising as catalytic centres in artificial ribonucleases.

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