JOURNAL OF PHYSICAL ORGANIC CHEMISTRY, VOL. 6, 347–356 (1993)

METAL IONS THAT PROMOTE THE HYDROLYSIS OF NUCLEOSIDE PHOSPHOESTERS DO NOT ENHANCE INTRAMOLECULAR PHOSPHATE MIGRATION

SATU KUUSELA AND HARRI LÖNNBERG*

Department of Chemistry, University of Turku, SF-20500 Turku, Finland

The effects of several metal ions and metal ion complexes on the hydrolysis and interconversion of uridylyl(2′,5′)uridine and its 3′,5′-isomer were studied as a function of pH and metal ion concentration. The hydrolysis was shown to be markedly accelerated by Zn^{2+}, Cd^{2+} and trivalent lanthanide ions and by tri- and tetraaza complexes of Zn^{2+}. In contrast, none of these species appreciably promotes the interconversion of the 2′,5′- and 3′,5′-isomers, in spite of the fact that this reaction proceeds through the same pentacoordinated intermediate as the hydrolysis. Lanthanide ions also promote the hydrolytic dephosphorylation of uridine 2′- and 3′-monophosphates, but have a barely noticeable effect on their interconversion. The mechanisms of the metal ion-promoted reactions are discussed.

INTRODUCTION

The metal ion-promoted hydrolysis of phosphoesters has recently attracted considerable interest for two reasons: (i) several enzymes catalysing the transfer of the phosphate group require metal ions as cofactors[1] and (ii) metal-based catalysts offer a versatile tool to hydrolyse the phosphodiester bonds of nucleic acids.[2] Since ribonucleic acids (RNA) are hydrolytically much less stable than deoxyribonucleic acids (DNA),[3] most of the latest studies have been focused on reactions of RNA.[4] The model compounds used to elucidate the mechanism of metal ion action include 4-nitrophenyl 1-(2-hydroxypropyl)phosphate,[5,6] dinucleoside mono-[5,7,8] and diphosphates,[9–11] trinucleoside di- and triphosphates[9,10,12] and oligoribonucleotides.[4,13] Furthermore, the metal ion-promoted hydrolysis of nucleoside 2′,3′-cyclic monophosphates, appearing as intermediates of RNA hydrolysis, has been studied.[14] Among various metal species, the aquo ions and/or aza complexes of Cu^{2+}, Zn^{2+}, Pb^{2+} and lanthanide ions have been shown to cleave RNA and its model compounds most effectively.[4–13]

The metal ion-promoted hydrolysis of internucleosidic phosphodiester bonds of RNA proceeds by the intermediate formation of a 2′,3′-cyclic monophosphate, indicating that the neighbouring 2′-hydroxyl function acts as an intramolecular nucleophile.[9,11,12] Two alternative mechanisms, consistent with this reaction pathway, have been proposed to explain the rate-accelerating effect of metal ions. Butzow and Eichhorn[9] suggested that binding of a metal ion to the negatively charged phosphodiester group electrostatically facilitates the attack of the 2′-hydroxyl function. More recently, a bifunctional mechanism has been preferred: a hydroxo ligand of the phosphate bound metal ion is assumed to act as a general base, deprotonating the 2′-hydroxyl group concerted with its nucleophilic attack on phosphorus.[5,6,8,13]

Whereas the metal ion-promoted hydrolysis of the internucleosidic phosphodiester bond has been extensively studied, virtually no attention has been paid to the isomerization of the 3′,5′-bond to the 2′,5′-bond. However, the latter reaction competes with the phosphoester hydrolysis under acidic conditions and is even the predominant reaction in neutral solutions.[15,16] Since the phosphodiester hydrolysis and 3′,5′ → 2′,5′ migration proceed via a common pentacoordinated intermediate, quantitative data on the effects that metal ions have on the phosphate migration would undoubtedly further the understanding of the action of metal ions on the solvolytic reactions of nucleoside phosphoesters. For this purpose, we report here on the effect of metal ions and metal ion complexes on the following reactions: (i) hydrolysis of uridylyl-(2′,5′)-

---

* Author for correspondence.

0894–3230/93/060347–10$10.00

Received 16 November 1992
Revised 4 February 1993

and -(3′,5′)-uridine (2′,5′- and 3′,5′-UpU) to uridine 2′,3′-cyclic monophosphate (2′,3′-cUMP) and uridine, (ii) interconversion of 2′,5′- and 3′,5′-UpU, (iii) hydrolytic dephosphorylation of uridine 2′- and 3′-monophosphates (2′- and 3′-UMP) and (iv) interconversion of 2′- and 3′-UMP.

2′,5′-UpU and 3′,5′-UpU were selected as model compounds in spite of the fact that metal ions are known to accelerate the hydrolysis of dinucleoside diphosphates, such as uridylyl-(3′,5′)-uridine 3′-monophosphate, much more efficiently than that of dinucleoside monophosphates.[9,11] However, RNA molecules do not bear a 3′-terminal monophosphate function, and hence dinucleoside monophosphates appear to be more appropriate model compounds. Metal ions have been suggested to undergo in RNA a monodentate binding to phosphodiester bonds, whereas in dinucleoside diphosphates bidentate coordination to the 3′-monophosphate group and the adjacent 3′,5′-phosphodiester bond takes place.[10] Moreover, comparative kinetic studies with short homooligomers have indicated that a dinucleoside monophosphate is already a sufficient model of oligoribonucleotides; the increased hydrolytic instability of oligomers compared with dinucleoside monophosphates may simply be attributed to the increased number of phosphodiester bonds.[9] Interconversion of nucleoside monophosphates was included in this study to elucidate the susceptibility of the final hydrolysis products of UpUs to the presence of metal ions and to establish whether the mechanistic conclusions drawn using mutual isomerization of phosphodiesters may be extended to the isomerization of the corresponding monoesters.

RESULTS AND DISCUSSION

Effect of divalent metal ions on hydrolysis and interconversion of 2′,5′- and 3′,5′-UpU

Table 1 shows the effect of various divalent metal ions on the hydrolysis of 2′,5′-UpU (**1**) and 3′,5′-UpU (**2**) to uridine (**6**) and a mixture of 2′-UMP (**4**) and 3′-UMP (**5**) (Scheme 1). As suggested previously,[9,12] the reaction undoubtedly proceeds via a 2′,3′-cyclic monophosphate (**3**); high-performance liquid chromatographic (HPLC) analyses show an intermediate appearance of **3**, and **4** and **5** are produced in exactly the same ratio as in the hydrolysis of **3**.[14] Of the metal ions studied, Zn^{2+} promotes the hydrolysis UpUs most effectively, resulting in an 80- and 140-fold acceleration ($[M^{2+}] = 5 \times 10^{-3}$ mol dm^{-3}) on the hydrolysis of **1** and **2**, respectively. With the other metal ions the rate enhancement ranges from 2- to 40-fold. The effects of the latter cations on hydrolysis of the 2′,5′- and 3′,5′-isomers must be almost equal, since the disappearance of **1** + **2** strictly obeyed first-order kinetics (as in the absence of metal ions[16]), in spite of the fact that the starting material underwent isomerization concurrent with hydrolysis.

The rate-accelerating effects observed are parallel but smaller than those obtained with 4-nitrophenyl 1-(2-hydroxypropyl)phosphate (**7**).[5,6] The Zn^{2+}-promoted reaction of the latter compound, for example, is 150 times faster than the uncatalysed reaction at $[Zn^{2+}] = 5 \times 10^{-4}$ mol dm^{-3} and pH 7, whereas with UpUs a tenfold higher metal ion concentration is needed to result in a comparable rate enhancement. Similarly, Mg^{2+} at a concentration of

Table 1. Effect of divalent metal ions (5×10^{-3} mol dm^{-3}) on the interconversion and hydrolysis of 2′,5′-UpU (**1**) and 3′,5′-UpU (**2**) at pH 5·6 and 363·2 K[a]

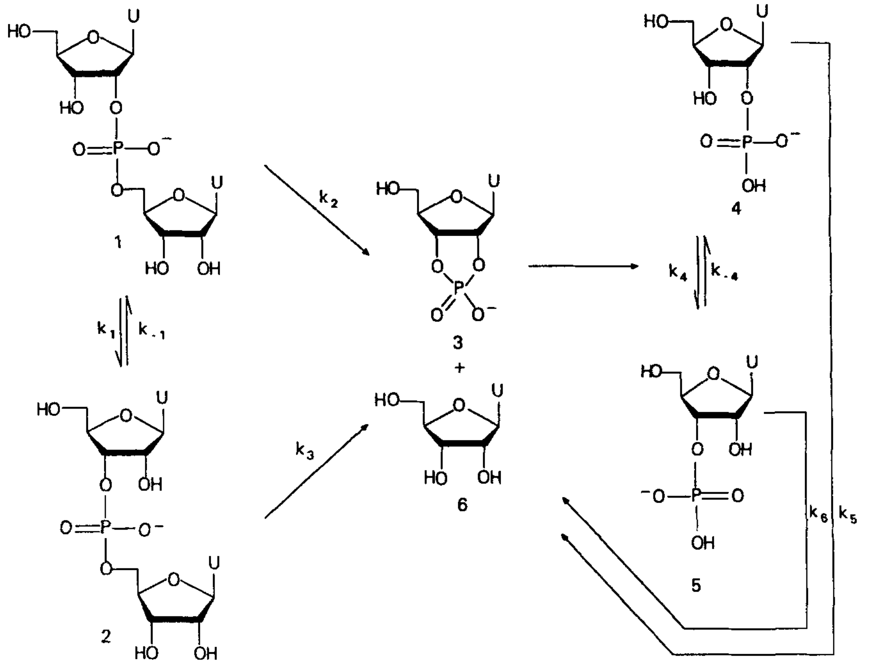
M^{z+}	$k_1/10^{-6}$ s^{-1}	$k_{-1}/10^{-6}$ s^{-1}	$k_2/10^{-6}$ s^{-1}	$k_3/10^{-6}$ s^{-1}
None	0·73	0·65	0·052 ± 0·001	[d]
Mg^{2+}[b]	0·73	0·65	0·104 ± 0·008	[d]
Mn^{2+}[c]	0·79	0·76	0·650 ± 0·017	[d]
Co^{2+}[c]	0·74	0·68	0·455 ± 0·009	[d]
Ni^{2+}[c]	0·74	0·66	0·181 ± 0·011	[d]
Zn^{2+}[c]	0·46	0·81	4·25 ± 0·04	7·22 ± 0·17
Cd^{2+}[c]	0·35		2·00 ± 0·01	

[a] Obtained in a HEPES buffer {$[HA]/[A^-] = 0·1/0·01$ mol dm^{-3}; the pK_a value of HEPES extrapolated to 363·2 K is 6·6 (Ref. 17)}. The ionic strength was adjusted to 0·1 mol dm^{-3} with $NaClO_4$. For the rate constants see Scheme 1.

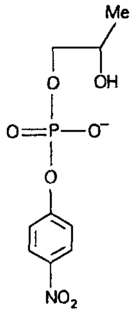
[b] Added as nitrate.

[c] Added as perchlorate.

[d] The disappearance of **1** + **2** strictly obeyed first-order kinetics, in spite of the fact that $[1]/([1] + [2])$ changed from 1·00 to less than 0·50 during a kinetic run. Accordingly, k_2 must be approximately equal to k_3.



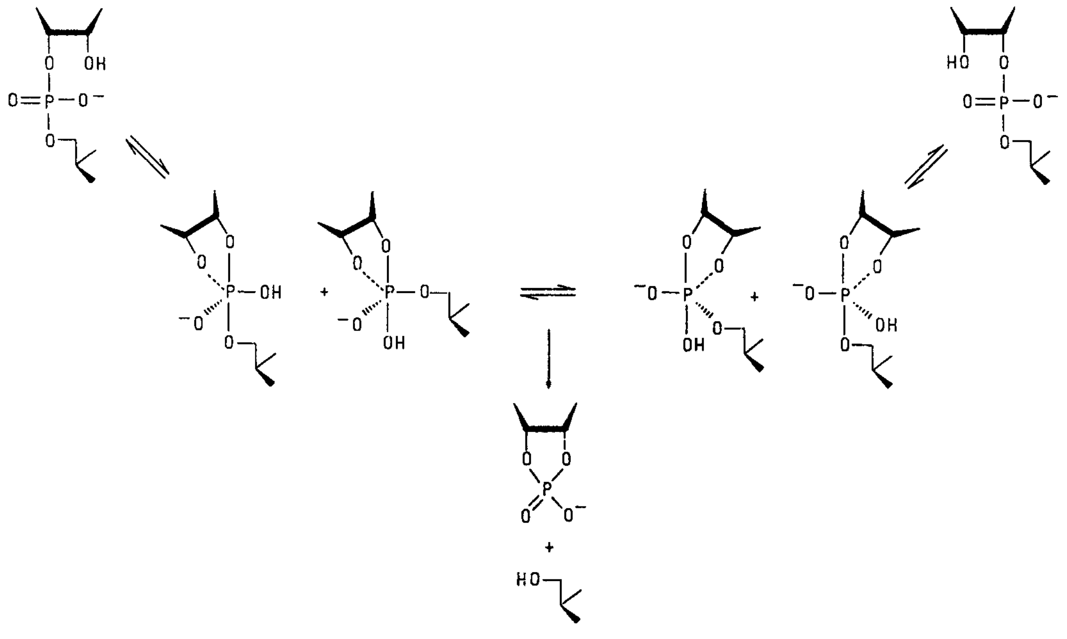
Scheme 1.



7

5×10^{-4} mol dm^{-3} accelerates the hydrolysis of 7 by a factor of 46,[5] but a tenfold higher Mg^{2+} concentration has a barely noticeable effect on the hydrolysis of UpUs. In the absence of metal ions both the UpUs and 7 may be expected to hydrolyse through a pentacoordinated phosphorane intermediate obtained by an intramolecular attack of the neighbouring hydroxyl group on phosphorus,[5,15,18] as depicted in Scheme 2. With 7 the rate-limiting step is undoubtedly the formation of the phosphorane intermediate, since the 4-nitrophenoxide ion is such a good leaving group that its departure is always faster than the reverse of the formation step.[5] The observed metal ion catalysis may thus simply be attributed to enhanced formation of the phosphorane intermediate. With UpUs both the formation and breakdown of the phosphorane intermediate contribute to the observed hydrolysis rate. In the absence of metal ions the interconversion of **1** and **2** is considerably faster than their hydrolysis to **3**, suggesting that the reverse of the formation step is faster than the departure of the 5′-nucleoside.[16] Accordingly, the decomposition of the phosphorane intermediate is rate limiting under these conditions, and hence the observed rate-accelerating effect of metal ions does not result entirely from the enhanced formation of the phosphorane intermediate, but may also contain a contribution of the breakdown of this species. Anyway, the hydrolysis of UpUs is considerably less susceptible to the precence of metal ions than the hydrolysis of **7**. Evidently the use of aryl esters as model compounds may give an overestimated picture of the potential of metal ions as catalysts of RNA hydrolysis.

As seen from Figure 1, the Zn^{2+}-promoted reaction is first order in metal ion concentration at [Zn^{2+}] $< 5 \times 10^{-3}$ mol dm^{-3}. At higher concentrations the reaction order is diminished, probably owing to saturation of the starting material with the metal ion. Accordingly, it appears clear that only one metal ion participates in the hydrolysis reaction before the transition state. Another mechanistically relevant finding is that although metal ions markedly accelerate the hydrolysis of UpUs, they do not enhance the interconversion of **1** and **2** (Table 1). Since both reactions occur through



Scheme 2.

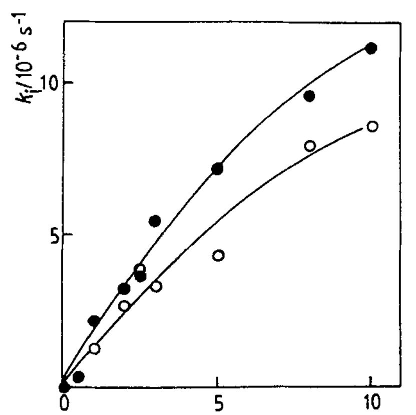
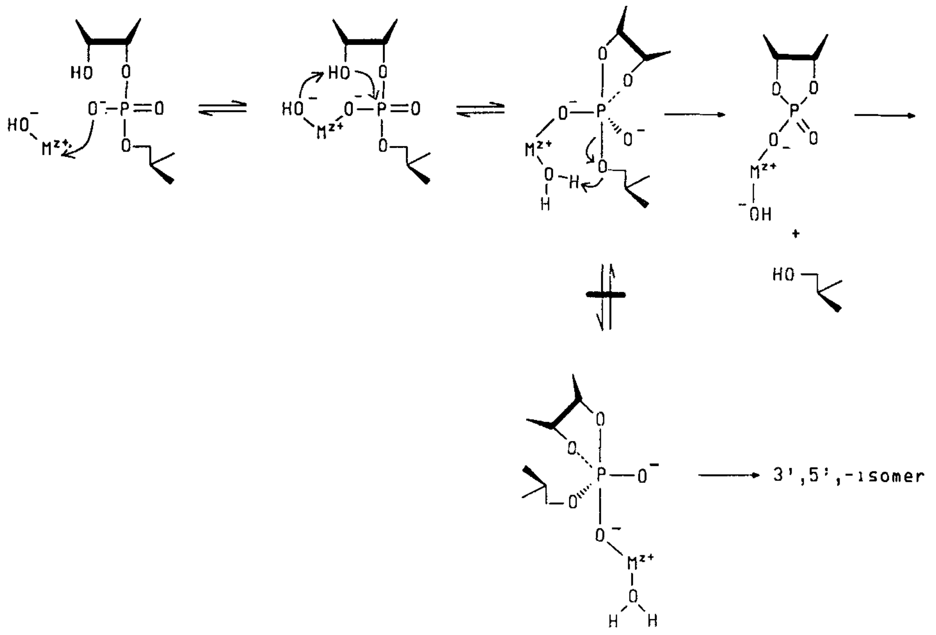


Figure 1. Effect of Zn^{2+} ion concentration on hydrolysis of (○) 2′,5′-UpU (**1**) and (●) 3′,5′-UpU (**2**) at pH 5·6 (adjusted with HEPES buffer) and 363·2 K ($I = 0{\cdot}1$ mol dm^{-3}, adjusted with $NaClO_4$)

a common phosphorane intermediate,[15,18] this means that metal ions steer the breakdown of this intermediate to hydrolysis products. In other words, the cleavage of the P—O-5′ bond is favoured over that of the P—O-2′ bond, whereas the situation is opposite in the absence of metal ions. Analogously to hydrolysis of **7**,[5,6] metal ions undoubtedly accelerate the formation of phosphorane intermediate from UpUs. However, they also must affect the breakdown of this intermediate, since they result in a change in the product distribution.

All these observations may be accounted for by the mechanism depicted in Scheme 3 for 2′,5′-UpU (**1**). This mechanism is consistent with the bifunctional mechanism suggested earlier for the metal ion action.[5,6,8,13] A rapid initial coordination of the metal ion to the phosphodiester monoanion facilitate the formation of phosphorane intermediate by lowering the electron density on phosphorus. Simultaneously, a hydroxo ligand of the phosphate bound metal ion acts as a general base deprotonating the attacking 3′-hydroxyl group concerted with formation of the P—O-3′ bond. According to the rules of Westheimer, the entering nucleophile (O-3′) adopts an apical position in the phosphorane intermediate. Hence O-2 must be equatorial, since it belongs to the same five-membered ring as O-3′. The two unsubstituted oxygen ligands become negatively charged. As strongly electropositive groups they take equatorial positions, leaving O-5′ apical. Accordingly, the P—O-5′ bond may cleave (groups may leave from apical positions only). An aquo ligand of the phosphate bound metal ion facilitates the cleavage by protonating the developing alkoxide ion. This is consistent with the principle of microscopic reversibility.

The formation of the phosphorane intermediate is assumed to be facilitated by general base catalysis, and hence its breakdown must be susceptible to general acid catalysis. Cleavage of the P—O-2′ bond, which would



Scheme 3.

lead to phosphate migration, is possible only after pseudo-rotation of the phosphorane intermediate. Since metal ions do not promote migration, it appears evident that the metal ion binding to the phosphorane intermediate retards the pseudo-rotation. A prerequisite for pseudo-roration is that one of the originally equatorial unsubstituted oxygen ligands may take an apical position. This is probably possible only if one of them becomes protonated. In all likelihood, metal ions prevent this by competing with proton for the negatively charged oxygen ligands. For comparison, in aqueous alkali the phosphorane intermediate remains in the dianionic form, and no migration, only hydrolysis, takes place.[18]

One should bear in mind that mechanisms in which one metal ion coordinates to the phosphodiester group and a second one acts as an intermolecular general acid–base catalyst may be rejected on the basis of formal kinetics; the reaction is first order in metal ion concentration. Mechanisms involving metal aquo ions as mere intermolecular general acid–base catalysts without initial coordination to the starting material also appear unlikely. Organic bases (HEPES$^-$, triethanolamine, glycine$^-$), the basicity of which is comparable to that of the hydroxo form of metal aquo ions (pK_a 7–9), do not enhance the hydrolysis of UpUs under the experimental conditions employed in studies with metal ions (Table 2). One may also argue that metal ions act simply as electrophiles without participation in intracomplex proton transfer. Although this mechanism is difficult to exclude definitely, we prefer the bifunctional alternative for the following reasons. First, the rate of

Table 2. Effect of organic bases on uncatalysed and Zn^{2+}-promoted hydrolysis of 3',5'-UpU (**2**) at pH 5·6 and 363·2 K[a]

$[Zn^{2+}]$/mol dm^{-3}	[HEPES]/mol dm^{-3}	[HEPES$^-$]/mol dm^{-3}	[Base]/mol dm^{-3}	$k_3/10^{-6}$ s^{-1}
–	0·050	0·005	0·050 (TEA)[b]	0·088 ± 0·008
–	0·050	0·005	0·050 (Gly$^-$)[c]	0·19 ± 0·01
0·005	0·020	0·002	–	9·6 ± 0·1
0·005	0·050	0·005	–	7·8 ± 0·1
0·005	0·100	0·010	–	7·1 ± 0·1
0·005	0·200	0·020	–	6·0 ± 0·3

[a] The ionic strength was adjusted to 0·1 mol dm^{-3} with $NaClO_4$.
[b] TEA = triethanolamine.
[c] Gly = glycine.

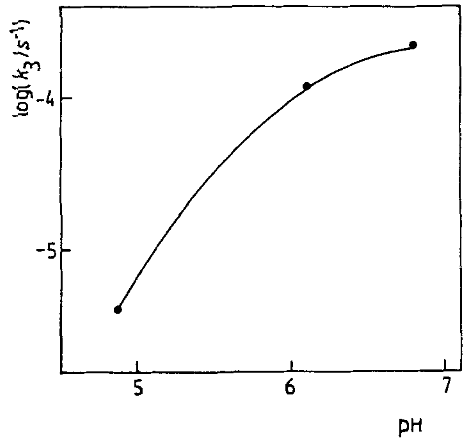
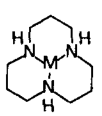
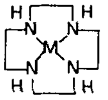


Figure 2. Effect of pH on the Zn^{2+}(1,5,9-triazacyclododecane) (**8**; [**8**] = 0·01 mol dm^{-3})-promoted hydrolysis of 3′,5′-UpU (**2**) at 363·2 K. The pH was adjusted with HEPES buffer and the ionic strength was maintained at 0·1 mol dm^{-3} with $NaClO_4$

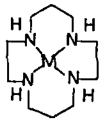
Zn^{2+}(1,5,9-triazacyclododecane) (**8**)-promoted hydrolysis initially increases with increasing pH and levels off to a constant value under conditions where the hydroxo form of the aquo complex becomes the predominant species (Figure 2; pK_a of **8** is 7·51 at 298·2 K and $I = 0·1$ mol dm^{-3}).[19] This kind of behaviour may be accounted for by intracomplex general acid–base catalysis; general base and general acid catalysis in consecutive steps result in a bell-shaped pH–rate profile when the total concentration of the acid and base catalysts remains constant. Mere electrostatic facilitation of the attack of a 2′-oxyanion would, in turn, result in a linear dependence of $\log(k_2/s^{-1})$ on pH. Second, the rate-enhancing effects obtained with various macrocyclic aza complexes of Zn^{2+} (**8**–**11**) at a fixed pH correlate with the pK_a values of their aquo ions (Figure 3).



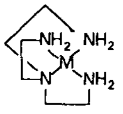
8; M=Zn^{2+}



9; M=Zn^{2+}



10; M=Zn^{2+}



11; M=Zn^{2+}, Ni^{2++}

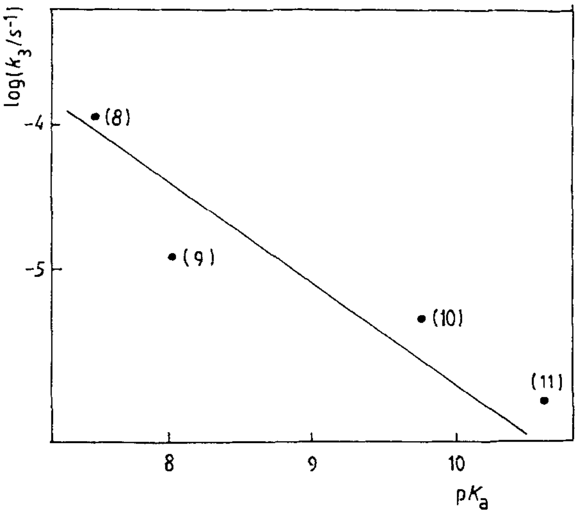


Figure 3. Logarithmic first-order rate constants for the Zn^{2+} complex-promoted hydrolysis of 3′,5′-UpU (**2**) at pH 6·1 and 363·2 K plotted against the pK_a values of the aquated complexes (kinetics refer to [complex] = 0·01 mol dm^{-3}, $I = 0·1$ mol dm^{-3} with $NaClO_4$; pK_a values[19–21] refer to 298·2 K)

Increasing acidity increases the mole fraction of intracomplex general base catalyst at pH < pK_a, and simultaneously the proton-donating ability of the intracomplex general acid catalyst is enforced. Third, the effects of various metal ions and metal complexes on

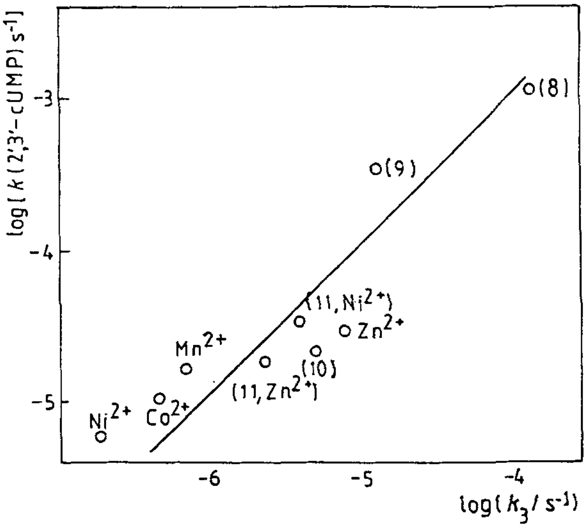


Figure 4. Comparison of the rates of metal ion-promoted hydrolysis of 3′,5′-UpU (**2**) and 2′,3′-cUMP (**3**) at pH 5·6, 298·2 K and $I(NaClO_4) = 0·1$ mol dm^{-3}. The data for **3** refer to Ref. 14

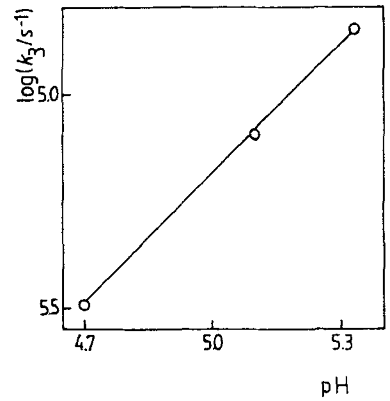


Figure 5. Effect of pH on the Zn^{2+} promoted hydrolysis of 3′,5′-UpU (**2**) ($[Zn^{2+}] = 5 \times 10^{-3}$ mol dm^{-3}) at 363·2 K. The pH was adjusted with HEPES buffer and the ionic strength was maintained at 0·1 mol dm^{-3} with $NaClO_4$

the hydrolysis of 3′,5′-UpU are very similar to those that the same species have on the hydrolysis of 2′,3′-cUMP[14] (Figure 4). The latter reaction has been established to proceed by intracomplex participation of the aquo ligand of the phosphate-bound metal ion. Finally, the rate of Zn^{2+}-promoted hydrolysis increases with increasing pH (Figure 5), consistent with both of the mechanistic alternatives. Either the mole fraction of intracomplex general base, i.e. the hydroxo form of the phosphate-bound metal ion, or the mole fraction of the 2′-oxyanion is proportional to the concentration of hydroxide ion.

Effect of lanthanide ions on hydrolysis and interconversion of 2′,5′- and 3′,5′-UpU

It has been reported recently that lanthanide ions are extremely efficient catalysts of the hydrolysis of dinucleoside monophosphates.[5,8] The experimental data available are, however, limited to measurements at one pH only. In order to understand the chemical basis of the action of lanthanide ions, we carried out more extensive studies on the hydrolysis and interconversion of UpUs. The results indicate that in several respects the effect of lanthanide ions resemble that of Zn^{2+}, but seems to be mechanistically more complicated. As with divalent metal ions, the phosphate migration is accelerated to a much lesser extent than the hydrolysis. In fact, the migration can only be detected at low lanthanide ion concentrations, i.e. under conditions where the hydrolysis is only moderately accelerated.

Figure 6 shows the effect of pH on the Gd^{3+}-promoted hydrolysis of 3′,5′-UpU (**2**). All the points refer to HEPES buffers, the buffer concentration being always less than 0·1 mol dm^{-3}. Under these conditions the hydrolysis rate was observed to be independent of the buffer concentration. As can be seen, the pH–rate profile is non-linear, the reaction order in hydroxide ion concentration gradually increasing with increasing pH. Similarly, the dependence of the hydrolysis rate on

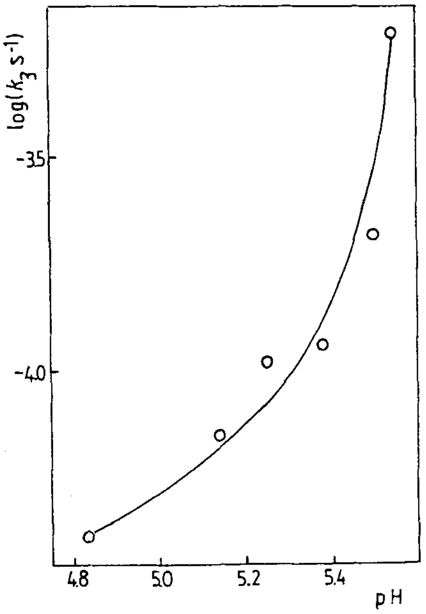


Figure 6. Effect of pH on the Gd^{3+}-promoted hydrolysis of 3′,5′-UpU (**2**) at 363·2 K. The pH was adjusted with HEPES buffer and the ionic strength was maintained at 0·1 mol dm^{-3} with $NaClO_4$

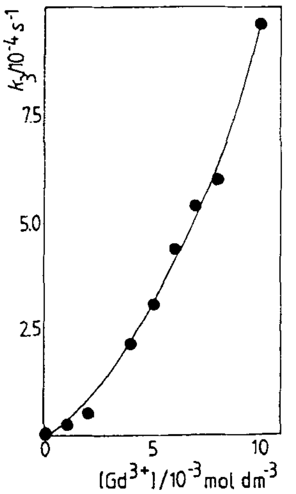


Figure 7. Effect of Gd^{3+} ion concentration on hydrolysis of 3′,5′-UpU (**2**) at pH 5·3 (adjusted with HEPES buffer). Temperature = 363·2 K and $I(NaClO_4) = 0{\cdot}1$ mol dm^{-3}

Gd^{3+} concentration shows an upward curvature (Figure 7). These findings suggest that the catalytically active species is a polynuclear structure formed on approaching the concentration limits where precipitation takes place. In fact, the observation that lanthanum hydroxide gel catalyses the hydrolysis of phosphoesters was made many years ago.[22] It is worth noting however, that La^{3+} ion has recently been reported to retain a considerable part of its catalytic activity on binding to a neutral aromatic hexaaza ligand,[4,23] and this type of chelate may therefore be applicable in developing artificial catalysts for sequence-specific strand cleavage of RNA.

Effect of metal ions on interconversion and hydrolytic dephosphorylation of 2′- and 3′- UMP

Table 3 summarizes the effects of a number of metal ions on the interconversion and hydrolytic dephosphorylation of 2′-UMP (**4**) and 3′-UMP (**5**). As can be seen, none of the divalent metal ions studied appreciably accelerates either the dephosphorylation or interconversion of **4** and **5** at pH 4·7 or 5·6. Trivalent lanthanide ions (and Y^{3+}), in turn, markedly promote the dephosphorylation of **4** and **5** at pH 5·6, whereas at pH 4·7 no rate acceleration is detected. As with UpUs (**1** and **2**), the effect of lanthanide ions on the phosphate migration, i.e. interconversion of **4** and **5**, is barely noticeable.

Figure 8 shows the effect of Gd^{3+} ion concentration on the dephosphorylation of 3′-UMP (**5**) at pH 5·3 and Figure 9 shows the dependence of the reaction rate on pH. At low metal ion concentrations ($[Gd^{3+}] < 5 \times 10^{-3}$ mol dm^{-3}) the dephosphorylation is first order in $[Gd^{3+}]$, but at higher concentrations the reaction order is gradually decreased. The dependence of dephosphorylation rate on pH exhibits a marked upward curvature, the reaction order in $[OH^-]$ falling between 2 and 3 on approaching the limit of precipitation of Gd^{3+} ion as a hydroxide. The concentration of HEPES buffers used to adjust the pH had virtually no effect on the dephosphorylation rate.

The observed dependence of rate on pH strongly suggests that Gd^{3+} does not simply act as an electrophile,

Table 3. Effect of metal ions (5×10^{-3} mol dm^{-3}) on the interconversion and dephosphorylation of 2′- and 3′-UMP at 363·2 K[a]

M^{z+}	pH	$k_4/10^{-6}\ s^{-1}$	$k_{-4}/10^{-6}\ s^{-1}$	$k_5/10^{-6}\ s^{-1}$	$k_6/10^{-6}\ s^{-1}$
None	4·7	7·5 ± 0·1	4·3 ± 0·1	12·9 ± 0·1	[e]
	5·6	5·1 ± 0·1	3·0 ± 0·1	8·7 ± 0·2	6·4 ± 0·1
Mg^{2+}[b]	4·7	7·2 ± 0·1	3·7 ± 0·1	12·1 ± 0·2	[e]
	5·6	5·5 ± 0·1	3·0 ± 0·1	9·2 ± 0·2	[e]
Mn^{2+}[b]	4·7	7·4 ± 0·1	4·7 ± 0·1	11·0 ± 0·1	[e]
Co^{2+}[c]	4·7	7·2 ± 0·1	4·8 ± 0·1	12·4 ± 0·1	[e]
	5·6	4·5 ± 0·1	2·7 ± 0·1	6·5 ± 0·1	4·8 ± 0·1
Ni^{2+}[c]	4·7	7·0 ± 0·1	3·0 ± 0·1	13·1 ± 0·1	[e]
	5·6	4·2 ± 0·1	2·2 ± 0·1	6·9 ± 0·1	[e]
Cu^{2+}[c]	4·7	7·0 ± 0·1	5·7 ± 0·1	10·7 ± 0·2	[e]
Zn^{2+}[c]	4·7	7·4 ± 0·1	4·9 ± 0·1	11·6 ± 0·2	[e]
	5·6	4·7 ± 0·1	2·9 ± 0·1	6·9 ± 0·2	[e]
Cd^{2+}[c]	4·7	7·5 ± 0·1	5·2 ± 0·1	11·2 ± 0·1	[e]
	5·6	3·0 ± 0·1	1·3 ± 0·1	4·9 ± 0·1	[e]
Pb^{2+}[b]	4·7	7·9 ± 0·1	2·7 ± 0·1	14·6 ± 0·8	[e]
Y^{3+}[b]	4·7	5·6 ± 0·1	3·5 ± 0·1	6·6 ± 0·1	3·5 ± 0·1
	5·7	7 ± 1	6 ± 1	167 ± 6	126 ± 3
La^{3+}[b]	4·7	5·7 ± 0·2	4·0 ± 0·1	9·3 ± 0·3	5·7 ± 0·1
	5·7	7 ± 1	5 ± 1	73 ± 6	53 ± 3
Eu^{3+}[b]	4·7	5·3 ± 0·2	4·1 ± 0·2	8·3 ± 0·3	4·8 ± 0·2
	5·7	7 ± 1	8 ± 1	180 ± 9	84 ± 3
Gd^{3+}[b]	4·7	6·1 ± 0·1	3·8 ± 0·1	5·7 ± 0·1	4·6 ± 0·1
	5·7	6 ± 1	7 ± 1	156 ± 6	85 ± 2
Tb^{3+}[d]	4·7	5·7 ± 0·2	3·5 ± 0·1	7·8 ± 0·2	4·1 ± 0·1

[a] See footnote a in Table 1.

[b] Added as nitrate.

[c] Added as perchlorate.

[d] Added as chloride.

[e] The disappearance of **4** + **5** strictly obeyed first-order kinetics, in spite of the fact that [**4**]/([**4**] + [**5**]) changed from 1·00 to less than 0·40 during a kinetic run. Accordingly, k_4 must be approximately equal to k_5.

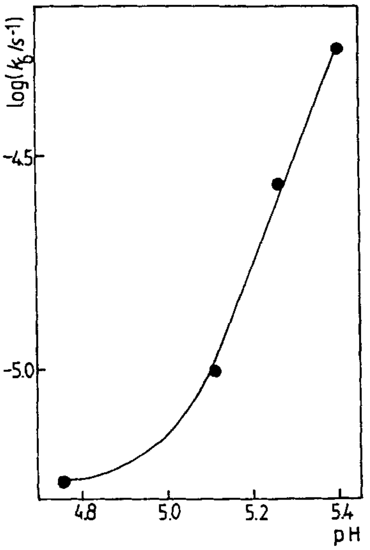


Figure 8. Effect of pH on the Gd^{3+}-promoted dephosphorylation of 3′-UMP (**5**) at 363·2 K ($[Gd^{3+}] = 2 \times 10^{-3}$ mol dm^{-3}). The pH was adjusted with HEPES buffer and the ionic strength was maintained at 0·1 mol dm^{-3} with $NaClO_4$

but in all likelihood hydroxo ligands of the phosphate-bound metal ion participate in the dephosphorylation either as an intracomplex nucleophile or a general base catalyst, resulting in the formation of a pentacoordinated intermediate. Accordingly, the lanthanide ion-promoted dephosphorylation appears to proceed by an associative mechanism, whereas in the absence of metal ions a dissociative mechanism, i.e. release of a metaphosphate ion preassociated with a solvent molecule, is followed.[24] However, the detailed mechanism of the action of metal ions is far from clear. Most likely the catalytically effective species is not a clear-cut mononuclear aquo ion, but rather a polynuclear gel-like structure. Whatever the composition of this species may be, it is important to note that only dephosphorylation, not phosphate migration, is enhanced.

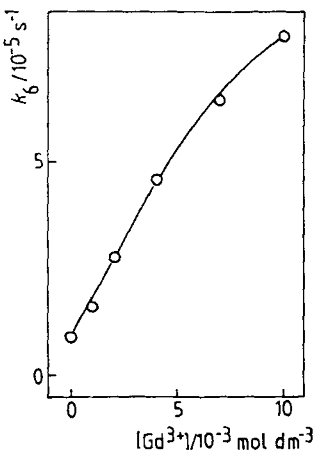


Figure 9. Effect of Gd^{3+} ion concentration on dephosphorylation of 3′-UMP (**5**) at 363·2 K. The pH was adjusted to 5·3 with HEPES buffer and the ionic strength was maintained at 0·1 mol dm^{-3} with $NaClO_4$

EXPERIMENTAL

Materials. The nucleoside and dinucleoside monophosphates (**1–5**) and uridine (**6**) were obtained from Sigma. They were used as received after checking their purity by HPLC. Ligands **8–11** were obtained from Aldrich. The other reagents were of reagent grade.

Kinetic measurements. Kinetic measurements were carried out by the HPLC technique described previously.[24] The pH values of the reaction solutions were measured at 298·2 K before and after a kinetic run. These two values agreed within 0·1 pH unit. The pH values measured at 298·2 K were extrapolated to 363·2 K by using the known temperature dependence of the pK_a value of HEPES.[17] The reaction solutions were allowed to stand at least overnight before the initiation of kinetic measurements to ensure that the complex formation equilibrium had settled. Aliquots withdrawn from the reaction mixture were immediately cooled in an ice-bath and analysed on a Hypersil RP-18 column (250 × 4 mm i.d., 5 μm). A mixture of acetate buffer (0.025 mol dm^{-3}, pH 4·3) and acetonitrile (4% with UpUs and 1% with UMPs), containing 0·1 mol dm^{-3} NH_4Cl, was used as the eluent. Before analysis the metal ions were removed by shaking the aliquots with Chelex 100 resin.

Calculation of rate constants. In most of the cases studied, the hydrolysis was fast compared with phosphate migration ($k_2 > k_1$ and $k_3 > k_{-1}$, or $k_5 > k_4$ and $k_6 > k_4$). Accordingly, the reverse reaction of the phosphate migration did not appreciably affect the kinetics of the disappearance of the starting material during the first half-life, and this reaction was observed to obey first-order kinetics within the limits of experimental errors. The rate constant obtained in this manner for the disappearance of the starting material ($k_1 + k_2, k_{-1} + k_3, k_4 + k_5$ or $k_{-4} + k_6$) was bisected to the rate constants of the two parallel first-order reactions (hydrolysis and forward migration) by using the concentration ratio observed for the products of the hydrolysis and migration during the early stage of the reaction. In cases where the rate of phosphate migration was comparable to that of hydrolysis, the first-order rate constants were obtained as described previously.[16]

ACKNOWLEDGEMENTS

Financial aid from the Foundation of Jenny and Antti Wihuri and the Research Council for Natural Sciences, the Academy of Finland is gratefully acknowledged.

REFERENCES

1. C. Walsh, *Enzymatic Reaction Mechanisms*, Freeman, San Francisco (1979).
2. T. D. Tullius, *Metal–DNA Chemistry*, ACS Symposium Series, No. 402. American Chemical Society, Washington, DC (1989).
3. D. M. Brown, *Basic Princ. Nucleic Acid Chem.* **2**, 1–90 (1974).
4. J. R. Morrow, L. A. Buttrey, V. M. Shelton and K. A. Berback, *J. Am. Chem. Soc.* **114**, 1903–1905 (1992), and references cited therein.
5. R. Breslow and D.-L. Huang, *Proc. Natl. Acad. Sci. USA* **88**, 4080–4083 (1991).
6. J. R. Morrow, L. A. Buttrey and K. A. Berback, *Inorg. Chem.* **30**, 16–20 (1992).
7. Y. Matsumoto and M. Komiyama, *J. Chem. Soc., Chem. Commun.* 1050–1051 (1990).
8. M. Komiyama, Y. Matsumoto and K. Matsumura, *J. Chem. Soc., Chem. Commun.* 640–641 (1992).
9. J. J. Butzow and G. L. Eichhorn, *Biochemistry* **10**, 2019–2027 (1971).
10. H. Ikenaga and Y. Inoue, *Biochemistry* **13**, 577–582 (1974).
11. V. M. Shelton and J. R. Morrow, *Inorg. Chem.* **30**, 4295–4299 (1991).
12. A. George, P. Draganac and W.-R. Farkas, *Inorg. Chem.* **24**, 3627–3631 (1985).
13. K. Stern, J. K. Bashkin and E. O. Sall, *J. Am. Chem. Soc.* **112**, 5357–5359 (1990).
14. S. Kuusela and H. Lönnberg, *J. Phys. Org. Chem.* **5**, 803–811 (1992).
15. R. Breslow, E. Anslyn and D.-L. Huang, *Tetrahedron* **47**, 2365–2376 (1991).
16. P. Järvinen, M. Oivanen and H. L. Lönnberg, *J. Org. Chem.* **56**, 5396–5401 (1991).
17. N. E. Good, G. D. Winget, W. Winter, T. N. Connolly, S. Izawa and R. M. M. Singh, *Biochemistry* **5**, 467–477 (1966).
18. M. Oivanen and H. Lönnberg, *Trends Org. Chem.* **2**, 183–198 (1991).
19. L. J. Zompa, *Inorg. Chem.* **17**, 2531–2536 (1978).
20. E. Kimura, T. Shiota, T. Koike, M. Shiro and M. Kodama, *J. Am. Chem. Soc.* **112**, 5805–5811 (1990).
21. S. P. Dagnall, D. N. Hague, M. E. McAdam and A. D. Moreton, *J. Chem. Soc. Farday Trans. 1* **81**, 1483–1494 (1985).
22. W. W. Butcher and F. H. Westheimer, *J. Am. Chem. Soc.* **77**, 2420–2424 (1955).
23. R. W. Hay and N. Govan, *J. Chem. Soc., Chem. Commun.* 714–715 (1990).
24. M. Oivanen and H. Lönnberg, *J. Org. Chem.* **54**, 2556–2560 (1989).