# Short Communication

Hydrolysis of Isomeric Cytidylyl-(3',5')-5'-C-methyluridines by Acids, Bases and Metal Ions: Steric Effects in the Hydrolysis of the Phosphodiester Bonds of RNA

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The metal ion promoted hydrolysis of the phosphodiester bonds of RNA has recently received increasing interest, since metal-based catalysts tethered to molecules that are able to recognize the base-sequence may possibly be exploited in the chemical tailoring of RNA, or even in selective inhibition of gene expression.[1] A bifunctional mechanism has been proposed for the metal ion action:[2–5] the metal ion is coordinated to the phosphodiester bond, and its hydroxo ligand acts as a general base deprotonating the attacking 2'-hydroxy group (Scheme 1). Owing to the pre-equilibrium binding of the
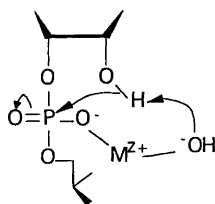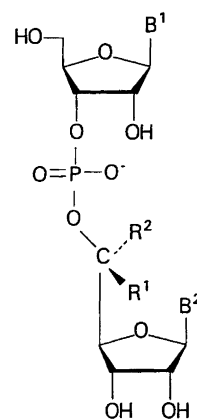

Scheme 1.

metal ion to phosphate, and the bifunctional nature of the catalysis, the reaction may be expected to be more sensitive to steric obstacles than the hydronium or hydroxide ion catalyzed hydrolysis. To elucidate the importance of steric factors, we now report on the hydrolysis of two diastereomeric 3',5'-dinucleoside monophosphates bearing a methyl substituent in place of either the pro-R (1a) or pro-S (1b) hydrogen at C5' of the 5'-esterified nucleo-

side. The hydrolysis is promoted by hydronium, hydroxide, Zn^{2+} and Gd^{3+} ions and some macrocyclic tri- and tetra-aza chelates of Zn^{2+}. The results are compared with those obtained with unsubstituted 3',5'-dinucleoside monophosphates (1c).

It has been shown previously that 3',5'-dinucleoside monophosphates (1c) undergo under acidic and neutral
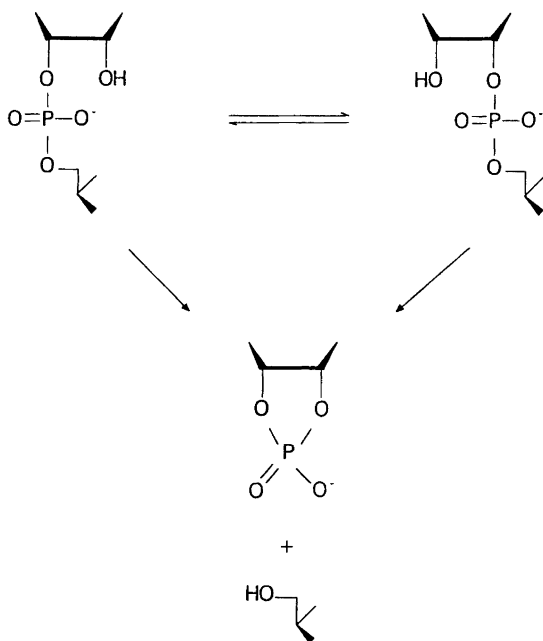

1a: B¹= cytosine, B²= uracil
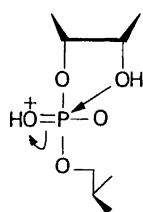   R¹= H, R²= Me

1b: B¹= cytosine, B²= uracil
    R¹= Me, R²= H

1c: R¹= R²= H

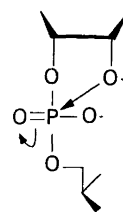† To whom correspondence should be addressed.

Scheme 2.

Scheme 4.

conditions intramolecular transesterification to their 2',5'-counterparts concurrent with the hydrolysis to a 2',3'-cyclic monophosphate (Scheme 2).[6,7] In all likelihood the diastereomeric cytidylyl-(3',5')-5'-C-methyluridines, CpRMeU (**1a**) and CpSMeU (**1b**), used in the present study behave similarly. When their acid-catalyzed hydrolyses were followed by RP HPLC, an intermediate was observed to accumulate. This intermediate (i) was UV-spectroscopically identical with the starting material, (ii) was hydrolyzed to cytosine 2',3'-cyclic monophosphate approximately as readily as the starting material, and (iii) exhibited the expected chromatographic behaviour (t_R slightly shorter than that of the starting material). Under alkaline conditions no such intermediate appeared, consistent with the fact that the alkaline hydrolysis of dinucleoside monophosphates is not accompanied by phosphate migration.[8] By analogy to the known behaviour of **1c**,[6,7] the intermediate observed in acidic solutions was assigned to the 2',5'-isomer of the starting material. The first-order rate constant, $k_f + k_r$, for the interconversion, 3',5'-Cp(R/S)MeU $\rightleftharpoons$ 2',5'-Cp(R/S) MeU, was independent of the stereochemistry at C5', being 3.95×10^{-4} s^{-1} and 3.97×10^{-4} s^{-1} ([H$^+$] = 0.1 M, $T = 363.2$ K) with CpRMeU and CpSMeU, re-

spectively. With both compounds, the 3',5'-isomer was slightly favoured at the equilibrium [x_{eq}(**1a**) = 0.56, x_{eq}(**1b**) = 0.60].

Table 1 records the first-order rate constants obtained for the hydrolysis of the phosphodiester bond of **1a** and **1b**. Under acidic conditions, where the hydrolysis proceeds by the attack of the unionized 2'-hydroxy function on the monocationic phosphodiester (Scheme 3),[7] the hydrolysis rate is completely independent of the configuration at C5', being about one fifth of that of UpU (**1c**, B^1 = B^2 = uracil). On making comparisons, one should bear in mind that replacement of the 3'-linked uridine with cytosine may be expected to retard the acid-catalyzed hydrolysis by 30%.[9] Accordingly, the hydrolysis rate of dinucleoside monophosphates methylated at C5' of the 5'-linked nucleoside appears to be 30% of that of their unsubstituted counterparts. This reactivity difference is almost equal to that between the isopropyl and methyl esters of adenosine 3'-monophosphate.[10]
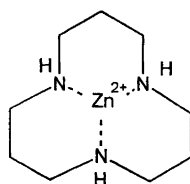
In aqueous alkali, the hydrolysis proceeds by the attack of the 2'-oxyanion on monoanionic phosphodiester (Scheme 4).[8] This reaction is more susceptible to the configuration at C5' than the acid catalyzed hydrolysis; CpRMeU is hydrolyzed in 0.1 M aqueous sodium hy-
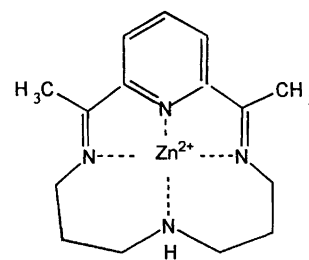

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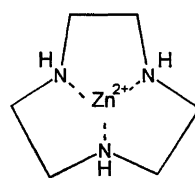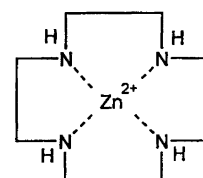
Scheme 3.

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Table 1. First-order rate constants for the hydrolysis of the phosphodiester bond of cytidylyl-(3′,5′)-5′-(R)- and -(S)-me-thyluridines (1a, b) at 363.2 K.

| Solution | $k/10^{-4}\,s^{-1}$ | | | | |
	CpRMeU	CpSMeU	UpU	a	b
0.10 M HCl	1.50(4)	1.59(2)	6.81[c]	5.40	1.67
0.05 M NaOH[d]	3.69(3)			7.59	
0.10 M NaOH	6.34(2)	3.94(2)	45.1[c]	17.9	
0.50 M NaOH	24.8(2)				
0.005 M Gd³⁺, pH 5.5[e]	3.09(3)	2.61(5)	8.65(15)		
0.005 M Zn²⁺, pH 5.6[e]	0.0228(4)	0.0129(4)	0.072[f]		
0.010 M ([9]aneN₃)Zn²⁺ (2), pH 6.1[e]	0.0344(7)	0.0100(2)	0.128(2)		
0.010 M ([12]aneN₄)Zn²⁺ (3), pH 6.1[e]	0.0130(3)	0.0032(2)	0.127(3)		
0.010 M ([12]aneN₃)Zn²⁺ (4), pH 6.1[e]	0.131(6)	0.023(2)	1.20[f]		
0.001 M 5, pH 6.1[e,g]	0.0157(6)	0.00301(6)	0.0140(4)		

[a] Methyl ester of 3′AMP; Ref. 10. [b] Isopropyl ester of 3′-AMP; Ref. 10. [c] From Ref. 7. [d] The ionic strenght adjusted to 0.1 M with sodium chloride. [e] The pH adjusted with a HEPES buffer. The ionic strength adjusted to 0.1 M with sodium perchlorate. [f] From Ref. 5. [g] Some precipitation occurred during the hydrolysis.

droxide 1.6 times as fast as CpSMeU, the reaction rate being 14% of that of unsubstituted UpU. Again part of the latter retardation may result from replacement of 3′-linked uridine with cytidine.[9]

Metal ions have been shown[5] to accelerate only the hydrolysis of dinucleoside monophosphate, not their intramolecular transesterification. Consistent with this finding, the Gd³⁺-promoted hydrolysis of CpRMeU and CpSMeU proceeded without any chromatographic indication of phosphate migration, and with Zn²⁺ and its chelates (2–5) the hydrolysis was much faster than migration. In each kinetic run the first-order rate constant $(k_f + k_r)$ of the migration was $<1 \times 10^{-6}\,s^{-1}$, i.e., smaller than the value determined earlier[7] for the uncatalyzed interconversion of 2′,5′- and 3′,5′-UpU $(1.3 \times 10^{-6}\,s^{-1})$. With Zn²⁺ and Gd³⁺, the rate-retarding effect of the 5′-C-methyl group on phosphodiester hydrolysis is comparable to that observed for the hydronium and hydroxide ion catalyzed reactions. Interestingly, the effect depends on the configuration at C5′ analogously to the hydroxide ion catalyzed hydrolysis: CpRMeU is hydrolyzed at [Zn²⁺] = 5 mM (pH = 5.6, T = 363.2 K, I = 0.1 M) 1.8 times as fast as CpSMeU, and at [Gd³⁺] = 5 mM 1.2 times as fast as CpSMeU. On replacing the Zn²⁺ ion with its more bulky macrocyclic aza complexes, the stereoselectivity, however, becomes more prominent, and seems to depend on the size of the complex ion: CpRMeU is hydrolyzed 3.4, 4.1, 5.7 and 5.2 times as fast as CpSMeU, when chelates 2–5, respectively, are employed as catalysts. With cyclododecane chelates, 3 and 4, the hydrolysis rates of CpRMeU and CpSMeU are only about 10 and 2% of that of UpU. However, since 5 promotes the hydrolysis of 1a and 1b even more effectively than that of UpU, interpretation of the steric effects is not straightforward. The stereospecificity of the hydrolysis, although clearly shown, is also difficult to rationalize. According to molecular mechanics calculations by Chem-X both stereoisomers, CpRMeU and CpS-MeU, prefer a conformation where the distances of the 5′-C-methyl group from the groups playing an essential role in the reaction, i.e., 2′-OH and the unesterified oxygen ligands, are approximately equal ranging from 0.4 to 0.5 nm.

CpRMeU and CpSMeU have previously[11] been employed to elucidate the mechanism of the action of various RNases. It was shown that CpRMeU is hydrolyzed by these enzymes 60 times as fast as CpSMeU, in spite of the fact that both stereoisomers exhibit a comparable affinity to the enzyme. The difference was attributed to the fact that the 5′-C-methyl substituent of CpSMeU reduces the mole fraction of the most reactive conformation of the dinucleoside monophosphate in the enzyme-substrate complex, i.e., the one having P–O3′ and O5′–C5′ bonds eclipsed, and hence neither of the O5′ lone electron pair orbitals would be app to the P–O3′ bond (which would according to the stereoelectronic argumentation stabilize the P–O5′ bond). It is interesting to note that although this kind of reasoning is hardly applicable when non-enzymatic reactions of flexible acyclic phosphodiesters are concerned, the effect of the C5′-configuration on the hydrolysis by enzymes, metal-ion species and hydroxide ion are all parallel.

Experimental

Materials. The preparation of CpRMeU and CpSMeU has been described earlier.[12] Uridylyl(3′,5′)uridine was a commercial product from Sigma. The polyazamacrocycles of chelates 2–4 were purchased from Aldrich. Complex 5 was prepared as described earlier.[13]

Kinetic measurements. The HPLC method utilized for kinetic measurements has been described earlier.[5,7] The rate constants of the phosphodiester hydrolysis were calculated by applying the integrated first-order rate constant to the appearance of the appropriate 5′-C-methyl-uridine.

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