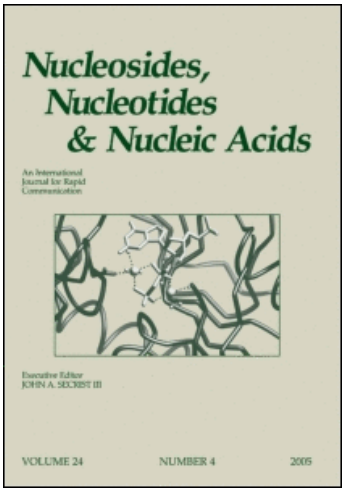


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Zn^{2} -Promoted Hydrolysis of 3',5'-Dinucleoside Monophosphates and Polyribonucleotides. The Effect of Nearest Neighbours on the Cleavage of Phosphodiester Bonds

Satu Kuusela[a]; Harri Lönnberg[a]
[a] Department of Chemistry, University of Turku, Turku, Finland

To cite this Article Kuusela, Satu and Lönnberg, Harri(1996) 'Zn^{2} -Promoted Hydrolysis of 3',5'-Dinucleoside Monophosphates and Polyribonucleotides. The Effect of Nearest Neighbours on the Cleavage of Phosphodiester Bonds', Nucleosides, Nucleotides and Nucleic Acids, 15: 10, 1669 — 1678
To link to this Article: DOI: 10.1080/07328319608002466
URL: http://dx.doi.org/10.1080/07328319608002466

NUCLEOSIDES & NUCLEOTIDES, 15(10), 1669-1678 (1996)

NOTE

Zn^{2+}-PROMOTED HYDROLYSIS OF 3',5'-DINUCLEOSIDE MONOPHOSPHATES AND POLYRIBONUCLEOTIDES. THE EFFECT OF NEAREST NEIGHBOURS ON THE CLEAVAGE OF PHOSPHODIESTER BONDS

Satu Kuusela* and Harri Lönnberg

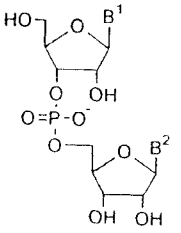
Department of Chemistry, University of Turku, FIN-20014 Turku, Finland

Abstract: Pseudo first-order rate constants for the Zn^{2+}-promoted cleavage of 15 different dinucleoside monophosphates, 4 different ribo homopolymers and RNA III from baker's yeast have been determined. Furthermore, the distribution of various nucleosides at the 3'- and 5'-terminus of the oligomeric hydrolysis products of RNA has been quantified. On these bases, the effect of nearest neighbours on the metal-ion-promoted hydrolysis of the internucleosidic phosphodiester bonds of RNA is discussed.

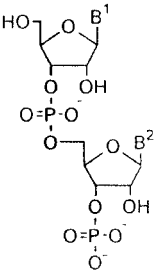
The metal-ion-promoted hydrolysis of the phosphodiester bonds of RNA has been extensively studied during the past two decades.[1,2] The aim of these studies ranges from the mechanistic understanding of the action of metal ions themselves[3] to elucidation of the role of metal ions as constituents of artificial cleaving agents,[4,5] or cofactors of protein enzyme[6] and ribozyme catalysis.[7]

The existing knowledge on the mechanism of metal-ion-promoted hydrolysis of RNA phosphodiester bonds is largely based on reactions of small molecular non-nucleosidic model compounds,[5,8] nucleoside 2',3'-cyclic monophosphates,[9,10] and dinucleoside mono- (**1**) and diphosphates (**2**).[11-13] Most of these studies suggest that a phosphate bound metal ion accelerates the nucleophilic attack of the 2'-hydroxy group on phosphorus by facilitating the deprotonation of this function concerted with its attack. Whether this facilitation results from a direct coordination of the metal ion to the attacking oxygen (*Scheme 2*), or from an intracomplex general base catalysis by the metal ion hydroxo ligand (*Scheme 1*), still remains open.[3]

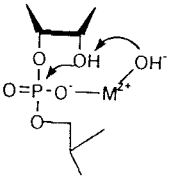
Our recent studies on hydrolysis of polyuridylic acid[14] and short chimeric ribo/deoxy-ribo oligonucleotides[15,16] suggest that the essential features of the metal-ion-catalysis remain unchanged on going to polymeric substrates. However, the molecular environment markedly affects the reactivity of an individual phosphodiester bond: neighbouring phosphodiester bonds, and especially a neighbouring 3'-terminal monophosphate group accelerate the



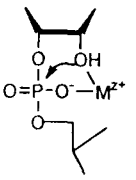
1



2



Scheme 1



Scheme 2

hydrolysis by even two orders of magnitude.[12,15,17,18] This rate-acceleration most likely results from two-dentate binding of the metal ion to two different phosphate groups, which enhances the interaction between the metal ion and the scissile bond.[15] Whether the base moieties possibly play any role in the formation of reactive oligomer/metal ion macrochelates remained an open question on the basis of these studies, since the oligonucleotides employed entirely consisted of uridine and thymine units, which are known to bind metal ions only weakly under neutral conditions.[19] With the other nucleosides, coordination of metal ions to the base moiety is more probable, in particular to N7 of purines, bearing in mind that metal ions have been shown to form aquo ligand mediated N7/5'-phosphate macrochelates.[20] In principle, additional binding to the base moiety may either enhance or decrease the catalytic activity of the metal ion: it may increase the effective concentration of metal ion in the vicinity of a scissile bond, but also hamper the interaction of the metal ion with the 2'-hydroxy function, and hence lead to reduced catalytic activity.

The existing data on the effect of base moiety structure on the metal-ion-promoted hydrolysis of RNA are puzzling. Up to 10-fold differences have been reported for the rates of Zn^{2+}-promoted hydrolysis of dinucleoside diphosphates (NpNp; **2**), the dimers derived from adenosine and/or uridine being usually more reactive than those derived from guanosine and/or cytidine.[18] By contrast, the homopolymers poly(A), poly(C) and poly(U) have been shown to be cleaved by Zn^{2+} approximately as rapidly, and only 2 times as rapidly as poly(I), the structural analogue of poly(G).[21] Finally, the distribution of 3'-terminal nucleotides after 20 to 25 % cleavage of the phosphodiester bonds of yeast RNA has been observed to some extent deviate from the statistical one: the amount of 3'-terminal GMP is reduced by 70 % and that of CMP and UMP increased by 40 % compared to a random result.[21] To learn more about the reasons for these apparent contradictions and the importance of nearest neighbours in general, we now report on the effect of base moiety structure on the Zn^{2+}-promoted hydrolysis 15 different 3',5'-dinucleoside monophosphates (NpN; **1**). With these compounds, the only potential coordination site, in addition to the

base moieties, is the scissile phosphodiester bond. With NpNp, the primary binding site is the 3'-monophosphate group, and the monophosphate bound metal ion then interacts with the scissile bond *via* intramolecular macrochelate formation between the phosphodiester and phosphomonoester group.[15,17,22] Accordingly, the significance of metal ion binding to bases neighbouring the scissile phosphodiester bond may be evaluated in a more straightforward manner with NpN than with NpNp. Furthermore, the Zn^{2+}-promoted hydrolysis of 4 different homopolymers, RNA III from baker's yeast, and some NpNp has been reexamined, in the first place to obtain a set of results under similar experimental conditions, but also to verify the early results[21] by using a different experimental approach and modern HPLC technique.

RESULTS AND DISCUSSION

The hydrolysis of 15 different 3',5'-dinucleoside monophosphates (NpN; **1**) was followed by RP-HPLC in 10 mM solution of $Zn(NO_3)_2$ at pH 5.1 and T = 363.2 K (I = 0.1 M). As reported previously for 3',5'-UpU,[13] isomerization to 2',5'-dinucleoside monophosphates was not observed to take place under these conditions. Accordingly, the starting material was quantitatively decomposed to nucleoside 2',3'-cyclic monophosphate (2',3'-cNMP) with release of the 5'-linked nucleoside, and 2',3'-cNMP was then rather rapidly hydrolyzed to a 1:2 mixture of nucleoside 2'- and 3'-monophosphates (2'/3'-NMP). Only with NpNs derived from cytidine, hydrolytic deamination of the cytosine base was observed to compete with this reaction. Table 1 records the pseudo first-order rate constants for the Zn^{2+}-promoted transesterification of NpN to 2',3'-cNMP. As seen, the rate of this reaction is markedly less susceptible to the base moiety structure than that of the same reaction of dinucleoside diphosphates (NpNp; **2**). The largest rate constant obtained with 3',5'-CpA is only 1.9-fold compared to the smallest one obtained with 3',5'-ApU. As also seen from Table 1, NpNps (**2**) exhibit up to 11-fold reactivity differences. The Zn^{2+}-promoted transesterification of NpN to 2',3'-cNMP is also less susceptible to the base moiety structure than the corresponding hydronium- and hydroxide-ion-catalyzed reactions.[23,24] Although the reactivity differences are small, NpNs containing a 5'-*O*-linked purine nucleoside are invariably cleaved by Zn^{2+} more readily than those having a pyrimidine nucleoside as a leaving group. The hydronium- or hydroxide-ion-catalyzed reactions do not exhibit a similar tendency.[23,24] One might speculate that phosphate/N7 macrochelate formation slightly enhances the binding of Zn^{2+} to the phosphodiester bond, resulting in a slight rate-acceleration. The intramolecular base-stacking that has been proposed[18] as a possible source of reactivity differences with NpNps, does not seem to play any significant role. The extent of intramolecular base-stacking estimated by theoretical calculations for various NpNs[25] is also indicated in Table 1. As seen, no correlation with the kinetic data appear to exist.

The fact that the base moieties exert a lesser effect on the Zn^{2+}-promoted transesterification of NpNs (**1**) than on the same reaction of NpNps (**2**) is somewhat surprising. To find

Table 1: The pseudo first-order rate constants for the hydrolysis of the phosphodiester bonds of dinucleoside monophosphates[a] (**1**) and dinucleoside diphosphates (**2**)[b], and the mole fractions of the stacked form of **1**.

B^1	B^2	$k(\mathbf{1})/10^{-6}$ s^{-1}	c	$k(\mathbf{2})^b$/s^{-1}
Ade	Ade	6.4±0.1	0.102	0.049
Ade	Cyt	4.6±0.1	0.100	0.039
Ade	Gua		0.138	0.059
Ade	Ura	4.2±0.1	0.156	0.10
Cyt	Ade	7.9±0.2	0.529	0.054
Cyt	Cyt	5.1±0.2	0.011	0.026
Cyt	Gua	6.7±0.1	0.070	0.012
Cyt	Ura	4.8±0.1	0.017	
Gua	Ade	5.4±0.4	0.044	0.036
Gua	Cyt	5.1±0.1	0.129	0.010
Gua	Gua	5.9±0.1	0.021	0.009
Gua	Ura	4.4±0.1	0.119	0.044
Ura	Ade	6.0±0.3	0.017	0.096
Ura	Cyt	5.7±0.1	0.021	
Ura	Gua	6.2±0.1	0.318	0.041
Ura	Ura	5.4±0.1	0.004	0.056

[a] The rate constants for the hydrolysis of NpN determined at $[Zn^{2+}]$ = 10 mM, pH 5.1 and T = 363.2 K. The pH was adjusted with a HEPES buffer, and the ionic strength (0.1 M) with $NaNO_3$.
[b] The rate constants for the hydrolysis of NpNp taken from *Ref.* 18. They refer to pH 7.0 (not adjusted) and T = 335.3 K. $[\mathbf{2}]/[Zn^{2+}]$ = 0.5.
[c] Mole fractions of the stacked form of **1** calculated at the distance of 4.5 Å. The data are taken from *Ref* 25.

out whether this difference could possibly result from different experimental conditions, such as different ratio of $[Zn^{2+}]$ and [substrate] or different temperature, employed in the present work and in the previous study of Ikenaga and Inoue,[18] the transesterification of two NpNps, *viz.* 3',5'-ApUp and 3',5'-GpUp, was reexamined under the conditions of the present work. The rate constants obtained were (7.71±0.09)x10^{-4} s^{-1} and (3.74±0.09)x10^{-4} s^{-1}, respectively. The 2.1-fold reactivity difference observed is consistent with that of 2.3 determined by Ikenaga and Inoue.[18] Accordingly, the structural effects in the transesterification of NpNs and NpNps really seem to be dissimilar.

Tentatively one may argue that the markedly different sensitivity of the cleavage of NpN and NpNp to the base moiety structure reflects different Zn^{2+} binding modes. With NpNp, the primary coordination site is undoubtedly the 3'-monophosphate dianion. The logarithmic stability constants for the Zn^{2+} complexes of nucleoside 3'-monophosphates are about 2.7.[26]

Coordination to the base moiety is weaker, as long as N1 of guanine and N3 of uracil remain fully protonated, *i.e.* at pH < 7.[19] However, the 3'-phosphate bound metal ion serves as a catalyst only if it interacts with the internucleosidic phosphodiester bond and/or the 2'-hydroxy function of the 3'-linked nucleoside. In other words, a macrochelate formation is a prerequisite for catalysis. The stability of this kind of a macrochelate may be sensitive to sugar-ring puckering, which is, in turn, influenced by the nature of the base moieties and base-base interactions. With NpN, monodentate binding to the scissile phosphodiester bond takes place, the logarithmic stability constant for the 1:1 complex being < 1.[27] The much weaker binding of Zn^{2+} to NpN than to NpNp is reflected as a slower hydrolysis of the internucleosidic phosphodiester bond.[15] However, the monodentate binding to NpN is not as sensitive to ligand conformation as the macrochelate formation with NpNp, and hence the sugar-ring puckering does not play a similar role. It is also worth noting that although Zn^{2+}-binding to the base moieties undoubtedly compete with the binding to the phosphate group of NpN,[19,27] this seems to have only a minor effect, if any, on the metal-ion-promoted hydrolysis. We feel that NpN constitutes a better model for an internucleosidic phosphodiester bond of RNA than NpNp. Intact RNA does not contain monophosphate functions. Consequently, the macrochelate binding mode suggested for NpNp may play a role only after cleavage of an internucleosidic phosphodiester bond and subsequent hydrolysis of the resulting 3'-terminal 2',3'-cyclic monophosphate group. Since the 2',3'-cyclic monophosphate groups are known to accumulate during the Zn^{2+}-promoted hydrolysis of polyribonucleotides,[14] NpNp constitutes a good model for RNA hydrolysis only during the late stages of depolymerization.

The results presented above for NpN strongly suggest that the nearest neighbours themselves have no significant effect on the Zn^{2+}-promoted cleavage of the internucleosidic phosphodiester bond. Consistent with this view, various homopolyribonucleotides have been reported to be depolymerized at comparable rates by Zn^{2+}.[21] By contrast, it is difficult to understand on these bases why Zn^{2+}-promoted hydrolysis of RNA would occur with considerable differentiation among phosphodiester linkages, as suggested previously.[21] In other words, why cleavage at the 3'-side of cytidine and uridine would be favoured, and that at 3'-side of guanosine retarded. To have a comlete set of results determined under similar experimental conditions, we reexamined the Zn^{2+}-promoted cleavage of 4 homopolymers and RNA type III from baker's yeast. We also felt this relevant, because the earlier results[21] were based on the assumption that the cleavage of internucleosidic phosphodiester bonds of RNA yields 3'-terminal monophosphate groups. As mentioned above, the 3'-terminal nucleosides, however, predominantly exist as 2',3'-cyclic monophosphates. For this reason, we have now followed the RNA hydrolysis by detecting the formation of 5'-terminal hydroxy functions and 3'-terminal 2',3'-monophosphate groups, instead of formation of 3'-monophosphate groups. The results of the present study are thus complementary to those reported earlier.

The measurements were carried out at pH 5.1, in 1 or 2 mM solution of $Zn(NO_3)_2$ at 363.2 K. At this temperature, the polymers can be expected to be in a fully denaturated form. Hence the cleavage observed is random, and not induced by a formation of any specific conformation that favours the cleavage at a certain site. The temperature is also high enough to inactivate the protein enzymes. The metal-ion-promoted cleavage of an internucleosidic phosphodiester bond of a polynucleotide gives two polymeric products, one of which has a 3'-terminal 2',3'-cyclic monophosphate group and the other a 5'-terminal 5'-hydroxyl function.[14] To determine the resulting 5'-terminal nucleosides, the aliquots were treated with phosphodiesterase I, which cleaved the intact phosphodiester bonds yielding nucleoside 5'-monophosphates. At sites where the phosphodiester bond was already chemically cleaved, a nucleoside was released.[14,28] Accordingly, the 5'-cleavage sites could be detected by RP-HPLC. The 3'-terminal nucleosides were, in turn, detected as 2',3'-cyclic monophosphates, which were obtained by the digestion of aliquots with a mixture of phosphodiesterase I and alkaline phosphatase. Upon this treatment, phosphodiesterase I released the 3'-terminal monophosphate groups as 3',5'-bisphosphates that were further dephosphorylated by alkaline phosphatase. Since neither of the enzymes cleaves cyclic monophosphate groups, the 3'-cleavage sites appeared as nucleoside 2',3'-cyclic monophosphates on HPLC separation. This method suffers from the shortcoming that the 2',3'-cyclic monophosphate groups are hydrolyzed further, although the hydrolysis is slower than the cleavage of the internucleosidic phosphodiester bonds. Our previous results[14] on Zn^{2+}-promoted hydrolysis of poly(U) at T = 333.2 K showed that about 60 % of the 3'-terminal nucleotides exist in a 2',3'-cyclic form at the early stage of the reaction. The subsequent hydrolysis of the cyclic monophosphate groups does not, however, significantly bias the results, since the 2',3'-cyclic monophosphates of different nucleosides are hydrolyzed at approximately the same rate. Under the experimental conditions employed in the polymer studies ($[Zn^{2+}]$ = 1 mM), the rate constants determined for 2',3'-cAMP, 2',3'-cCMP, 2',3'-cGMP and 2',3'-cUMP were $11.5x10^{-6}$ s^{-1}, $12.0x10^{-6}$ s^{-1}, $9.20x10^{-6}$ s^{-1} $7.5x10^{-6}$ s^{-1}, respectively.

The results obtained for the cleavage of the phosphodiester bonds of RNA are presented in FIG. 1, where the mole fraction of the 3'- and 5'-terminal nucleosides is plotted against the reaction time. The mole fractions of the 3'-terminal nucleosides in FIG 1B are slightly smaller than those of 5'-terminal nucleosides in FIG 1A. This inconsistency results from the subsequent hydrolysis of the 3'-terminal cyclic monophosphate groups discussed above. The data (FIG 1B) partly verify the previous observation of Eichhorn *et al.*[21], according to which the cleavage at the 3'-side of guanosine is somewhat slower than that at the 3'-side of pyrimidine nucleosides. The differences are, however, smaller than those observed previously. Among the 5'-*O*-linked nucleoside, adenosine appears to be slightly favoured as a leaving-group (FIG 1A), while Eichhorn *et al.*[21] reported cleavage at the 5'-side of uridine being preferred. The preferable cleavage at the 5'-side of adenosine receives some additional support from the studies on the Zn^{2+}-promoted hydrolysis of various

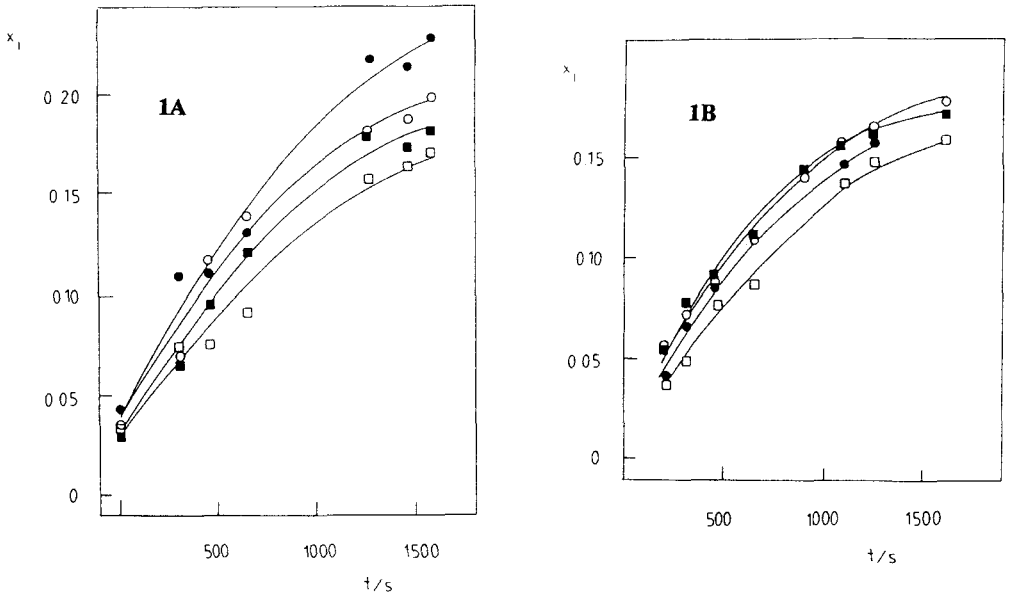


FIG 1: Time-dependent distribution of various nucleosides at the 5'-terminus (FIG 1A) and 3'-terminus (FIG 1B) of the oligomeric products of the Zn^{2+}-promoted hydrolysis of RNA III from baker's yeast ($[Zn^{2+}]$ = 1 mM, pH = 5.1, T = 363.2 K, I = 0.1 M with $NaNO_3$). x_i is the amount of a given 5'-terminal (1A) or 3'-terminal (1B) nucleoside at moment t divided by the total amount of the same nucleoside in RNA (varies from 0 to 1 in the course of complete depolymerization). Notation: A (filled circles), C (open circles), U (filled squares), G (open squares).

homopolymers. The pseudo first-order rate constants obtained for the hydrolysis of poly(A), poly(C), poly(G) and poly(U) at $[Zn^{2+}]$ = 2 mM (for the other conditions FIG 1) were $(12.7\pm0.7)\times10^{-5}$ s^{-1}, $(8.3\pm0.3)\times10^{-5}$ s^{-1}, $(8.1\pm0.4)\times10^{-5}$ s^{-1} and $(8.8\pm0.5)\times10^{-5}$ s^{-1}, respectively. In other words, the cleavage rates of poly(C), poly(G) and poly(U) are almost equal, but that of poly(A) is about 50 % higher. For comparison, the pseudo first-order rate constant for the cleavage of RNA under the same conditions, except at $[Zn^{2+}]$ = 1 mM, was $(13\pm1)\times10^{-5}$ s^{-1}.

In summary, the results of the present study strongly suggest that the Zn^{2+}-promoted hydrolysis of the internucleosidic phosphodiester bonds is not appreciably susceptible to the base moiety structure of nearest neighbours.

EXPERIMENTAL SECTION

Materials. Ribonucleic acid type III from baker's yeast, polyadenylic, polycytidylic, polyguanylic and polyuridylic acids, all dinucleoside monophosphates, adenylyl- and

guanylyl(3',5')uridine-3'-monophosphates, and the nucleosides and nucleotides used as reference compounds were products of Sigma. They were used as received after checking the purity by HPLC. Phosphodiesterase I (lyophilized powder) was purchased from USB and alkaline phosphatase (a concentrated solution) from Boehringer Mannheim. All the other reagents were of analytical grade.

Kinetic measurements. The kinetic runs with dinucleoside monophophates (**1**) were performed as described earlier.[29] The pH of the reaction solutions was measured before the reaction at 273.2 K and extrapolated to 363.2 K with the aid of the known temperature dependence of HEPES buffer.[30] The aliquots withdrawn were analyzed by RP-HPLC. The column employed was Hypersil RP-18 (250 x 4 mm i.d., 5 μM), and the eluent a mixture of acetic acid buffer (0.1 M, pH 4.3, 0.1 M NH_4Cl) and acetonitrile. The acetonitrile content varied from 2.5% to 5% depending on the compound studied. In general, compounds with pyrimidine bases required less acetonitrile than those containing purine bases. The initial concentration of the starting material was of the order of 10^{-4} M.

Kinetic runs with dinucleoside diphosphates (**2**) were carried out as described above for dinucleoside monophosphates, but the aliquots were dephosphorylated with alkaline phosphatase before the HPLC analysis.

The first-order rate constants for the hydrolysis of **1** and **2** were calculated as described previously.[13] With compounds containing a cytosine residue, the rate constant for the disappearance of the starting material was bisected to the rate constants for the hydrolysis and for the deamination of cytosine moiety by using the kinetic equations for concecutive and parallel first-order reactions.[31]

The kinetic runs with polynucleotides were performed with extreme care to avoid the uncontrolled cleavage of the starting material. The reaction solutions were first heated to 373.2 K for an hour to denaturate the possibly existing contaminating enzymes. After this the reaction solution was cooled on an ice bath. The solid substrate was carefully introduced to the reaction solution. Initially a small amount of a buffer (50-100 μl), not containing Zn^{2+}, was added on the solid substrate, and the polymer was allowed to absorb the liquid within an hour. After this, a small amount of water was added and the solution was gently shaken to dissolve all the material. The cold aqueous solution of the starting material was then added into the cold reaction solution, and the solution obtained was slowly heated to 373.2 K during about 5 minutes to avoid too drastic changes.

The aliquots (750 μl) withdrawn were cooled on an ice bath and then divided into two portions, one of which was treated with phosphodiesterase I, and the other with a mixture of phosphodiesterase I and alkaline phosphatase. The enzyme was added in 1:1 Tris-HCl buffer containing Mg^{2+} ions that are required as an enzyme cofactor. The volume of the enzyme solution was 50 μl. The final concenration of the buffer in an aliquot was 0.05 M and that of Mg^{2+} ions 10 mM. The aliquots were let to stand 10-14 hours to make sure that the enzyme digestion was complete, after which their pH was adjusted with 10 μl 1 M HCl

to 5-6. This was done, since Zn^{2+} may, under the alkaline conditions used in the enzyme digestion, cause a slow hydrolysis of the 2',3'-cyclic phosphate groups. At pH 5-6 this reaction is slower. The aliquots were filtered to remove the enzyme.

The Zn^{2+} ions were not removed with chelex as has been done previously,[14] since it turned out that the treatment induces the deamination of cytidine moieties to uridine in an extent that disturbs the analysis. The presence of Zn^{2+} during the enzyme treatment complicated the situation, since under alkaline conditions, Zn^{2+} tends to precipitate as a hydroxide. To avoid this, the kinetic measurements were carried out at a lower metal ion concentration (1-2 mM) than with **1** and **2**.

The aliquots were analyzed as described above for the dinucleoside monophosphates, but the acetate buffer used contained 0.3 M NH_4Cl. A higher ionic strength was required to improve the separation of the products. A gradient was applied to elute all the possible products. The initial eluent was pure acetic acid buffer, which elutes the pyrimidine nucleosides and their monophosphates during 10 minutes. After 6 minutes eluation, a very gently sloping gradient was applied to change the acetonitrile content of the eluent to 3.5% during 3 minutes. With this composition the total time required to elute all the possible components was 25 minutes. The order of appearance of the products was as follows. For an aliquot digested with phosphodiesterase I: 5'-CMP, 5'-UMP, and 5'-GMP (3-5 min), cytidine (6.5 min), uridine (9 min), 5'-AMP (10 min), guanosine (18 min) and adenosine (23 min). For an aliquot digested with a mixture of phosphodiesterase I and alkaline phosphatase: 2',3'-cCMP (5 min), 2',3'-cUMP (6 min), 2',3'-cGMP (15 min) and 2',3'-cAMP (18 min). The nucleosides were eluted as described above.

The area of the signals were converted to concentrations with the aid of calibration solutions of known concentrations. The 5'-terminal nucleosides were detected as nucleosides after the digestion with phosphodiesterase I. A small amount of 2',3'-cyclic monophosphates formed by the cleavage of the 3'-terminal phosphodiester bond also appeared. To obtain the total amount of the 5'-terminal nucleosides, the amount of these products were added to those of nucleosides. The 3'-terminal nucleosides were detected as nucleoside 2',3'-cyclic monophosphates after the digestion with a mixture of phosphodiesterase I and alkaline phosphatase. The rate constants were calculated by comparing the total mole fraction of the 5'-terminal nucleosides at moment t to the amount of all nucleoside units of the starting material.

REFERENCES

1. Morrow, J.R. *Metal Ions Biol. Syst.* **1996**, *33*, 561.
2. Bashkin, J.K.; Jenkins, L.A. *Comments Inorg. Chem.* **1994**, *16*, 77.
3. Kuusela, S.; Lönnberg, H. *Metal Ions Biol. Syst.* **1996**, *32*, 271.
4. Kimura, E., *Progr. Inorg. Chem.* **1994**, *41*, 443.

5. Young, M.J.; Chin, J., *J. Am. Chem. Soc.* **1995**, *117*, 10577.
6. Reichwein, A.M.; Verboom, W.; Reinhoudt, D.N. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 343.
7. Dahm, S.C.; Uhlenbeck, O.C. *Biochemistry* **1991**, *30*, 9464.
8. Morrow, J.R.; Buttrey, L.A.; Berback, K.A. *Inorg. Chem.* **1992**, *31*, 16.
9. Kuusela, S.; Lönnberg, H. *J. Phys. Org. Chem.* **1992**, *5*, 801.
10. Bashkin, J.K.; Jenkins, L.A. *J. Chem. Soc. Dalton Trans.* **1993**, 3631.
11. Breslow, R.; Huang, D-L.; Anslyn E. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 1746.
12. Morrow, J.R.; Shelton, V.M. *Inorg. Chem.* **1991**, *30*, 4295.
13. Kuusela, S.; Lönnberg, H. *J. Phys. Org. Chem.* **1993**, *6*, 347.
14. Kuusela, S.; Lönnberg, H. *J. Chem. Soc. Perkin Trans. 2* **1994**, 2301.
15. Kuusela, S.; Azhayev, A.; Guzaev, A.; Lönnberg, H. *J. Chem. Soc. Perkin Trans.2* **1995**, 1197.
16. Kuusela, S.; Guzaev, A.; Lönnberg, H. *J. Chem. Soc. Perkin Trans. 2* in the press.
17. Butzow, J.J.; Eichhorn, G.L. *Biochemistry* **1971**, *10*, 2019.
18. Ikenaga, H.; Inoue, Y. *Biochemistry* **1974**, *13*, 577.
19. Lönnberg, H., in *Biocoordination Chemistry, Coordination Equilibria in Biologically Active Systems*; Burger, K., Ed., Ellis Horwood, Chichester, **1990**, p.308.
20. Sigel, H.; Massaud, S.S.; Tribolet, K. *J. Am. Chem. Soc.* **1988**, *110*, 6857.
21. Eichhorn, G.L.; Tarien, E.; Butzow, J.J. *Biochemistry* **1971**, *10*, 2014.
22. Shelton, V.M.; Morrow, J.M. *Inorg. Chem.* **1992**, *30*, 4295.
23. Witzel, H. *J. Liebigs Ann. Chem.* **1960**, *635*, 182.
24. Järvinen, P.; Oivanen, M.; Lönnberg, H. *J. Org. Chem.* **1991**, *56*, 5396.
25. Norberg, J.; Nilsson, L. *J. Am. Chem. Soc.* **1995**, *117*, 10832.
26. Smith, R.M.; Martell, J.E. *Pure Appl. Chem.* **1991**, *63*, 1015.
27. Askolin, C.-P.; Mattinen, J.; Kuusela, S.; Lönnberg, H. unpublished results.
28. Corcoran, R.; LaBelle, M.; Czarnik, A.W.; Breslow, R. *Anal. Biochem.* **1985**, *144*, 563
29. Oivanen M.; Lönnberg, H. *J. Org. Chem.* **1989**, *54*, 2556.
30. Good, N.E.; Winget, G.D.; Winter, W.; Connolly, T.N.; Izawa, S.; Singh, R.M.M. *Biochemistry* **1966**, *5*, 467.
31. Rodiguin, N.M.; Rodiguina, E.N. in *Concecutive Chemical Reactions - Mathematical Analysis and Development;* Schneider, R.F., Ed.; van Nostrand Inc., Princeton, **1964**, p.49
20. Sigel, H.; Massaud, S.S.; Tribolet, K. *J. Am. Chem. Soc.* **1988**, *110*, 6857.

Received May 28, 1996
Accepted July 11, 1996