Non-Enzymatic RNA Hydrolysis Promoted by the Combined Catalytic Activity of Buffers and Magnesium Ions

Mounir G. AbouHaidar[a] and Ivan G. Ivanov[b]*

[a] Department of Botany, Virology Group, University of Toronto, 25 Willcocks Street, Toronto, Ontario, Canada M5S 3B2

[b] Institute of Molecular Biology, Department of Genetic Engineering, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria. Fax: (+3592) 736227; 723507. E-mail: iivanov@bg400.bg; iviv@obzor.bio21.bas.bg

* Author for correspondence and reprint requests

Z. Naturforsch. **54c,** 542–548 (1999); received January 20/March 23, 1999

RNA, RNA Degradation, RNA Hydrolysis, Acid-Base Catalysis, Magnesium Catalysis

Although Mg^{2+} is an important cofactor for the specific degradation of RNA by ribozymes, it is not considered as a typical chemical nuclease. In this study we show that in combination with common buffers such as tris(hydroxymethyl)aminomethane and sodium borate, Mg^{2+} is a powerful catalyst for the degradation of RNA. pH and temperature are found to be the principal factors for the efficient degradation of RNA. Whereas in Tris-HCl/Mg^{2+} the efficient cleavage starts at pH values higher than 7.5 and temperatures higher than 37 °C, in sodium borate RNA degradation begins at pH 7.0 and at 37 °C. RNA hydrolysis promoted under the combined catalytic activity of buffer/Mg^{2+} results in partially degraded RNA and negligible amounts of acid-soluble material. Reaction is insensitive to the concentration of monovalent cations but is completely prevented by chelating agents (EDTA and citrate) at concentrations exceeding that of Mg^{2+}. Borate-magnesium reaction is inhibited also by some polyvalent alcohols (glycerol) and sugars.

Introduction

The presence of a 2′-OH group in the ribose moiety is the main reason for chemical instability of RNA in comparison with DNA. Unlike DNA, RNA is easily hydrolysed in aqueous solutions in the presence of alkali or heavy metal ions (Morrow, 1994). On the other hand, RNA as a single-stranded biopolymer, binds naturally Mg^{2+} and other metal ions, which are necessary for the maintenance of its higher ordered structures (Morrow, 1994; Van Atta and Hecht, 1994). Whereas Mg^{2+} is not a co-factor for the enzymatic hydrolysis of RNA (Morrow, 1994), Mg^{2+} or Mn^{2+} are indispensable for the activity of catalytic RNAs (introns and ribozymes; for review see Dahm and Uhlenbeck, 1991; Dahm *et al.,* 1993; Van Atta and Hecht, 1994).

Due to the increased interest in the mode of action of ribozymes, the role of Mg^{2+} and other divalent metal ions for the specific non-enzymatic hydrolysis of RNA has been extensively studied (Dahm and Uhlenbeck, 1991; Dahm *et al.,* 1993; Long and Uhlenbeck, 1993; Uchimaru *et al.,* 1993; Van Atta and Hecht, 1994).

As a result, the necessity of divalent ions for the ribozyme reaction is commonly accepted but the exact role of magnesium in the transesterification reaction accompanying the cleavage of the phosphate diester bond is still disputable. It should be mentioned that the Mg^{2+} catalysed hydrolysis is considered as independent of the buffer components and it is mainly discussed in the context of sequence specific cleavage of RNA. Therefore, Mg^{2+} itself is not considered as a degrading agent for RNA at neutral pH values (Breslow and Huang, 1991; Morrow, 1994) but as a secondary structure stabiliser.

In our laboratories we have observed an increased instability of RNA in certain buffers in the presence of magnesium salts. Surprisingly, some of them like tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) and sodium borate, are among the most commonly used buffers in the RNA research. In this report, we provide data about the factors affecting RNA hydrolysis in buffers containing Mg^{2+} and discuss the possible mechanism of that reaction.

0939–5075/99/0700–0542 $ 06.00

Materials and Methods

Preparation of RNA

Tobacco mosaic virus (TMV) RNA was prepared by phenol extraction of purified TMV. Total RNA from *E. coli* was prepared according to current protocols (Sambrook *et al.*, 1989). RNA preparations were extracted consecutively with phenol/chloroform and chloroform. RNA was used only if no detectable degradation was found after an overnight incubation in TE buffer (10 mM Tris-HCl, 1 mM EDTA) at 37 °C. *E. coli* rRNAs and tRNAs were either purchased from Boehringer Manheim or purified from total bacterial RNA.

RNA fragments of defined length were prepared by *in vitro* transcription of DNA fragments cloned downstream of the T7 RNA polymerase promoter in pBS M13$^+$ (Stratagene) using T7 RNA polymerase and ribonuclease inhibitors (New England BioLabs). Template DNA was digested by DNase (RNase free) and RNA samples were extracted extensively with phenol, phenol/chloroform and chloroform. All RNA preparations were stored at −20 °C in TE buffer at a concentration of 1 mg/ml.

Hydrolysis and electrophoresis of RNA

Conditions for degradation of RNA in buffers are given in the figure legends. Agarose gel (1% or 1.5%) electrophoresis was carried out in Tris/borate, EDTA, pH 7.5 buffer containing 0.5 μg/ml ethidium bromide at room temperature. Values for pH were determined for each buffer at the indicated temperature.

Terminal nucleotide analysis

^{32}P-labelled RNA preparations were prepared for 5′- or 3′-terminal nucleotide analysis as follows: TMV RNA and rRNA were partially degraded in: 1) 50 mM Tris-HCl, pH 8.0, 20 mM $MgCl_2$ at 55 °C for 2 h; 2) 50 mM sodium borate, pH 7.5, 20 mM $MgCl_2$ at 55 °C for 2 h; 3) 10 mM KOH at 37 °C for 30 min. RNA was precipitated with 5% (final concentration) ice-cold trichloro acetic acid (TCA) for 15 min and the precipitate was washed consecutively with ice-cold 90% ethanol, 70% ethanol and then dissolved in TE buffer at a concentration of 0.5–1.0 mg/ml. One half of the RNA sample was treated with alkaline phosphatase (0.5 U/mg RNA) at 37 °C for 30 min and the other half was extensively extracted with phenol, phenol/chloroform and chloroform, precipitated with ethanol and dissolved in TE buffer (0.5–1.0 mg/ml). For 5′-end labelling, samples of 3 μl were treated with T4 polynucleotide kinase in the presence of γ-[^{32}P]-ATP (2 μCi) in 50 μl reaction buffer at 37 °C for 1 h and for 3′-end labelling the same amount of RNA was incubated overnight with RNA ligase and [^{32}P]-CPC (2 μCi) in 50 μl at 4 °C. Reactions were stopped by adding an equal volume of ice-cold 10% TCA. After 15 min incubation on ice precipitates were collected by centrifugation at 4 °C and washed 3 times with ice-cold 5% TCA. Precipitates were dissolved in 50 μl 1 N KOH and incubated for 1 h at 60 °C. Samples were chilled on ice and 50 μl of ice-cold 1 N $HClO_4$ were added. After 5 min the white precipitate ($KClO_4$) was removed by centrifugation and the water phase was concentrated (if necessary) in a Speed-Vac centrifuge to about 10,000 cpm/μl of ^{32}P radioactivity (counted by Cerenkov). ^{32}P-labelled terminal nucleotides were separated by electrophoresis on a Whatman #1 paper in a pyridine (0.5%) /acetic acid (10%) pH 3.5 buffer at 40 V/cm for 30 min. The radioactive spots were visualised by autoradiography, cut out and ^{32}P-radioactivity was measured by Cerenkov in a Beckman scintillation counter.

HPLC analysis of ribonucleotides

Three di-ribonucleoside monophosphates (UpU, CpU and ApU) were hydrolysed overnight in both buffers 50 mM Tris-HCl, pH 8.0, 20 mM $MgCl_2$ and 50 mM sodium borate, pH 7.5, 20 mM $MgCl_2$ under the conditions described above and the degradation products were analysed by HPLC (Waters). Two type of samples were used as referents for identification of the degradation products: 1) the same di-nucleoside monophosphates hydrolysed in 10 mM KOH at 37 °C for 2 h, and 2) commercial nucleoside (U) and ribonucleotides (3′-UMP, 3′-CMP, 3′-AMP, 2′-UMP, 2′-AMP, 2′,3′-UMP, 2′,3′-CMP and 2′,3′-AMP). The chromatographic conditions for separation of reaction products were the following: amino column (Spherisorb-NH_2, 5 μl particles), flow rate 0.8 ml/min, isocratic elution with 10 mM KH_2PO_4/acetonitrile (30/70 v/v), pH 4.6.

The difference between the retention times of the nucleoside-2′-monophosphates and nucleoside-3′-monophosphates was approximately 5 min (i.e. 17 min for 2′-UMP and 22 min for the 3′-UMP).

Chemicals and labware

All reagents used in this study were of ultra pure grade practically free from metal ions and RNase free. Buffers were prepared with diethylpyrocarbonate (DEPC) treated and autoclaved water. All reactions and manipulations were carried out in DEPC pre-treated glass and plastic wear followed by autoclaving.

Results

We have observed that ultra pure preparations of RNA (free of RNases) were unstable at storage or heating in certain buffers. RNA stability increased in the presence of EDTA or sodium citrate and decreased drastically when Tris-HCl, sodium borate and other buffers were supplemented with magnesium salts. The aim of the present study is to investigate this phenomenon.

Degradation of RNA in buffers supplemented with magnesium salts

Ultrapure (deproteinised) TMV RNA is stable in neutral solutions at 55 °C for many hours. Since this RNA is homogeneous in size (6395 nt) and because it is of high molecular weight, it is easy to detect any partial degradation simply by agarose gel electrophoresis. That is why TMV RNA was chosen as a substrate for preliminary screening of hydrolytic activity of the buffers. The following buffers were tested alone and also in combination with 20 mM $MgCl_2$: threonine, glycine, sodium borate, Tris-HCl and Tris-borate. Solutions (50 mM) of most of the L-amino acids were also tested.

Surprisingly, we found that the most commonly used buffers such as Tris-HCl, Tris-borate and sodium borate were among the most aggressive in the presence of Mg^{2+}. As seen in Fig. 1, TMV RNA was stable when incubated in water in the presence of 20 mM $MgCl_2$ and also in buffers in the absence of $MgCl_2$. However, in the presence of Mg^{2+} the degradation of RNA started at 37 °C and became extensive at 55 °C. Since the degradation of TMV RNA in sodium borate and Tris-borate was essentially the same (but always higher in comparison with Tris-HCl) the latter one was abandoned. All further experiments were carried out with Tris-HCl and sodium borate buffers, designated as buffers **A** and **B**. Experiments with *E. coli* rRNA carried out under the same conditions showed identical (data not shown).

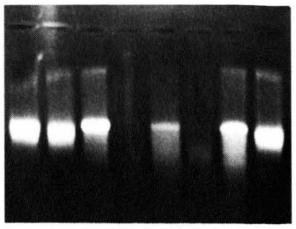

Fig. 1. Stability of TMV RNA in different buffers at pH 8.0. TMV RNA (1 μg) was incubated at 55 °C for 2 hours in distilled water (lane 8) or 50 mM buffers (lanes 1–7) supplemented with 20 mM $MgCl_2$ (lanes 2, 4, 6, 8) or free of Mg^{2+} (lanes 1, 3, 5, 7). Buffers: threonine (1); glycine (2, 3); sodium borate (4, 5) and Tris-HCl (6, 7). Samples were loaded on 1% agarose gel containing 0.5 mg/ml ethidium bromide. The lesser amount of RNA in lane 5 is probably due to degradation under these conditions.

Effect of buffer and monovalent ion concentrations

To study the effect of buffer concentration on the degradation of RNA, Mg^{2+} concentration and pH values were kept constant (20 mM and pH 8.0 respectively) and buffer concentration was varied between 20 and 200 mM for buffer A and 20 to 70 mM for buffer B. The results showed that the degradation of RNA was practically independent of buffer concentration (data not shown). Monovalent ions, Na^+ and K^+, used at concentrations between 0 and 300 mM in buffers A and B supplemented with Mg^{2+}, did not have a noticeable effect on the hydrolytic reaction (data not shown).

Effect of Mg^{2+} concentration and pH

Although increased Mg^{2+} concentration (between 2 and 100 mM) accelerated the degradation of RNA, it tended to aggregate into insoluble complexes preventing its migration in agarose gels at concentrations higher than 50 mM $MgCl_2$ (Fig. 2A).

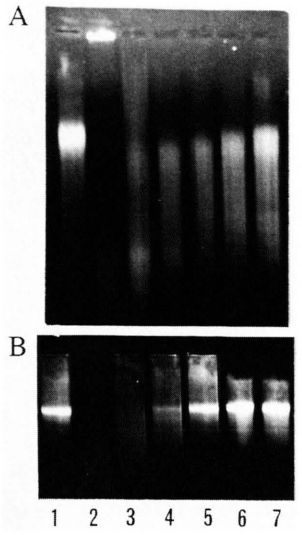

Fig. 2. Effect of $MgCl_2$ concentration (A) and pH (B) on the TMV RNA hydrolysis catalysed by buffers supplemented with $MgCl_2$. (A) TMV RNA (1 μg) was incubated at 55 °C for 2 h in 50 mM Tris-HCl, pH 8.0 supplemented with the following molarities of $MgCl_2$: (lane 1) 0 mM; (lane 2) 100 mM; (lane 3) 50 mM; (lane 4) 20 mM; (lane 5) 10 mM; (lane 6) 5 mM; (lane 7) 2 mM. (B) TMV RNA (1 μg) was incubated at 55 °C for 2 h in 50 mM Tris-HCl buffer at different pH values, supplemented with 20 mM $MgCl_2$: (lane 1) pH 8.0 plus 20 mM EDTA; (lane 2) pH 9.0; (lane 3) pH 8.5; (lane 4) pH 8.0; (lane 5) pH 7.0; (lane 6) pH 6.0; (lane 7) pH 5.0.

As expected, the pH value was one of the most critical parameters for the degradation of RNA. Fig. 2B shows that the hydrolysis of RNA in presence of Mg^{2+} sharply increased at pH values higher than pH 7.0 for buffer B and pH 7.5 for buffer A.

Incubation time

Time dependence of RNA degradation in buffers A and B supplemented with Mg^{2+} was determined by agarose gel electrophoresis and from the analysis of acid soluble material. The obtained results showed that detectable (by electrophoresis) degradation of TMV RNA appeared after 15 min at 55 °C in buffer B/Mg^{2+} and after 1 h in buffer A/Mg^{2+}. The two buffers differed also in the formation of acid soluble material. The latter amounted to 15% for buffer B/Mg^{2+} and 1–2% for buffer A/Mg^{2+} after incubation at 55 °C for 4 h and increased linearly up to 10% for buffer A and 60% for buffer B respectively after an overnight incubation under the same conditions.

Inhibitors

To shed light on the mechanism of degradation of RNA in buffers A and B supplemented with Mg^{2+} we investigated the interference of various inorganic and organic compounds with the hydrolytic reaction. Our results showed that in both buffers the hydrolytic reaction was inhibited by EDTA concentrations higher than those of Mg^{2+} (Fig. 2). Similar results were obtained also with sodium citrate (data not shown). Surprisingly, both compounds prevented also the slight (background) degradation of RNA observed in buffer A (at pH >8.0) and in buffer B (at pH >7.5) alone in the absence of $MgCl_2$ (Fig. 1). From this observation we concluded that the minor degradation is probably due to the catalytic effect of traces of polyvalent metal ions naturally occurring in the RNA preparations.

Another unexpected result relates to the effect of polyvalent alcohols and carbohydrates on the degradation of RNA in buffer B (Fig. 3). Some compounds like glycerol (but not ethylene or propylene glycols), glucose (but not sucrose or inositol) inhibited the hydrolytic reaction of RNA in buffer B/Mg^{2+} with the same efficiency as the EDTA and citrate. None of these compounds, however, inhibited the degradation of RNA in buffer A/Mg^{2+}. This observation clearly suggests that the degradation of RNA in the two buffer systems follows different mechanisms.

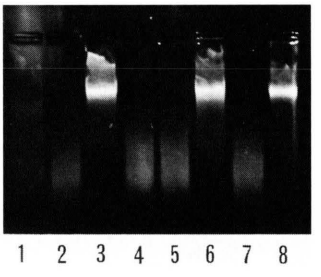

Fig. 3. Inhibitory effect of polyvalent alcohols and sugars on the degradation of RNA in sodium borate buffer containing $MgCl_2$. TMV RNA (1 μg) was incubated at 55 °C for 2 h in 50 mM sodium borate, pH 7.5 supplemented with 20 mM $MgCl_2$ and 10% of the following compounds: inositol, sucrose, glucose, propyleneglycol, ethyleneglycol, glycerol and no supplement (lanes 1–7) respectively. Lane 8: 20 mM $MgCl_2$ in H_2O.

Sequence specificity of the hydrolytic reaction

Figures 1–4 show that the hydrolysis of natural RNAs (TMV and rRNA) promoted by the catalytic activity of both buffers A and B in combination with Mg^{2+} resulted in generation of a smear material, i.e. RNA species of random molecular size. The lack of distinct bands on the agarose gels implies a sequence non-specific reaction (e.g. ribozyme). The same result was obtained also with *in vitro* transcribed RNA molecules (data not shown).

Base specificity of the hydrolytic reaction and position of the terminal phosphate group

The sequence non-specificity of the hydrolytic reaction does not exclude base preference. Hypothetically, the hydrolysis of the phosphate diester group in RNA might result in the attachment of the phosphate group to the 5′-OH, 3′-OH, 2′-OH or both 3′-OH and 2′-OH (3′,2′-cyclic phosphate). Although the 5′-O-P product was unexpected, all these possibilities were checked experimentally. To this end TMV RNA and rRNA were partially hydrolysed in buffer A/Mg^{2+} and buffer B/Mg^{2+} and the degradation products were labelled with ^{32}P at either the 5′- or 3′-end (see Materials and Methods). In order to determine which one (5′ or 3′) OH group is phosphorylated, labelling of both 5′- and 3′-ends of the RNA fragments was carried out before and after treatment with alkaline phosphatase.

As expected, the alkaline phosphatase treatment did not interfere significantly with the incorporation of ^{32}P-radioactivity at the 5′-terminus but drastically increased the radioactivity of the 3′-labelled products (data not shown). Based on this result, we concluded that the 3′-OH is phosphorylated either as a 3′-phosphate or 3′,2′-cyclic phosphate diester. To specify the exact position of the terminal phosphate group a model experiment was performed with three nucleoside monophosphates: UpU, CpU and ApU. They were hydrolysed in both buffers A/Mg^{2+} and B/Mg^{2+} and analysed by HPLC. Our results showed that in both cases, a free uridine was released and the nucleotide component consisted of (almost) equimolar mixture of 3′- and 2′-ribonucleoside monophosphates. The same elution profile was obtained with the other three compounds hydrolysed with KOH.

To study base specificity of the RNA hydrolysis promoted by buffer A/Mg^{2+} and buffer B/Mg^{2+}, both 5′- and 3′-labelled products were hydrolysed completely with KOH and the distribution of the ^{32}P-radioactivity between the free nucleotides was determined by electrophoresis. The results presented in Table I show that the distribution pattern of ^{32}P radioactivity was almost the same for both 5′-labelled samples (treated and non-treated with alkaline phosphatase) whereas it was totally different for the 3′-labelled RNA preparations. As seen in Table I mainly the natural 3′-terminal nucleosides (A for TMV RNA and A and U for rRNAs) were labelled before the phosphatase treatment.

Based on these results, we conclude definitely that RNA hydrolysis in buffer A/Mg^{2+} and buffer B/Mg^{2+} is not sequence or base specific and generate RNA species containing both 3′ and 2′ monophosphates.

Table I. Terminal nucleotide analysis of RNAs partially hydrolysed in Tris-HCl, pH 8.0 buffer containing 20 mM $MgCl_2$[a].

RNA	Base	5′-[^{32}P]-labelling (% ^{32}P-radioactivity)		3′-[^{32}P]-labelling (% ^{32}P-radioactivity)	
		Alkaline phosphatase treatment			
		(−)	(+)	(−)	(+)
TMV	A	30.5	28.8	88.2	28.2
rRNA		25.3	25.8	47.6	24.4
TMV	G	24.1	24.6	2.4	24.1
rRNA		29.5	32.6	5.4	31.2
TMV	C	18.6	18.7	4.0	19.1
rRNA		24.0	22.8	7.8	23.3
TMV	U	26.8	27.9	5.4	28.6
rRNA		21.2	18.8	39.2	21.1

[a] TMV RNA and *E. coli* total rRNA were partially hydrolysed in 50 mM Tris-HCl, pH 8.0 containing 20 mM $MgCl_2$ at 55 °C for 2 h and labelled either at the 5′- or the 3′-end. Labelled RNAs were completely hydrolysed in 10 mM KOH and samples of 2×10^5 cpm were used for electrophoresis. Data represent average values from three independent experiments.

Discussion

In our experiments with buffer A/Mg^{2+} the Tris-cation should be considered as a Lewis acid promoting the nucleophilic attack of the phosphorus and stabilising the intermediate phosphorane state. This assumption is supported by the fact that

most of the organic compounds shown to promote transesterification of RNA contain nitrogenous bases (Morrow, 1994). A nitrogenous base may act both as a general base (in the neutral form) deprotonating the 2′-OH group and as a general acid (in the ionised form) to relieve the electrostatic effects accompanying the cyclization step.

The role of the borate anion in the buffer system B/Mg^{2+} however, is not as clear as that of the Tris-cation in buffer A/Mg^{2+}. As an anion, i.e. as a general (Lewis) base it might participate in the deprotonation of the 2′-OH group. However, we postulate another role of the sodium borate. It is well known that borates and boronates interact with cis-diols under mild alkaline conditions forming cyclic complexes which dissociate easily at lower pH (see for review Singhal and DeSilva, 1992). It is known also that carbohydrates, devoid of 1,2-cis-diol groups, can still react with borates because of ring flexibility and metarotation. Due to this, furanose rings are more reactive with borates than pyranose (Singhal and DeSilva, 1992). This assumption is supported by the results showing that certain sugars and polyvalent alcohols (glycerol, glucose, etc.) inhibit the cleavage reaction as efficiently as the chelating agents like EDTA and citrate (see Fig. 3).

Although the role of the two buffer components, Tris-cation and borate anion, in the hydrolysis of RNA might be explained in the light of the general base/general acid catalysis theory, they are both extremely weak hydrolytic agents in the absence of Mg^{2+}. The Mg^{2+} itself (in the absence of the two buffers) has a stabilising effect on RNA. This observation raised the question: What is the role of Mg^{2+} in the cleavage reactions of RNA in buffers A/Mg^{2+} and B/Mg^{2+}?

Catalytic activity of metal ions in hydrolysing RNA, RNA analogues and modelling organic compounds has been widely studied (see for review Morrow, 1994). Except for few reports (Breslow and Huang, 1991) in most of these studies the reaction solution (buffer) is considered as an inert medium and the hydrolytic activity is ascribed to the corresponding ion only. The same approach is employed also in numerous studies dedicated to the mechanism of ribozyme reaction (Breslow and Huang, 1991; Dahm *et al.*, 1993; Long and Uhlenbeck, 1993; Pyle, 1993; Uchimaru *et al.*, 1993).

Comparative studies on the effect of metal ions on the cleavage of RNA and RNA model compounds have shown that the earth metal ions (Ca^{2+} and Mg^{2+}) are much weaker as catalysts than Zn^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+} and they are much weaker (up to several orders of magnitude) than the lanthanides La^{3+}, Nd^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Yb^{3+}, and Lu^{3+} (Breslow and Huang, 1991; Morrow, 1994). In spite of that Mg^{2+} is indispensable for the ribozyme activity and for the introns self-splicing (Uchimaru *et al.*, 1993). This paradox is explained by the low ionic radius and the high chelating potency of Mg^{2+} which is able to form strong metal-ligand bonds with oxygen containing groups. The Mg^{2+} usually co-ordinates six ligands in an octahedral geometry and has a low affinity to the nitrogen ligands. These properties of Mg^{2+} together with its (relative) high concentration in the cytoplasm predetermine the natural affinity of RNA and other cellular components for Mg^{2+}. As mentioned above, in the absence of hydrolytic conditions (low pH values and non-aggressive buffers) Mg^{2+} has a stabilising effect on RNA but it becomes a catalyst in certain buffers at higher pH. Under such conditions the Mg^{2+} is converted into a metal-aqua ion

$$Mg^{2+} + H_2O = Mg(H_2O)^{2+} = Mg(OH)^{+} + H^{+}$$

whose action resembles that of a hydroxide ion in respect to the 2′-OH deprotonation.

Taking into consideration that the ribozyme reaction results in cyclic 2′,3′-monophosphate formation and that RNA dissolved in a typical ribozyme buffer (Tris/Mg^{2+}) is degraded non-specifically, we are tempted to assume a different role of Mg^{2+} in the specific ribozyme reaction. We consider Mg^{2+} as playing a bifunctional role in the specific RNA cleavage. First, it employs its stabilising effect on the secondary structure of RNA and second, it is a non-specific catalysts in the form of magnesium-aqua ion ($Mg(OH)^{+}$). In this form (occurring at pH higher than 7.0) Mg^{2+} together with the buffer component (i.e. the Tris cation in the role of a Lewis acid) is able to cleave any phosphate diester bond in RNA. The hydrolytic rate constant for the different phosphate diester bonds, however, is different and depends on the tension at the corresponding bond. In a typical ribozyme structure one of the bonds is much weaker than the others and it is hydrolysed prefer-

entially. That is why the products of a ribozyme reaction usually consist of one or two main bands plus heterogeneous (smear) fragments generated by non-specific Mg^{2+}/buffer catalysed hydrolysis of RNA. In the latter case, the hydrolytic activity of Mg^{2+} is not necessarily related to the co-ordination of the transition (phosphorane) state.

Besides Tris and sodium borate, combined catalytic activity in the presence of Mg^{2+} is observed also with some other nitrogen containing buffers such as glycine, glycylglycine, glycineamide, Bicine [N-tris(hydroxymethyl)methylglycine], Tricine [N-tris(hydroxymethyl)methylglycine], etc. and even with some α-L-aminoacids (results to be published elsewhere). Taking into consideration that both Mg^{2+} and nitrogen compounds are naturally occurring in any living cell, one can speculate that they play the role of important factors determining chemical stability and turnover of RNA (including mRNA) *in vivo*.

Acknowledgements

This work is partially supported by research grants from NSERC, Canada to M. G. AbouHaidar and from Copernicus, EU to I. G. Ivanov.

Breslow R. and Huang D.-L. (1991), Effects of metal ions including Mg^{2+} and lanthanides on the cleavage of ribonucleotides and RNA model compounds. Proc. Natl. Acad. Sci. USA **88**, 4080–4083.

Breslow R. and Xu R. (1993), Recognition and catalysis in nucleic acid chemistry. Proc. Natl. Acad. Sci. USA **90**, 1201–1207.

Breslow R. (1993) Kinetics and mechanism in RNA cleavage. Proc. Natl. Acad. Sci. USA **30**, 1208–1211.

Dahm S. C. and Uhlenbeck O. C. (1991), Role of divalent metal ions in the hammerhead RNA cleavage reaction. Biochemistry **30**, 9464–9469.

Dahm S. C., Derrick W. B. and Uhlenbeck O. C. (1993), Evidence for the role of solvated metal hydroxide in the hammerhead cleavage mechanism. Biochemistry **32**, 13040–13045.

Eftink M. R. and Robertson H. D. (1987), in: Hydrolytic Enzymes (Neuberger A. and Brocklehurst K., eds.). Elsevier, New York, pp. 333–345.

Fersht A. (1985), in: Enzyme Structure and Mechanism. Freeman, New York, pp. 426–436.

Long D. M. and Uhlenbeck O. C. (1993), Self-cleaving catalytic RNA. FASEB J. **7**, 25–30.

Morrow J. R. (1994), Artificial ribonucleases. Adv. Inorg. Biochemistry **9**, 41–47.

Pyle A. M. (1993), Ribozymes: a distinct class of metalloenzymes. Science **261**, 709–714.

Sambrock, J., Fritsch, E. F. and Maniatis, T. (1989), A Laboratory Manual, 2nd edit. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Singhal R. P. and DeSilva S. S. M. (1992), Boronate affinity chromatography. Adv. Chromatogr. **31**, 293–335.

Uchimaru T., Uebayasi M., Tanabe K. and Taira K. (1993), Theoretical analyses on the role of Mg^{2+} ions in ribozyme reactions. FASEB J. **7**, 137–142.

Van Atta R. B. and Hecht S. (1994), A ribozyme model: site specific cleavage of an RNA substrate by Mn^{2+}. Adv. Inorg. Biochemistry. **9**, 2–40.