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Depolymerization of the Interferon-Induced 2′,5′-Oligoadenylate by Metal Ions

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Cells treated with interferon produce an oligoadenylate that contains 2′,5′- rather than 3′,5′-phosphodiester bonds. We have studied the effect of multivalent cations on these oligoadenylates and found that many of the cations (plumbous and ferric ions were the ones most extensively studied) catalyzed the cleavage of the 2′,5′-phosphodiester bond. The biologically active forms of the oligoadenylates are phosphorylated at their 5′-termini, and we found that the oligoadenylates containing 5′-phosphate groups were more labile by 2 magnitudes than their unphosphorylated derivatives. The results with ferric ion were surprising since ferric ion is essentially insoluble under physiological conditions. Furthermore, ferric ion did not depolymerize poly A or tRNA.

Introduction

In an aqueous solution of RNA, the phosphodiester bonds of RNA are broken at elevated temperature (90 °C).[1] This reaction is accelerated by multivalent cations.[2-5] Plumbous ion, which is the most efficient of all the ions tested, rapidly depolymerized RNA at physiological pH and temperature.[5] On the basis of the fact that Pb^{2+} inactivated mRNA at concentrations below those at which clinical manifestations of lead intoxication are observed, it was suggested that depolymerization of RNA is as important a factor in the toxicity of Pb^{2+} as the binding of this metal to the sulfhydryl groups of enzymes.[6] This idea was recently reinforced by Brown et al.[7] in a study carried out to explain the observation that when yeast $tRNA^{Phe}$ was treated with Pb^{2+}, one of the phosphodiester bonds in the dihydro U loop was more susceptible to Pb^{2+}-catalyzed cleavage than any of the other phosphodiester bonds in the polynucleotide.[7,8,22] The study, which was based on X-ray crystallographic analysis of a Pb^{2+}–$tRNA^{Phe}$ adduct, showed that the Pb^{2+} was bound preferentially to the O-4 of a uridine residue and N-3 of a cytidine in the TΨC loop. In this position the O–H group from a water of hydration bound to the Pb^{2+} is positioned in proximity to the phosphodiester bond on the ribose of the susceptible bond located in the dihydro U loop that is so susceptible to cleavage. Since the p*K* of the water of hydration is 7.4 and therefore partially ionized at physiological pH, the ionized water of hydration removes the proton from the 2′-hydroxyl group. The negatively charged 2′-oxygen now initiates cleavage of the phosphodiester bond by a nucleophilic attack on the phosphorus atom leading to the breakage of the 3′,5′-phosphodiester bond with concomitant formation of a cyclic 2′,3′-phosphodiester bond and a 5′-hydroxyl group.[7]

One of the early biochemical events that occurs in cells exposed to interferon is the synthesis of oligomers of adenylic acid in which the adenylic acid residues are linked to one another by 2′,5′-phosphodiester linkages rather than the 3′,5′-linkages found in RNA and DNA. These 2′,5′-oligoadenylates have the general formula $pppA(2'p5'A)_n$ where *n* varies from 0 to about 14.[9] The most common sizes of these oligomers are the dimer, trimer, and tetramer. The trimer and its higher derivatives are biologically active, but the dimer is inactive.[10-12] The oligoadenylates are usually phosphorylated at the 5′-position, as is indicated in the general formula. The 5′-triphosphate and -diphosphates are active, but the monophosphorylated and unphosphorylated derivatives are not.[13] The biological role of the 2′,5′-oligoadenylates is to bind to a ribonuclease that is latent until it is activated by the 2′,5′-oligoadenylates.[14,15]

The purpose of the present study was to determine if metal-catalyzed depolymerization occurred in the 2′,5′-linked oligoadenylates as well as in the 3′,5′-linked polynucleotides.

In studies carried out with $(2',5')A_3$ (which is not phosphorylated at the 5′-end we found the 2′,5′-phosphodiester bond was broken by the same cations that degrade the 3′,5′-phosphodiester bonds in RNA. One of the surprises that came out of this study was that Fe^{3+}, which had no effect on macromolecular RNA, did catalyze the depolymerization of $(2',5')A_3$. Ferric ion also degraded $(3',5')A_3$.

The biologically active forms of the interferon-induced oligoadenylates are phosphorylated on their 5′-end $pppA(2',5')A_n$, and we felt that it was important to determine the effect of the metals on the phosphorylated compounds. Rather than protecting the oligoadenylate by binding the Pb^{2+} and holding it away from the internal phosphodiester bonds, the presence of terminal 5′-phosphates caused a 200-fold increase in the rate of degradation of the oligoadenylates by Pb^{2+} and Fe^{3+}.

Materials and Methods

Incubation of Oligonucleotides with Metals. The method for carrying out the depolymerization reaction was a modification of one previously described.[5] In summary, the assay consisted of incubating the metal (1.0 mM), oligonucleotide (70 μM), p-aminobenzoic acid (0.49 μM; PABA), and imidazole (pH 7.4; 10 mM). In some experiments the concentration of the metals or the oligonucleotides was varied as indicated. The PABA served as an internal standard during the assay for the oligonucleotide by HPLC (see below). The solutions were filter sterilized into sterile tubes and incubated at 37 °C unless otherwise indicated. At the indicated times aliquots of the reaction mixtures were analyzed for oligoadenylate and degradation products by HPLC.

Analysis of Oligoadenylates by HPLC. (2′,5′)ApApA, (3′,5′)ApApA, (2′,5′)pppApApA, and polyadenylic acid (poly A) were each analyzed under different chromatographic conditions that gave optimal separation of these compounds from the internal standard and from the degradation products that formed during the depolymerization reaction. (2′,5′)-ApApA was resolved from its degradation products with a linear gradient that started with 0.05 M potassium phosphate, pH 3.15, and ended with 20% acetonitrile dissolved in the same buffer. The stationary phase was Spherisorb 5-μm micrometer spherical C-18 material packed into a 250 mm × 4.6 mm analytical column, which was coupled to a 50 mm × 4.6 mm guard column, packed with the same material. The flow rate was 0.5 mL/min. (3′,5′)ApApA was analyzed as above except that acetonitrile was replaced by methanol. In the case of (2′,5′)pApApA the column was developed with a linear O–15% acetonitrile gradient in 0.05 M phosphate buffer, pH 2.75, for 25 min. Polyadenylic acid and tRNA

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were depolymerized as above without adding an internal standard and were analyzed on the same C-18 column, which was developed for 20 min with a 0–20% methanol gradient in 0.05 M ammonium phosphate, pH 3.15. The degradation of poly A and tRNA was monitored by the decrease in the area of the peak in which the undegraded polynucleotides were eluted and by the appearance of degradation products that were eluted earlier than the intact polynucleotides. (2′,5′)pppApApA was analyzed on a column packed with 5-μm spherical rosil HLDA C-18 packing. The gradient consisted of 0–25% methanol in 0.10 M potassium phosphate, pH 6.50, and the separation was run for 25 min. The HPLC apparatus used in these studies was a Beckman 334 liquid chromatograph equipped with two Model 110A pumps and a Model 421 gradient controller. Injections were made on an Altex 210 injector equipped with a 20-μL loop. The oligonucleotides and their degradation products were detected by their absorbance at 254 nm with a Beckman 153 detector. HPLC grade acetonitrile and methanol were obtained from Burdick and Jackson Laboratories. HPLC grade potassium phosphate was from Fisher Scientific, and water was processed through a Millipore Corp. cartridge system. After adjustment of each solution to the indicated pH and concentration, the solutions were filtered through a Millipore 0.45 μM filter and degassed prior to use. Imidazole was purchased from Sigma and recrystallized from boiling ethyl acetate. The metallic compounds and PABA were reagent grade from several different suppliers and were used without further purification. The oligonucleotides (2′,5′)ApApA, -pApApA, and -pppApApA and (3′,5′)ApApA were from PL Biochemicals. The polyadenylic acid, *E. coli* tRNA, and (3′,5′)IpIpI were from Sigma. The (2′,5′)IpIpI was a generous gift from Dr. Robert J. Suhadolnik, Temple University School of Medicine.

Preparation of Calibration Curves. In order to quantitate the oligonucleotides under study, six solutions were prepared with the same concentrations of imidazole buffer and PABA as in the experimental solutions, but the concentration of oligonucleotide was varied from 0–105 nmol/mL. Samples of each solution were injected into the HPLC, and the ratio of peak area of the oligonucleotide to that of the internal standard was plotted against the concentration of the oligonucleotide. The calibration curves were straight lines from which the peak ratios determined from the printout on the HPLC recorder were used to calculate the concentration of the oligonucleotides.

Identification of Degradation Products of the Oligoadenylates. A partial digest of (2′,5′)- or (3′,5′)ApApA showed multiple peaks on chromatography. In order to identify the degradation products, the reaction mixture was "spiked", by adding an authentic sample of one of the degradation products, either 2′AMP, 3′AMP, ApA, or adenosine and then observing which peaks on the chromatogram were enlarged.

Detection and Quantitation of Intermediates Containing Cyclic 2′,3′-Phosphodiester Bonds. The methodology was a modification of that described by Farkas and Marks.[16] (2′,5′)A_3 was incubated with Pb^{2+} or Fe^{3+} as described above. At the appropriate times, the metal was removed from the oligonucleotide by adding 120 mg of Dowex 50 (NH_4^+) to the sample. After gentle agitation to assure that the entire solution had been in contact with the resin, the resin was removed by centrifugation. Aliquots of 250 μL were removed and adjusted to pH 1.0 by addition of 6.0 M perchloric acid. The hydrolysis of the cyclic 2′,3′-phosphodiester bond at pH 1.0 was carried out at 0 °C for 1 h, after which the pH was brought back to 7.4 with 3.0 M ammonium hydroxide. Another aliquot of the reaction mix was kept at pH 7.4. At pH 7.4, the cyclic phosphodiester bond is stable. The solutions were then assayed for the presence of phosphomonoesters by addition of 60 μL of the oligonucleotide to 0.05 M Tris–HCl, pH 8.5, containing 5 μg of *E. coli* phosphomonoesterase in a final volume of 0.3 mL. The phosphomonoesterase reaction was carried out at 37 °C for 90 min. The amount of phosphate released from the oligonucleotides was determined by adding 0.7 mL of a solution containing 11.9 mg of ascrobic acid and 5 mg ammonium molybdate in 0.24 M sulfuric acid. The blue color that developed at 37 °C after 1 h was quantitated by determining the absorbance at 820 nm.[16]

Results

Assay for the Depolymerization of (2′,5′)- and (3′,5′)ApApA. The triadenylates were incubated with one of the cations, and the solution was monitored for the disappearance of the trimer by HPLC. The results observed when (2′,5′)ApApA was incubated with 1 mM Pb^{2+} are shown in Figure 1. The chromatogram of a sample that was injected at zero time is shown in Figure 1A. After the Pb^{2+}-catalyzed depolymerization proceeded for 48 h at 37 °C, another aliquot was injected into the HPLC. The results shown in Figure 1B indicate that the peak corresponding to ApApA was greatly reduced and four degradation products, which elute before ApApA, were formed. The degradation products were identified by "spiking" the reaction mixture with authentic compounds. The degradation products in order of their elution times were 3′AMP, 2′AMP, adenosine, and ApA. The presence of both 2′AMP and 3′AMP indicated that a cyclic intermediate of (2′,3′)AMP must have been formed, but no cyclic (2′,3′)AMP was observed in this chromatogram. Empirical proof for the existence of the cyclic intermediate is given below.

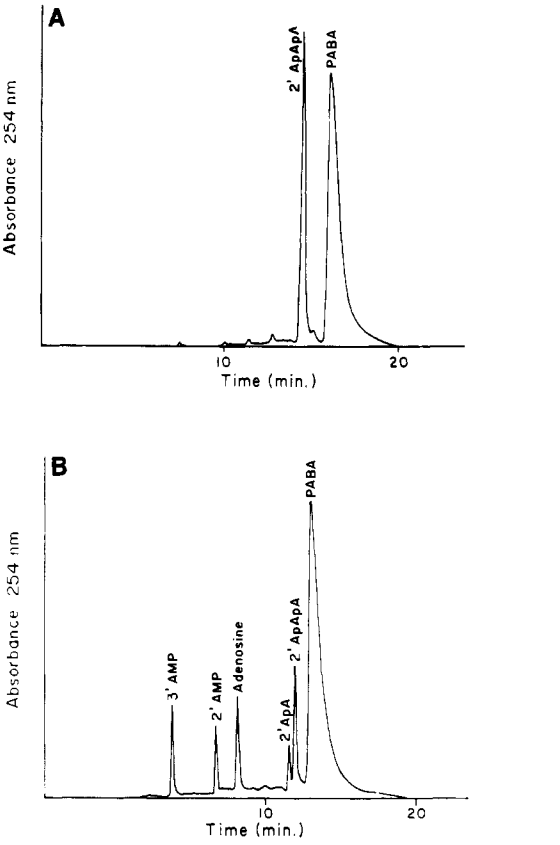

Figure 1. HPLC analysis of (2′,5′)ApApA and its depolymerization by Pb^{2+}. (A) An aliquot of the reaction mixture containing (2′,5′)ApApA, and PABA in imidazole buffer was injected into the HPLC immediately after the addition of the Pb^{2+} (at 0 time). The concentration of the components and the conditions for the HPLC analysis are described under Methods. (B) An aliquot of the same reaction mixture injected into the HPLC after 48 h at 37 °C. The identity of the degradation products which were determined in a separate experiment by "spiking" with authentic samples is indicated on the chromatogram.

Table I. Depolymerization of (2′,5′)- and (3′,5′)ApApA by Divalent Metals

	% degradation			% degradation	
metal	2′,5′	3′,5′	metal	2′,5′	3′,5′
Ba^{2+}	0	0	Mg^{2+}	0	0
Be^{2+}	0	0	Mn^{2+}	0	0
Ca^{2+}	0	0	Ni^{2+}	0	0
Cd^{2+}	0	0	Pb^{2+}	66	76
Co^{2+}	0	0	Sr^{2+}	0	0
Cu^{2+}	0	0	VO^{2+}	45	38
Hg^{2+}	21	19	Zn^{2+}	9	9

Comparison of the Depolymerization of (2′,5′)ApApA and (3′,5′)ApApA by Different Metals. It was of interest to determine if any metals catalyzed depolymerization of 2′,5′-phosphodiester bonds but not 3′,5′-phosphodiester bonds. The 2′,5′- and 3′,5′-triadenylates were incubated as described above for Figure 1. The results in Table I summarize the results for divalent cations; the data show that any metal that attacked the 2′,5′-type of phosphodiester bond also attacked the 3′,5′-bond. Where differences were seen in the rate at which 2′,5′- and 3′,5′-phosphodiester bonds were attacked, the differences were small and not significant. The

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Table II. Depolymerization of (2′,5′)ApApA by Trivalent Metals

metal	% degradation	metal	% degradation
Al^{3+}	59	La^{3+}	52
Au^{3+}	0	Nd^{3+}	100
Ce^{3+}	73	Pr^{3+}	35
Cr^{3+}	0	Sc^{3+}	94
Fe^{3+}	65	Sm^{3+}	100
In^{3+}	43	Y^{3+}	100

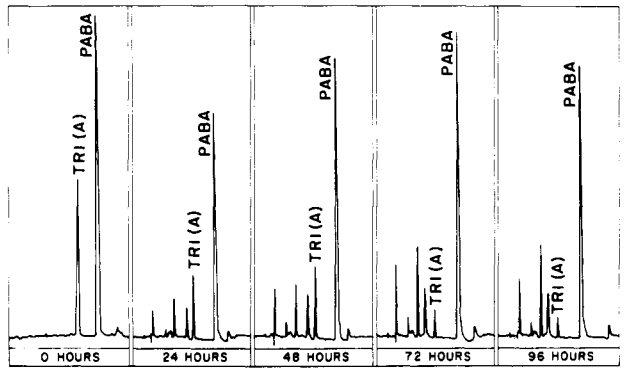

Figure 2. Analysis of the Nd^{3+}-catalyzed depolymerization of (2′,5′)-ApApA by HPLC. (2′,5′)ApApA was incubated with 1.0 mM Nd^{3+} under the standard incubation conditions (see text). At the indicated times, aliquots of the incubation mix were injected into the HPLC.

results with trivalent cations, which are shown in Table II, gave a major surprise, namely that Fe^{3+} depolymerized the triadenylates. This was surprising for two reasons: first, Fe^{3+} has not been reported to depolymerize macromolecular RNA, and second, at pH 7.4, the amount of Fe^{3+} in solution is inconsequential. At pH 7.4 the Fe^{3+} is present as a precipitate of $Fe(OH)_3$. The solubility product of $Fe(OH)_3$ is 1.1×10^{-36}. The $Fe(OH)_3$ gel and not the small amount of Fe^{3+} in solution depolymerized the oligonucleotides since after removal of the $Fe(OH)_3$ by centrifugation, the supernatant no longer caused depolymerization of the oligoadenylates. The result showing that Fe^{3+} depolymerized the oligoadenylates was so surprising that we felt it necessary to rule out the possibility that the ferric salt was contaminated by a nucleolytic enzyme. This was done by dissolving the $FeCl_3$ in 6 N HCl and heating it in a sealed tube at 110 °C for 24 h. The HCl was then removed by keeping the solution under vacuum over solid KOH. The dry ferric chloride was then redissolved in water, and its ability to depolymerize ApApA did not differ from that of untreated ferric chloride. The counterion to the Fe^{3+} ion did not make any difference since both ferric chloride and ferric sulfate depolymerized the oligoadenylates. Ferritin did not depolymerize either of the oligoadenylates.

The fact that Fe^{3+} was so effective at degrading the oligonucleotides led us to investigate if it was a general property of trivalent cations to depolymerize oligoribonucleotides. The results in Table II show that many but not all of the trivalent metals depolymerize the 2′,5′-core compound. Many of the metals that degraded the core compound have a hydrated radius of 9.0 Å. These metals include Fe^{3+}, La^{3+}, Ce^{3+}, Sc^{3+}, Y^{3+}, and In^{3+}.[17] Pb^{2+} has a hydrated radius of 4.5 Å. In order to determine if all cations with a hydrated radius of 9.0 Å would depolymerize the core compounds we tested tetramethylammonium chloride, which has a radius of 9 Å but is monovalent rather than trivalent; no degradation of oligonucleotide was observed after 48 h with this cation.

Order of the Metal-Catalyzed Depolymerization of the Oligoadenylates. To determine the order of the reaction the triadenylate was incubated with the metal as described in the Methods section and aliquots were removed at different times for analysis by HPLC (see Figure 2). The concentration of oligoadenylate at each time point was calculated, and its natural log was plotted against the time of incubation. The results summarized in Figure 3 show that

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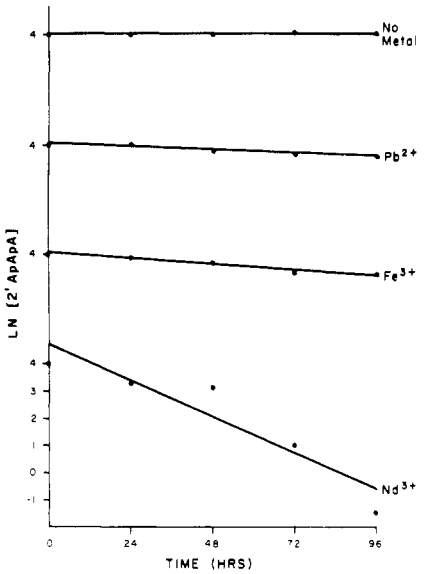

Figure 3. Metal-catalyzed depolymerization of (2′,5)ApApA, which follows first-order kinetics. The concentration of the oligoadenylate was calculated for each time point from HPLC chromatograms, such as those in Figure 5. The natural logarithm of the concentration of the oligonucleotide remaining at each time point is plotted against the reaction time.

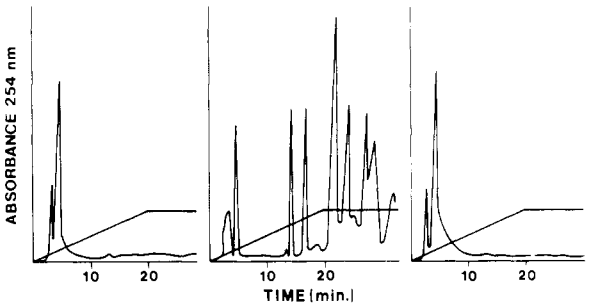

Figure 4. Comparison of the effect of Pb^{2+} and Fe^{3+} on polyadenylic acid. The depolymerization of the polyadenylic acid was carried out as described in the text. Poly A (0.32 mg/mL) was substituted for the trimer. The reaction was stopped after 1 h at 37 °C. Key to chromatograms: (A) incubation of poly A in the absence of metal; (B) poly A incubated with Pb^{2+}; (C) poly A incubated with Fe^{3+}. The reaction was stopped by the addition of ice-cold perchloric acid to a concentration of 0.58 M. The precipitate was removed and the supernatant assayed for degradation products by HPLC.

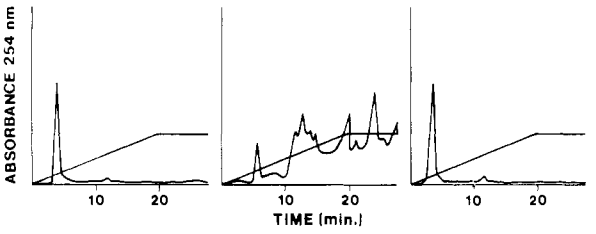

Figure 5. Comparison of the effect of Pb^{2+} and Fe^{3+} on tRNA. The experiment was carried out as in Figure 4, except that tRNA was substituted for poly A. Key: (A) tRNA alone; (B) tRNA incubated for 1 h with Pb^{2+}; (C) tRNA incubated for 1 h with Fe^{3+}.

the disappearance of ApApA followed first-order kinetics for the three metals tested.

Effect of Pb^{2+} and Fe^{3+} on High Molecular Weight Polynucleotides. Since Fe^{3+} and Pb^{2+} catalyzed the degradation of the oligoadenylate trimer at similar rates, it became of interest to compare the effects of these cations on high molecular weight RNA. In separate experiments, poly A and tRNA were incubated with either Pb^{2+} or Fe^{3+}. The results shown in Figures 4 and 5 indicate that both poly A and tRNA were degraded to many components by Pb^{2+} but not by Fe^{3+}. The fact that Fe^{3+} degraded

Table III. Amounts of Terminal 3′-Phosphate Residues Formed after Incubation of Poly A with Plumbous and Ferric Ions[a]

metal added	nmol of terminal phosphate generated/h
Pb^{2+}	6.70 ± 0.15
Fe^{3+}	0.0013 ± 0.0007

[a] Poly A was incubated at 37 °C with 1.0 mM Pb^{2+} or with 1.0 mol of $FeCl_3$ in 1.0 mL of reaction mix. The incubation with Pb^{2+} was for 1 h and with $FeCl_3$ the incubation time was 96 h.

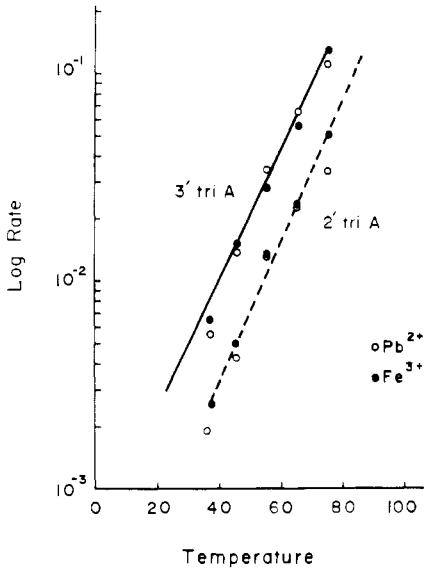

Figure 6. Effect of varying the temperature or the rate of degradation of (2′,5′)- and (3′,5′)ApApA by Fe^{3+} and Pb^{2+}. The (2′,5′)ApApA and (3′,5′)ApApA were incubated for the indicated times with either Fe^{3+} or Pb^{2+}. The temperatures of incubation were 22, 37, 45, 55, 65, and 75 °C. The time of incubation at each of these temperatures was 96, 48, 24, 12, 6, and 3 h, respectively. At 0 time and after the incubation the ratio of the trimer to the internal standard was determined by HPLC and the log of the decrease in this ratio was plotted in the diagram: (—) data for (3′,5′)tri A; (---) data for the (2′,5′)tri A. The open circles represent data from experiments run with Pb^{2+}. Closed circles represent experiments carried out with Fe^{3+}.

ApApA but not tRNA or poly A was unexpected, and we decided to use another method to show that Fe^{3+} did not depolymerize high molecular weight RNA. We incubated poly A with Pb^{2+} or Fe^{3+} as described above, but instead of analyzing for degradation products, we analyzed for the formation of 3′-linked terminal phosphate groups that would form if phosphodiester bonds were cleaved. The results summarized in Table III show that cleavage of poly A by Pb^{2+} occurred readily whereas the number of terminal 3′-phosphates generated by Fe^{3+} is only 1/5000th of that formed by Pb^{2+}.

Effect of Temperature on the Rate of Degradation of the Triadenylates. The effect of varying the temperature on the rate of degradation of the 2′,5′- and 3′,5′-triadenylates was studied, and the log of the reaction rate (disappearance of triadenylate) was plotted against the temperature. The results shown in Figure 6 show that both Pb^{2+} and Fe^{3+} were virtually equally effective when tested against the same compounds. The data in Figure 6 were also used to calculate the energies of activation for the depolymerization of the 2′,5′- and 3′,5′-core compounds by plotting the data as an Arrhenius plot (data not shown). The activation energies were 14 000 cal and 13 300 cal for the 2′,5′- and 3′,5′-compounds, respectively, and were identical for Pb^{2+} and Fe^{3+}.

Depolymerization of Oligoadenylates Phosphorylated at the 5′-End by Metals. The 2′,5′-oligoadenylates synthesized in response to interferon are phosphorylated at their 5′-ends. After we had shown that the 2′,5′-phosphodiester bond was susceptible to attack by Pb^{2+} and Fe^{3+}, we studied the rate of depolymerization of (2′,5′)pApApA and (2′,5′)pppApApA by different metals. In Table IV, the first-order rate constants for the depolymerization

Table IV. Comparison of the Susceptibility of the Core Compound, Its Mono- and Triphosphorylated Derivatives and (2′,5′)IpIpI and (3′,5′)IpIpI to Pb^{2+}, Fe^{3+}, and Nd^{3+}

	first-order rate constants, s^{-1}		
	Pb^{2+}	Fe^{3+}	Nd^{3+}
ApApA	2.8×10^{-6}	2.8×10^{-6}	1.4×10^{-5}
pApApA	3.3×10^{-4}	6.9×10^{-4}	2.8×10^{-6}
pppApApA	4.4×10^{-4}	1.7×10^{-5}	2.8×10^{-6}
(2′,5′)IpIpI	1.1×10^{-5}	2.8×10^{-6}	
(3′,5′)IpIpI	8.3×10^{-6}	2.8×10^{-6}	

Table V. Production of Cyclic 2′,3′-Phosphodiester Bonds during Metal-Catalyzed Depolymerization of (2′,5)ApApA and (3′,5′)ApApA[a]

	nmol of cyclic 2′,3′-phosphodiester	
metal	(2′,5′)ApApA	(3′,5′)ApApA
Pb^{2+}	1.34	1.32
Fe^{3+}	0.87	1.12
	0.35	0.36

[a] Samples were incubated at 85 °C with the indicated metals for 2 h and then assayed for cyclic 2′,3′-phosphodiester as described in the text.

of (2′,5′)ApApA, -pApApA, and -pppApApA are compared for Pb^{2+}, Fe^{3+}, and Nd^{3+}. The results show that with Pb^{2+} the rate of depolymerization increased 120-fold when the adenosine at the 5′-end had a single phosphate attached to the 5′-hydroxyl and that the rate increased even more for the 5′-triphosphorylated compound.

With Fe^{3+} the increase in rate between the monophosphorylated and core compounds was even greater than for Pb^{2+}. However, the triphosphate was only about 1/42 as susceptible to Fe^{3+} as the monophosphorylated compound. For Nd^{3+} the triphosphate and monophosphate were more stable than the core compound.

Comparison of Depolymerization of Triinosinates with Triadenylates by Pb^{2+} and Fe^{3+}. We felt that it was necessary to determine if the amino group of adenine was involved in the susceptibility of the triadenylates to metal-catalyzed degradation. In order to test this possibility we compared the susceptibility of the 2′,5′- and 3′,5′-triinosinates with that of the triadenylates. The data in Table V, which compares the first-order rate constants for degradation of the trinucleotides by Pb^{2+} and Fe^{3+} showed that there was no difference in the susceptibilities of the triinosinates and triadenylates to Fe^{3+}. With Pb^{2+} the triinosinates were actually less stable.

Detection of 2′,3′ Cyclic Intermediates. The strategy that we used for detecting the cyclic intermediates consisted of incubating the oligoadenylate with the metal and then dividing the sample into two aliquots. One aliquot was adjusted to pH 1.0 and incubated for 1 h in order to hydrolize any cyclic 2′,3′-phosphodiester intermediates. The other aliquot, which served as a control, was incubated at pH 7.4. At this pH the cyclic 2′,3′-phosphodiester bond is stable. Afterward both aliquots were adjusted to pH 8.5 and incubated with phosphomonoesterase, which hydrolyzes phosphomonoesters to form inorganic phosphate. The amount of inorganic phosphate found in the control is subtracted from the sample incubated at pH 1.0 to give the amount of cyclic 2′,3′-phosphate present. Phosphodiesters are not attacked by the phosphomonoesterase. The results in Table V show that the cyclic phosphate intermediate was detected by this method.

The presence of cyclic intermediates was also studied by trying to detect cyclic (2′,3′)AMP with HPLC. Using the elution buffer at pH 3.15, we did not detect the cyclic compound, presumably due to the hydrolysis of the cyclic phosphodiester bond under the acidic conditions of our HPLC system. When the buffer system was changed so that the pH was rasied to 6.50, cyclic (2′,3′)AMP was detected upon incubation with Pb^{2+} or Fe^{2+} at 37 and 85 °C but not in the absence of metal.

Discussion

We thought that direct binding of Pb^{2+} to the internal phosphate destabilized the 3′,5′-phosphodiester bond[5] by promoting attack

on the phosphate by OH^-. Therefore, we anticipated that the presence of one or more phosphates at the 5′-end of the triadenylates would decrease the rate of metal-catalyzed depolymerization, since the terminal phosphates would be more accessible than the internal phosphates and the metals would bind to the terminal phosphates rather than to the internal ones. A likely explanation as to why the oligoadenylates phosphorylated at the 5′-end are actually more susceptible to depolymerization by Pb^{2+} is that the agent that actually attacks the phosphodiester bonds is an ionized molecule of water in the hydration shell of the plumbous ion and that when Pb^{2+} is bound to the terminal 5′-phosphate the ribose residue positions the attacking water of hydration directly at one of the internal phosphodiester bonds.

We examined the products of Pb^{2+}-catalyzed degradation of ApApA, and we did not observe ApAp among the products. All of the other possible products, 2′- and 3′Ap, ApA, and adenosine were detected on the HPLC chromatogram. A likely explanation for the absence of ApAp is that phosphate at the 3′-end also makes the internal phosphodiester bond more susceptible to Pb^{2+}, and when ApAp was formed, it was rapidly converted to Ap.

When the molecular mechanism by which heavy metals exert their toxicity is explained, textbooks assume that the actions of the metals are identical. However, the fact that Pb^{2+} is 50 times more potent at inhibiting globin synthesis in reticulocytes than other heavy metals,[18] indicates that this may not be correct. In explaining the toxicity of metals, the ability of Pb^{2+} to depolymerize RNA is usually ignored but this mechanism may be more important than interactions between Pb^{2+} and proteins.[6,7] This is indicated both from evidence showing that the ability of mRNA's to program ribosomes is destroyed in the presence of Pb^{2+} at μmol concentrations[6] and studies by Werner et al. showing that there are particular phosphodiester bonds in an RNA molecule that are exquisitely susceptible to Pb^{2+}-catalyzed cleavage.[8]

Our data on comparing the effects of Nd^{3+}, Fe^{3+}, and Pb^{2+} on 5′-phosphorylated and unphosphorylated oligoadenylates showed that Nd^{3+} differed from the other metals in that the presence of a 5′-phosphate did not increase the susceptibility of the oligoadenylate to Nd^{3+}. Clearly Nd^{3+} must act differently than the other metals in catalyzing the depolymerization of oligonucleotides.

The metal-catalyzed cleavage of phosphodiester bonds in oligoadenylates differs from that in polynucleotides, e.g. ferric salts degraded tri A and tri I, but had little effect on poly A or tRNA. It is unlikely that chelation of the metals to the amino group at the 6-position of adenine plays a role in binding the metal since the 2′,5- and 3′,5-triinosinates were also degraded by Fe^{3+}. However, chelation at N7 cannot be ruled out. It would be of interest to determine if trinucleotides of 7-deazaadenine would be depolymerized by ferric salts.

The ability of Fe^{3+} to degrade the trimers is remarkable in that it proceeds by heterogeneous catalysis between the oligonucleotides in aqueous solution and a solid catalyst. This reaction should be reversible since cyclic 2′,3′-AMP is an intermediate, and it might be an example of prebiotic synthesis of polyribonucleotides. Deposits of ferric salts are not ordinarily found in vivo, but they do occur in patients who are hypertransfused to treat hematologic dyscrasias such as thalassemia.[19] Deposits of Fe^{3+} salts are also found in patients (usually children) who accidentally ingest iron pills and in patients with hemochromatosis. Since 2′,5′-oligoadenylates may be involved with the control of cell growth and macromolecule synthesis[20,21] as well as in the interferon response, it is possible that iron overload may exert metabolic effects that were not previously suspected.

In the experiments carried out in this study, the metals were present in the 0.1–1.0 mM range. These concentrations exceed the lethal concentrations of most of the metals, and it would be premature to construe that the interferon-induced response is compromised by these metals in vivo. The effect of Pb^{2+} and the other metals that degrade the oligoadenylates on the interferon response must be carried out either in vivo or with tissue culture cells. These studies are in progress. In our previous work on the degradation of RNA by Pb^{2+} we could not detect any effect of Pb^{2+} in the μM range by chemical means, either.[5] However, deleterious effects were readily detected when a biochemical assay for mRNA that had been exposed to Pb^{2+} was used.[6]

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Registry No. (2′,5′)ApApA, 70062-83-8; (3′,5′)ApApA, 917-44-2; (2′,5′)pppApApA, 65954-93-0; (2′,5′)pApApA, 61172-40-5; (3′,5′)IpIpI, 33403-48-4; (2′,5′)IpIpI, 84651-06-9; 2′-AMP, 130-49-4; 3′-AMP, 84-21-9; ApA, 2391-46-0; A, 58-61-7; (2′,3′)cAMP, 634-01-5; poly(A), 24937-83-5; Ba, 7440-39-3; Be, 7440-41-7; Ca, 7440-70-2; Cd, 7440-43-9; Co, 7440-48-4; Cu, 7440-50-8; Hg, 7439-97-6; Mg, 7439-95-4; Mn, 7439-96-5; Ni, 7440-02-0; Pb, 7439-92-1; Sr, 7440-24-6; VO, 20644-97-7; Zn, 7440-66-6; Al, 7429-90-5; Au, 7440-57-5; Ce, 7440-45-1; Cr, 7440-47-3; Fe, 7439-89-6; In, 7440-74-6; La, 7439-91-0; Nd, 7440-00-8; Pr, 7440-10-0; Sc, 7440-20-2; Sm, 7440-19-9; Y, 7440-65-5.

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Molecular Dipole Moment Charges from a New Electronegativity Scale

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A testing of Mande et al.'s new electronegativity scale is presented by comparing calculated dipole moment charges with those obtained from experimental data. An analysis is made of several equations in which the dipole moment charges are functions of the difference in electronegativity of the atoms forming the bond.

Introduction

The concept of electronegativity is a very convenient tool to understand chemical binding in molecules, alloys, and compounds.[1-12] Several attempts have been performed in order to obtain a quantitative measure of atomic electronegativities.[1,13-18] Unfortunately, it has proven very difficult to achieve a unique

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