Interaction of Metal Ions with Nucleic Acids and Related Compounds. XVI. Specific Cleavage Effects in the Depolymerization of Ribonucleic Acids by Zinc(II) Ions*

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ABSTRACT: Cleavage of RNA by zinc(II) occurs with considerable differentiation among phosphodiester linkages between different nucleosides. The cleavage pattern produced by partial (20–25%) depolymerization has been examined under various conditions. At neutral pH, small excess of zinc over phosphate (2:1), and elevated temperature (60° range), scission next to guanosine is depressed and scission next to uridine elevated, compared to the random expectation. At low temperature (30°), scission next to C is also reduced, with an increase in that at U, but the reaction is quite slow. Increasing the zinc concentration to very high levels, 10–20/phosphate, greatly accentuates the differentiation, scission being depressed next to G by as much as 70%, and elevated next to C and U by as much as 50 and 40%. Relatively small changes in the cleavage pattern with pH are overshadowed by those brought about by varying the zinc concentration; the highest degree of differentiation is obtained at pH 7 and high ratio (20:1) of zinc to phosphate. The cleavage pattern obtained in the absence of zinc is somewhat similar to that obtained at low and moderate zinc levels, under neutral and slightly alkaline conditions, although the reaction is much slower. It is greatly different from that obtained at higher zinc concentrations, showing that zinc ion can greatly modify intrinsic cleavage tendencies.

Various di- and trivalent metal ions are able to bring about the depolymerization of polyribonucleotides at neutral pH. We have previously compared the depolymerization effected by divalent ions of the various metals of the first transition series (Butzow and Eichhorn, 1965) and have found that zinc(II) causes a much more rapid reaction than any of the other metals in the series. The rate at which a homopolyribonucleotide is degraded depends greatly on the nature of the polymer (Butzow and Eichhorn, 1965), poly(rI) being attacked at a much lower rate than poly(rA), poly(rC), or poly(rU). Similar differentiation also occurs with lanthanum(III) (Eichhorn and Butzow, 1965). (Another order of reactivity has been reported for lead(II) (Farkas, 1968), poly(rI) being cleaved less rapidly than poly(rA) or poly(rU), but more rapidly than poly(rC).) If such a reaction can differentiate so well among homopolymers, it becomes of interest to find out whether the reaction can also differentiate among phosphodiester linkages between the different nucleosides in heteropolymers, such as RNA. We had attempted to detect this kind of differentiation with zinc by examining the nucleotide composition of the (high molecular weight) residual material from yeast RNA after 50% degradation to an acid-soluble state (Butzow and Eichhorn, 1965). There is no significant difference compared to the composition of the original RNA, but real differences in cleavage rates could have become obscured after an extent of reaction as high as 50%; in any event, analysis of the total nucleotide composition of residual material is not a sensitive method for detecting preferential cleavage.

Specific metal cleavage would provide a useful tool for RNA sequence analysis, in conjunction with the available enzymatic procedures. Metal cleavage of ribonucleic acids may be of advantage for other purposes, where the introduction of an enzyme is undesirable. Therefore, we decided to investigate more closely the sites of scission of RNA in the zinc reaction. (For this investigation, it is immaterial whether the RNA used is of defined sequence or not; from practical considerations we have studied the cleavage of unfractionated yeast RNA.) By stopping the reaction after less extensive degradation, then directly determining the nucleosides at which scission occurred, we have now shown that the zinc reaction does indeed differentiate among internucleoside linkages, and we have varied the cleavage conditions in order to ascertain requirements for maximum degree of differential cleavage.

Results and Discussion

Course of Depolymerization. In the zinc degradation of polyribonucleotides, the phosphodiester bonds are cleaved with retention of phosphate at the 3′ side (Butzow and Eichhorn, 1965). Just as with the acid-soluble oligomer assay used previously (Butzow and Eichhorn, 1965), poly(rI) is shown (Figure 1) by measurement of phosphate end groups (monoester phosphate) to be degraded by zinc much more slowly than poly(rA), poly(rC), or poly(rU). This is demonstrated at 64°, a temperature which has been conveniently employed in our previous investigation of the zinc reaction. The difference is more apparent in the inset of Figure 1, which covers only the early part of the reaction; specificity is, of course, lost as the reaction continues toward completion.

The effect of temperature on the course of monoester phosphate evolution during the reaction with yeast RNA is shown in Figure 2. Temperatures as low as 40° are already quite effective for partial depolymerization. The eventual leveling off of the reaction (Figures 1 and 2) will be discussed in the subsequent paper (Butzow and Eichhorn, 1971).

Detection of Specificity. The approach followed for detection of specific cleavage in the present studies is, after approxi-

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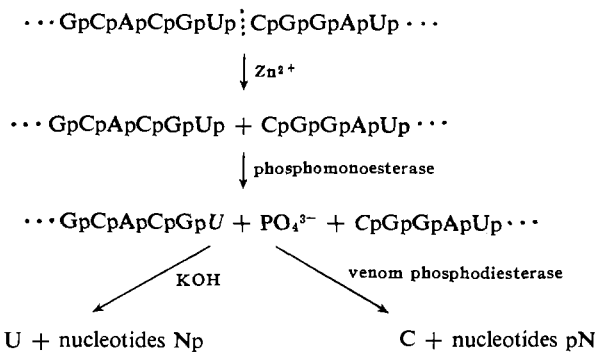
TABLE I: Time Required for 20–25% Depolymerization (Monoester Phosphate Production) of Yeast RNA under the Reaction Conditions Employed.

Temp (°C)	Zn/P	Time Required at pH 6	pH 7	pH 8	pH 9	pH 10	pH 13
30	2		3–5 weeks				
45	0.2		20–25 days				
	0.5		4–6 days				
	1		3.5–4 days				
	2	2–3 weeks		2–2.5 weeks			
	4		2–2.5 days				
64	0		10–20 days		3–6 days	15–30 hr	1–1.5 min
	2	5–6 days	7–9 hr			16–24 hr	
	10		2.5–4 hr	2.5–6 hr	8–30 hr	3–4 hr	
	20		1.5–4 hr	1–1.5 hr			

mately 20–25% depolymerization of yeast RNA in terms of evolution of monoester phosphate, to remove all the terminal (monoester) phosphate groups using a phosphomonoesterase, then to completely depolymerize the mixture to mononucleosides plus nucleosides, and finally to determine the amount of each species of nucleoside. The steps are illustrated[1] below for an arbitrary nucleoside sequence. For measurement of the nucleoside residues at the 3′ side of the cleavage of the phosphodiester bond, the total depolymerization is effected by KOH; for those at the 5′ side, venom phosphodiesterase is used



Times required for the various zinc degradations are given in Table I.

With such an approach, a considerable degree of differentiation is detected in the reaction under the conditions used previously (pH 7, 64°, 2 Zn/phosphate) in studies of the depolymerization of homopolymers (see Table II). While the amounts of A and C at the cleavage sites are as expected from the composition of the RNA, those of both G and U differ, U sites being cleaved more readily, and G sites less readily. The reduced degree of cleavage next to G might be predictable from the fact that poly(rI) is depolymerized more slowly than poly(rA), poly(rC), and poly(rU), on the basis of the analogous structure of guanine and hypoxanthine. The increased degree of cleavage next to U was not anticipated. In view of these results, it was decided to vary conditions for the cleavage reaction so as to increase, if possible, the observed differentiation.

Before considering the effects of various reaction conditions,

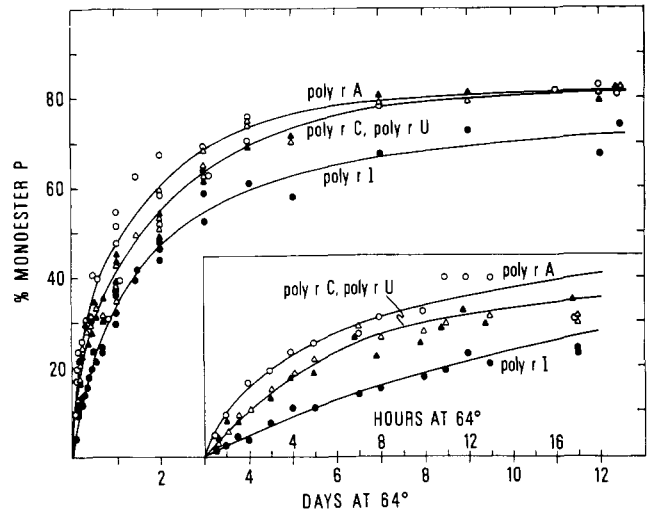


FIGURE 1: Course of zinc depolymerization of poly(rA) (○), poly(rI) (●), poly(rC) (△), and poly(rU) (▲), as followed by monoester phosphate production at pH 7, 64°, and 2 Zn/phosphate. Monoester phosphate was assayed as orthophosphate after treatment of reaction mixtures with phosphomonoesterase.

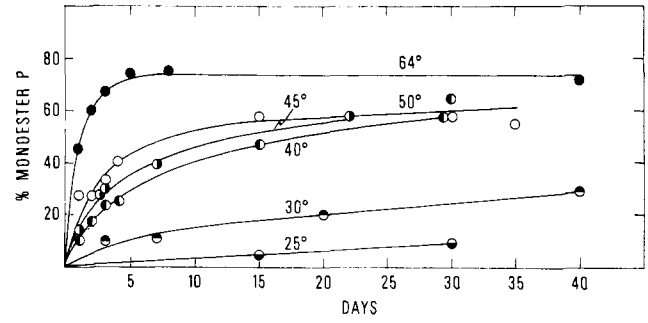


FIGURE 2: Course of zinc depolymerization of yeast RNA at pH 7 2 Zn/phosphate, and various temperatures, followed by monoester, phosphate production (as in Figure 1).

[1] Abbreviations used are: A, G, U, and C represent the ribonucleosides adenosine, guanosine, uridine, and cytidine; N represents any ribonucleoside. A phosphate in 3′ linkage is designated Np, and in 5′ linkage is designated pN.

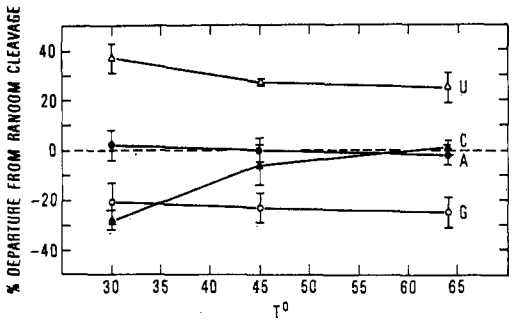


FIGURE 3: Effect of temperature on the departure from random cleavage, in limited (approximately 20–25%) zinc depolymerization of yeast RNA at pH 7 and 2 Zn/phosphate. The figure presents results of measuring the nucleosides at the 3′ side at the points of cleavage compared to the total composition of the RNA: G (○), A (●), U (△), C (▲). Vertical bars indicate mean deviations (+ and −) among repeated depolymerization experiments.

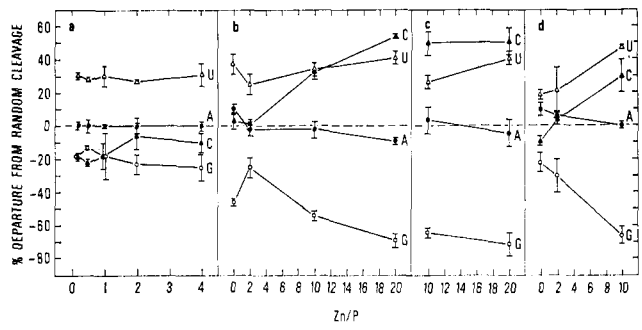


FIGURE 4: Effect of zinc level on the departure from random cleavage, in limited zinc depolymerization of yeast RNA (as in Figure 3) at (a) pH 7 and 45°, (b) pH 7 and 64°, (c) pH 8 and 64°, and (d) pH 10 and 64°.

we wish to point out that the examination of the residues at the 3′ side of the cleavage site, rather than at the 5′ side, need not lead to a bias in the differentiation found. This is shown by the similarity in the amounts of nucleosides at the 3′ and 5′ sides of the cleavage, demonstrated in experiments in which both were measured (pH 7, 45°, 2 Zn/phosphate) (see Table III). In fact, such results imply that the most likely cleavage point would be between two U residues and the least likely one between two G residues. In all the remaining experiments, only nucleosides at the 3′ side were measured.

Effect of Temperature. Variation of the reaction temperature (Figure 3) leads to essentially no change in the cleavage pattern over a broad range, 45–64°. The amount of C ends becomes less random as the temperature is lowered from 45 to 30°, down to the level of G, while U departs somewhat more positively from randomness. It might have been predicted that lower temperature could favor restriction of cleavage to the more reactive sites. This expectation obviously is not borne out by the results. Since the amounts of A and G remain unaffected (A remains random) while the changes in U and C are compensatory, there is no simple restriction controlled by temperature. Even so, decreasing the temperature can somewhat increase the degree of differentiation of the cleavage.

Effect of Zinc Concentration. Considering only the interaction of metal with phosphate (for withdrawal of electrons), it might be predicted that the use of zinc concentrations equal to or in excess of that required to saturate the phosphates could result in loss of a marginal specificity. Actually, the specificity is not improved at lower zinc levels. As the zinc level is reduced from 2 moles/mole of phosphate (Figure 4a,-b,d) there seems to be no very consistent change in the cleavage pattern, with G and C undergoing some compensatory changes. It appears that G becomes less random at pH 7 and 64° (Figure 4b), more random at pH 10 and 64° (Figure 4d), considering just the levels of 0 and 2 Zn per phosphate. At pH 7 and 45° (Figure 4a), data within the concentration range of 0–2 Zn/phosphate seem to show that the trend of G and C is also being altered in this range.

Some striking changes do take place, however, as the zinc level is *increased* from 2/phosphate (Figure 4b,c,d), G falling and both C and U rising very appreciably. At pH 7 (Figure 4b), C rises more sharply than U, with increasing zinc concentration; at pH 10, however, C and U rise in a parallel manner (Figure 4d).

In general, at high zinc levels, the nonrandomness of the cleavage pattern is appreciably accentuated compared to that found at moderate zinc levels.

Effect of pH. We have already begun to discuss results of pH variation in connection with the effect of zinc concentration. At lower temperature and moderate zinc concentration (45°, 2 Zn/phosphate; Figure 5a) there are some consistent changes with pH in the tendency of various sites to be cleaved, particularly with U departing more positively from randomness and G departing more negatively as the pH is increased. These changes do not take place or are obscured at higher temperatures or zinc concentrations (Figure 5b–d). At higher zinc concentrations, G remains much lower, and both C and U much higher (Figure 4c,d). The massive shifts in G, C, and U between moderate and high zinc level as seen in Figure 5 (see also Figure 4) dominate over smaller pH effects.

Optimal Conditions for Specific Cleavage. Specificity in a cleavage reaction is most useful if it represents exclusive cleavage, or lack of cleavage, at one type of site. Such exclusiveness is not obtained in the zinc reaction. What is obtained is a high

TABLE II: 3′-Cleavage Sites in Yeast RNA after 20–25% Depolymerization at pH 7, 64°, 2 Zn/P.

	3′-Cleavage Sites (%)	RNA Composition (%)
G	21	28
A	24	25
C	19	19
U	35	28

TABLE III: Comparison of 3′- and 5′-Cleavage Sites in Yeast RNA after 20–25% Depolymerization (at pH 7, 45°, 2 Zn/P).

	3′-Cleavage Sites (%)	5′-Cleavage Sites (%)	RNA Composition (%)
G	23	22	28
A	21	25	25
C	18	18	19
U	37	36	28

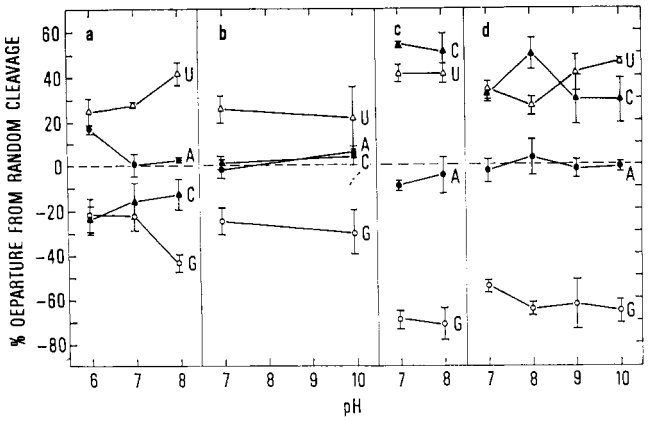


FIGURE 5: Effect of pH on the departure from random cleavage, in limited zinc depolymerization of yeast RNA (as in Figure 3) at (a) 2 Zn/phosphate and 45°, (b) 2 Zn/phosphate and 64°, (c) 20 Zn/phosphate and 64°, and (d) 10 Zn/phosphate and 64°.

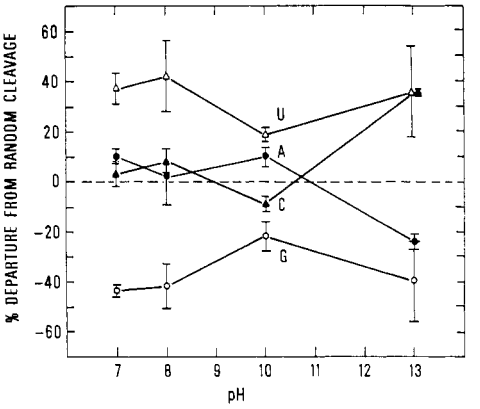


FIGURE 6: Departure from random cleavage in limited depolymerization of yeast RNA (as in Figure 3) without zinc, at various pH's and 64°.

degree of differentiation in cleavage—a large departure from randomness—in which cleavage at one or two nucleosides is elevated while cleavage at another is depressed. The highest degree of differentiation, as well as of depression of cleavage at G and elevation of cleavage at C and U, occurs at a moderate pH and a high ratio of zinc to phosphate (see Figures 4 and 5). At 20 zinc/phosphate, pH 7, and 64° (a high temperature was employed in order to avoid long reaction times), the cleavage pattern shown in Table IV is obtained. Under these conditions the cleavage at G has been reduced by nearly 70%, whereas cleavage at C and U has been increased by about 50 and 40%, respectively, compared to a random result. Let us compare the result at low temperature and moderate zinc concentration (see Figure 3): here, at 30°, cleavages at both G and C are reduced, but only by about 20 and 30% from random, while U is increased by 40% (the reaction rate, also, is quite low: see Figure 2, Table I).

Origins of the Specificity in Zinc Cleavage. At pH 7–10, the differentiation in cleavage obtained *without* zinc (Figure 6) is actually similar to that obtained at moderate zinc concentrations (Figure 5a,b), although the time required for the reaction is much longer (Table I). On increasing the pH to 13 (without zinc, Figure 6), the cleavage pattern becomes more similar to that usually noted for alkaline hydrolysis, namely, more cleavage at pyrimidine nucleosides than at purine nucleosides (Magasanik and Chargaff, 1951). At high zinc concentration, however, at pH 7–10, the amount of cleavage at G is depressed much more below random and that of C and U elevated above random, as discussed above; nor are the details of the pH response at moderate zinc concentration identical with those without zinc. But insofar as there are similarities between cleavage patterns obtained in the presence and in the absence of zinc, intrinsic tendencies governing cleavage ought to be considered.

Witzel (1960, 1963) has discussed the similarity among alkaline, acid, and lead hydrolysis of some 3′–5′-dinucleoside monophosphates, stressing faster cleavage next to pyrimidines than next to purines and relating this to possible hydrogen bonding of the 2′ hydroxyl to N-3 of purines or the oxygen of C-2 of pyrimidines. Also, due to the stronger mutual hydrogen bonding between the bases of G and C than between those of A and U, phosphodiester bonds adjacent to G and C could be especially stabilized against cleavage. A correlation with either of these possibilities is not really observed in our experiments. The order in which the nucleosides can be placed with regard to the ability of zinc to cleave the phosphodiester bond adjacent to them is, under most conditions except high pH or high zinc concentration, U > C > A > G; this order, indeed, happens to be the order often given for increasing π–π base-stacking interaction (from left to right; see Pullman, 1969). In terms of base stacking, the position of U in RNA represents a point of relative lability to cleavage, while the position of G represents a relatively stable point, because guanine would be much more strongly π bound than uracil to an adjacent base.

This explanation is complicated by the fact that the stacking order of the bases is similar to the order of their ability to complex metal ions—metal ions bind to the bases as well as to phosphate in polynucleotides (Eichhorn *et al.*, 1966; Eichhorn and Shin, 1968)—G complexing best and U poorest (Fiskin and Beer, 1965). By binding to bases, metal ions modify the structure of the bases, and thus can change the impact of the bases on reaction rates at affected phosphodiester bonds. We conclude that the main factors controlling cleavage specificity may involve base binding of zinc ions as well as base stacking.

Experimental Section

Yeast RNA, phosphomonoesterase (*Escherichia coli* alkaline phosphatase, EC 3.1.3.1, chromatographically purified) and venom phosphodiesterase (*Crotalus adamanteus* venom phosphodiesterase, EC 3.1.4.1) were obtained from Worthington Biochemical; poly(rA), poly(rI), poly(rC), and poly(rU) (Na, K, or NH_4 salts) were obtained from Miles Laboratories.

Degradation of RNA and homopolyribonucleotides by zinc

TABLE IV: 3′-Cleavage Sites in Yeast RNA after 20–25% Depolymerization at High Zn Level (20/P), at pH 7 and 64°.

	3′-Cleavage Sites (%)	RNA Composition (%)
G	9	28
A	23	25
C	30	19
U	40	28

was carried out by heating solutions containing 1.0×10^{-4} M (P) polymer, 1.0×10^{-2} M sodium nitrate, and zinc nitrate at 2.0×10^{-4} M (except in experiments where the zinc concentration was varied). The maximum extent of reaction (100% monoester phosphate) was established from the absorbance of the initial sample in 0.02 M NaOH. The molar (phosphate) extinction coefficients applied, which we determines through orthophosphate assay after ashing (Chen *et al.*, 1956), were as follows ($\times 10^{-3}$): 10.0 for poly(rA) at 256 nm, 14.4 for poly(rI) at 258 nm, 7.6 for poly(rU) at 260 nm, 6.3 for poly(rC) at 271 nm, and 9.2 for the yeast RNA at 258 nm. All reaction mixtures with 2 Zn/phosphate and below remained optically clear; at high zinc concentrations, however, the reaction does not remain homogeneous: at 10 Zn/phosphate, opalescence set in at a pH as low as 7 and turbidity was more pronounced at higher pH's, while at 20 Zn/phosphate, precipitation was evident at pH 7 and higher pH's.

For partial degradation of the yeast RNA, samples were heated for a period of time corresponding to 20–25% degradation as measured by monoester phosphate production. The time required under a given reaction condition was estimated from preliminary experiments. The actual extent of degradation obtained in the present experiments was confirmed by monoester phosphate measurements on aliquots, by treatment with phosphomonoesterase and subsequent orthophosphate determination (Chen *et al.*, 1956). Most samples were within the 20–25% range; data are included in the results only for samples which fell within 15–30%. The times required under the different reaction conditions employed are listed in Table I.

Phosphomonoesterase treatment was carried out in 0.01 M Tris-Cl buffer, pH 8.0 (sodium borate buffer, if venom phosphodiesterase depolymerization was to follow); 2 μg of phosphomonoesterase was added per μmole of RNA(P), and allowed to react about 20 hr at 40°. Afterward, the enzyme was inactivated by heating samples at 100° for 1 hr. Before phosphomonoesterase treatment, zinc was removed from samples by batch exchange on Dowex 50 (Na) (at least 5-fold equivalent excess).

Complete depolymerization of phosphomonoesterase-treated fragments was effected by (1) KOH (release of nucleosides from 3′ ends) or (2) venom phosphodiesterase (release of nucleosides from 5′ ends). (1) Reaction with KOH was carried out with 0.3 M KOH at 40° for 17 hr; samples were neutralized with $HClO_4$ to pH 5.5 and the excess $KClO_4$ allowed to precipitate on standing at 0° for 16–20 hr. The supernatants were desalted by batch adsorption and elution on activated charcoal. (2) Reaction with venom phosphodiesterase was carried out in 0.1 M sodium borate buffer, pH 8.6, containing 2.5×10^{-3} M $MgCl_2$; 1.4 mg of enzyme/μg of RNA(P) was allowed to react for 4 hr at 40°. Samples were then desalted on charcoal.

Desalting on charcoal proceeded as follows. After adjustment of the pH to 3.7 with HCl, acid-washed Norit was added (1 mg/μmole of RNA(P)), and the mixture shaken occasionally over a 2-hr period at 20–25°; the suspension was then filtered through a Millipore filter (GS, 0.22 μ; prewashed with elution solvent) and the charcoal washed on the filter with a few milliliters of ice-cold water. The charcoal was eluted by suspension in 60% aqueous ethanol containing 4% ammonium hydroxide and standing in the solvent for 2 hr at 40°; the eluate was recovered by filtration through the same filter. The elution procedure was repeated once. The combined eluate was taken to dryness by rotary vacuum evaporation at 40° (subsequent chromatography was improved by dissolving the solids in a small volume of water and evaporating again, twice; some impurities apparently are removed in this way). The selection of the pH for adsorption was made in such a way as to maximize overall recovery (adsorption of cytidine turned out to be the most pH dependent); tests with nucleosides and with ApApA gave recoveries of 90% or better for the entire procedure. Adsorption and total recovery (ultraviolet absorbance) were routinely monitored, and, if poor, the sample was discarded.

Amounts of nucleosides were measured after thin-layer chromatographic separation. Ascending chromatography was carried out on 10 × 20 cm glass plates coated with a mixture (1:4, wt) of DEAE-cellulose powder (Machery-Nagel, MN-300 DEAE) and unsubstituted cellulose powder (Machery-Nagel, MN-300), applied as a slurry of 15 g/90 ml of water to a thickness of 0.25 mm. A 6:1:3 (v/v) mixture of butanol–formic acid–water was equilibrated (24 hr); the plates with applied samples were equilibrated over the bottom layer for 1 hr, then developed in the top layer. The nucleotides remain at the origin, while the nucleosides move in the sequence: A > U > C > G. Adequate separation is obtained by repetition of the equilibration-development cycle three or four times (the nucleosides have low mobilities in this system). The positions of the nucleosides were visualized with short-wave ultraviolet illumination with the precaution of minimal exposure to avoid destruction of U. For confirmation of identity and for quantitation, the nucleoside spots and adjacent blanks of equal areas were scraped off the plates and eluted with 0.01 M HCl for 16–20 hr at 20–25°, the mixtures centrifuged at low speed to remove suspended cellulose, and spectra taken with a Cary-14 spectrophotometer (0–0.1 slidewire). The expected form of the spectrum and 260/280 nm ratio (corrected for blank) was always found. The quantity of each nucleoside was calculated from the (corrected) absorbance at 260 nm, except 280 nm for cytidine, using the following molar extinction coefficients: A, 14.3×10^3; U, 10.0×10^3; C, 12.4×10^3; G, 11.8×10^3.

The composition of the RNA was analyzed as nucleosides, after complete hydrolysis by KOH, neutralization of the KOH with $HClO_4$ and elimination of excess $KClO_4$, removal of terminal phosphate by phosphomonoesterase, and desalting on charcoal (as described above). The composition, measured as G + A + C + U, accounted for at least 97% of the ultraviolet-absorbing zones separated by chromatography (in terms of ultraviolet absorbance), the remaining material being found at the origin.

The nucleoside data represent averages from two or more independent experiments. The mean deviation of replicate experiments was approximately ±2% in determination of RNA composition and ±5–10% for the partial degradations.

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Interaction of Metal Ions with Nucleic Acids and Related Compounds. XVII. On the Mechanism of Degradation of Polyribonucleotides and Oligoribonucleotides by Zinc(II) Ions*

James J. Butzow and Gunther L. Eichhorn

ABSTRACT: Zinc(II) cleavage at neutral pH of the 3′–5′-phosphodiester bonds in homopolyribonucleotides and various oligoribonucleotides is found to occur with intermediate formation of 2′–3′-cyclic phosphate, whose subsequent opening to a mixture of the 2′ and 3′ forms is also promoted by zinc. In the degradation of trinucleoside diphosphates, there is a preference toward cleavage at the phosphodiester bond nearer the 5′ end. Susceptibility to zinc degradation increases with chain length to the tetramer level, where it becomes similar to that of the polymer. The course of degradation of homopolymers, the cleavage preference in a trinucleoside diphosphate, and the effect of chain length are all correlated with an accentuation of cleavage rate dependent on the existence, configuration, and charge of an adjacent phosphate group. The rates of cleavage of the internal phosphodiester bonds of adenosine dimers and ApApA fall in the order: ApA3′p ≫ ApApA > ApA2′p ≥ ApA2′–3′(cyclic)p ≥ ApApA ≥ ApA.

The degree of preference for cleavage at the phosphodiester bond nearer the 5′ end is greater for ApApA than ApApU, much greater for ApApG than ApApU, and much greater for UpUpG than UpUpU, reflecting the greater tendency to cleave next to U and the lesser tendency to cleave next to G found in the zinc degradation of RNA. Moreover, the presence of G at the 3′ end of these trinucleoside diphosphates leads to a marked increase in the actual cleavage rate of the phosphodiester bond nearer the 5′ end.

Zinc(II) depolymerization of polyribonucleotides can occur efficiently with a high nucleoside base specificity. At neutral pH and elevated temperature, and with a small excess of zinc ion over phosphate, poly(rI) is degraded much more slowly than poly(rA), poly(rC), or poly(rU) (Butzow and Eichhorn, 1965; Eichhorn *et al.*, 1971). With natural RNA there is very significantly reduced cleavage next to guanosine residues and increased cleavage next to uridine residues (Eichhorn *et al.*, 1971); different conditions of pH, temperature, and zinc concentration lead to a modified or accentuated cleavage pattern.

Although the zinc depolymerization is known (Butzow and Eichhorn, 1962) to operate by breakage of phosphodiester bonds with retention of phosphate at the 3′(2′) position of the ribose, the mechanism has not been well understood. The 2′ hydroxyl is required—DNA is not readily degraded by metal ions; thus, it was plausibly suggested (Bamann *et al.*, 1954; Eichhorn and Butzow, 1965) that degradation by metal ions proceeds through chelation of the metal between the phosphate and the 2′ hydroxyl.

Making use of simpler substrates, ribooligomers of defined nucleoside sequence, we now examine in some detail the breakage of the phosphodiester bond itself together with the influence of chain length and other phosphate groups in the chain, and of the nucleoside bases.

Experimental Section

Materials

Poly(rA), poly(rI), poly(rC), and poly(rU) (Na, K, or NH_4 salts) were obtained from Miles Laboratories, as were oligoadenylates $(Ap)_{1-5}A>p$[1] (Li salts).

ApApA, ApApG, ApApU, UpUpU, UpUpG, and CpCpC (Li salts) were from Miles Laboratories; another batch of UpUpG was kindly donated by Drs. Sober and Simpson.

IpIpI was prepared by KOH degradation of poly(rI), elimination of excess KOH as $KClO_4$ by neutralization with $HClO_4$, treatment with 0.1 M HCl (3 hr, 20–25°) as a precaution to open any residual 2′–3′-cyclic phosphates (see Heppel *et al.*, 1957; Simpkins and Richards, 1967a), phosphomonoesterase treatment, desalting on charcoal, and thin-layer chromatography on cellulose (30:70, 1 M aqueous NH_4OAc–95% ethanol) followed by extraction with H_2O and vacuum evaporation (30°) of the NH_4OAc from aqueous solutions. By two-dimensional thin-layer chromatography, the preparation contained 95.5% IpIpI, 0.3% IpI>p, and 4.1% IpI2′p plus IpI3′p.

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[1] Abbreviations used are: A, G, I, U, and C represent the ribonucleosides adenosine, guanosine, inosine, uridine, and cytidine; N represents any ribonucleoside. A diester phosphate group in 3′–5′ linkage is designated by NpN, a 3′-monoester phosphate by N3′p, a 2′-monoester phosphate by N2′p, and a 2′–3′-cyclic diester phosphate by N>p.