The Influence of the Size and Nature of Basic Activators on *Clostridium perfringens* Polynucleotide Phosphorylase-Catalysed Polyadenylic Acid Synthesis

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1. Basic oligo- and poly-(amino acids) stimulate polyadenylic acid synthesis by purified *Clostridium perfringens* polynucleotide phosphorylase (nucleoside diphosphate-polyribonucleotide nucleotidyltransferase, EC 2.7.7.8). 2. The effectiveness of the activators increases with chain length up to approx. 20–30 residues. 3. Polymers of the L and DL series are equally effective on a weight-for-weight basis. 4. L-Lysine, D-lysine, diethylamine and triethylamine, as hydrochlorides or hydrobromides, all stimulate the reaction markedly if their concentration is high enough. Their effect is similar to that of sodium chloride. 5. The size of the product depends primarily on the Mg^{2+} concentration and basic polymers have a relatively limited effect on it. 6. Polyadenylic acid itself undergoes an Mg^{2+} -catalysed non-enzymic hydrolysis.

The stimulation by polybases of *Clostridium perfringens* polynucleotide phosphorylase-catalysed ADP polymerization was first described by Dolin, Godiniaux & Grunberg-Manago (1961). Later studies showed that polylysine, polyornithine and polyvinylamine are all effective activators (Dolin, 1961, 1962). Polylysine interacts with and precipitates the polyA* formed during the reaction, thus displacing the equilibrium in the direction of polymer synthesis. However, no evidence of product inhibition could be detected, and concentrations of polylysine that displace the equilibrium only slightly stimulate the initial velocity to the maximum extent (Fitt, Dietz & Grunberg-Manago, 1968a). It was found that polylysine protected the enzyme against thermal inactivation, but this interaction appeared non-specific, since serum albumin was equally effective. The studies of Dolin (1962) and Fitt & Wille (1969) demonstrated that charge effects were important in this system, since inorganic salts were almost as effective activators as polylysine. Fitt & Wille (1969) showed that trypsin digestion of *Cl. perfringens* polynucleotide phosphorylase leads to a rapid, preferential, loss of the polybase-stimulated activity. Polyornithine protects the enzyme against the action of trypsin, but once again the interaction is not specific, since both *M. lysodeikticus* polynucleotide phosphorylase and pancreatic ribonuclease are also protected against proteolysis by low concentrations of polyornithine. Fitt & Wille (1969) concluded that the polybases

* Abbreviation: polyA, polyadenylic acid.

act directly on the polynucleotide phosphorylase itself and that the mechanism of activation does not depend on the interaction of the basic polymer with either the product or a hypothetical inhibitor present in the enzyme preparation. However, the detailed mechanism of the interaction remains obscure.

The present paper describes a study of the influence of the size and nature of basic activators on their ability to stimulate *Cl. perfringens* polynucleotide phosphorylase-catalysed polyA synthesis and of the factors affecting the size of the polyA formed in the presence and in the absence of polybases.

EXPERIMENTAL

Materials. Intermediates and enzymes were purchased from the following suppliers: diethylamine and triethylamine, Distillation Products Industries, Rochester, N.Y., U.S.A.; polyA, oligo-L-lysine hydrochlorides, poly-L-lysine hydrobromides (mol.wt. of free bases 56000 and 79000), poly-DL-lysine hydrobromide (mol.wt. of free base 116000), poly-DL-ornithine hydrobromide (mol.wt. of free base 3000–6000) and poly-L-ornithine hydrobromide (mol.wt. of free base 21000), Miles Chemical Co., Elkhart, Ind., U.S.A.; unlabelled ADP and [^{14}C]ADP, Schwarz BioResearch Inc., Orangeburg, N.Y., U.S.A.; poly-L-lysine hydrobromide (mol.wt. of free base 3000) and *Streptomyces griseus* protease (Pronase), Sigma Chemical Co., St Louis, Mo., U.S.A.

Polynucleotide phosphorylase. The isolation and purification of the *Cl. perfringens* polynucleotide phosphorylase used was described by Fitt & Wille (1969). It had a specific activity of ADP polymerization in the standard assay

system of 355 units/mg. with polylysine (mol.wt. 3000; 200 $\mu\text{g./ml.}$) and 20.3 units/mg. without polylysine. The enzyme was free from nucleases or phosphatases. In the absence of polylysine and in the standard incorporation assay, it gave a stable equilibrium at approx. 20% in the direction of synthesis measured either by ADP incorporation or by orthophosphate release.

Assay of *Cl. perfringens* polynucleotide phosphorylase. The method depends on the incorporation of [^{14}C]ADP into an acid-insoluble form. The standard reaction mixture (final volume 0.25 ml., contained: tris-HCl buffer, pH 8.1, 25 μmoles ; MgCl_2 , 3.75 μmoles ; EDTA, 0.25 μmole ; [^{14}C]ADP, 1 μmole (about 5000–10000 c.p.m./ μmole); activators, as indicated; enzyme. Incubation was at 37° for 30 min. The reaction was stopped by addition of 0.25 ml. of 7% (w/v) HClO_4 and the mixture was kept at 0° for 10 min. The precipitate was collected on a Whatman GF/C glass-fibre filter, washed four times with 1% (w/v) HClO_4 and once with aq. 50% (v/v) ethanol, dried and counted in a Nuclear-Chicago low-background thin-window counter. Suitable controls were carried out in all cases and their radioactivity was subtracted from the assay values.

Polyacrylamide-gel electrophoresis. Analytical polyacrylamide-gel electrophoresis of the products of the polymerization of ADP by polynucleotide phosphorylase was carried out in a 12-gel Canalco model 6 apparatus by the single-gel procedure previously described (Fitt, Fitt & Wille, 1968b). The concentration of acrylamide monomer was 10% (w/v). The gels and electrode baths contained 0.2M-tris-borate buffer, pH 8.3. A preliminary electrophoresis at the full operating voltage was carried out for 30 min. before the samples were layered on the gels. Sufficient sucrose was added to all samples to give a concentration of 10% (w/v). After electrophoresis, the gels were stained for polyA with 0.2% (w/v) methylene blue in 0.4M-sodium acetate buffer, pH 4.7 (Peacock & Dingman, 1967), and destained with running tap water.

RESULTS

Influence of basic oligo- and poly-(amino acids) on ADP polymerization by *Cl. perfringens* polynucleotide phosphorylase. The results in Table 1 show that oligolysines as small as tetralysine are able to stimulate polyA synthesis by *Cl. perfringens* polynucleotide phosphorylase provided that their concentration in the assay mixture is sufficiently high. Insufficient dilysine or trilysine was available for them to be tested at concentrations above those indicated in Table 1, at which they had no effect on the rate of reaction. However, it is clear from Fig. 1 and Table 1 that the effectiveness of the basic polymers as activators increases steadily with size to a maximum when a chain length of about 20–30 lysyl residues is reached (poly-L-lysine, mol.wt. 3000). All the polymers with mol.wt. 3000 or above were about equally effective. Polylysines of chain length 12–20 lysyl residues were not available, but the results suggest that the ability of the polymers to stimulate polyA synthesis is directly proportional to their chain length in the range from decalysine

Table 1. *Effect of oligolysines on ADP polymerization by *Cl. perfringens* polynucleotide phosphorylase*

The experiments were carried out as described in Fig. 1.

Oligolysine activator Concn. of activator ($\mu\text{g./ml.}$)...	Stimulation (%)				
	100	200	400	500	750
(Lys) $_4$	0	0	45	75	150
(Lys) $_8$	58	95	180	210	290
(Lys) $_{10}$	70	135	248	305	321
(Lys) $_{11}$	186	281	350	442	511

Fig. 1. Influence of oligo- and poly-lysines on ADP polymerization by *Cl. perfringens* polynucleotide phosphorylase. The reaction mixture (final volume 0.25 ml.) contained: tris-HCl buffer, pH 8.1, 25 μmoles ; MgCl_2 , 3.75 μmoles ; EDTA, 0.25 μmole ; [^{14}C]ADP, 1 μmole (about 5000–10000 c.p.m./ μmole); oligo- or poly-lysine, as indicated; *Cl. perfringens* polynucleotide phosphorylase, 0.034 unit. Incubation was performed at 37° for 30 min. The reaction was stopped by the addition of 0.25 ml. of 7% (w/v) HClO_4 and the mixture was kept at 0° for 10 min. The precipitate was collected on a Whatman GF/C filter, washed four times with 1% (w/v) HClO_4 and once with aq. 50% (v/v) ethanol, dried and counted. The results are expressed as percentage stimulation calculated by the formula: $100 \times (\text{incorporation with activator} - \text{incorporation without activator}) / \text{incorporation without activator}$. Polylysine used: $\blacktriangle$ — $\blacktriangle$, L-, mol.wt. 79 000; $\triangle$ — $\triangle$, DL-, mol.wt. 116 000; $\circ$ — $\circ$, L-, mol.wt. 56 000; $\bullet$ — $\bullet$, L-, mol.wt. 3000; $\circ$ — $\circ$, undecalysine; $\bullet$ — $\bullet$, decalysine.

to polylysine of mol.wt. 3000, which is approx. (Lys) $_{23}$.

Fig. 2 shows that polyornithine is as good an activator as polylysine, so that the nature of the amino acid is not critical. Further, Figs. 1 and 2 show that poly-DL-lysine (mol.wt. 116000) and

poly-DL-ornithine (mol.wt. 3000–6000) are as effective on a weight-for-weight basis as the polymers of the L series. Therefore the mechanism of the stimulation cannot depend on a stereochemically specific interaction between the basic polymer and the enzyme.

Effect of D-lysine, L-lysine, diethylamine, triethylamine and putrescine on ADP polymerization by Cl. perfringens polynucleotide phosphorylase. Dolin (1961, 1962) reported that spermidine and L-lysine had no effect on polyA synthesis by *Cl. perfringens* polynucleotide phosphorylase and concluded that only high-molecular-weight polybases were capable of stimulating the reaction. The results in Tables 1 and 2 show that this conclusion is only valid at the relatively low concentrations (maximum 240 $\mu\text{g./ml.}$) used by Dolin (1961, 1962). D- and L-Lysine, diethylamine and triethylamine all stimulate polyA synthesis significantly if present in

sufficiently high concentration. Putrescine also stimulates the reaction, provided that its concentration is not above 10 mg./ml., when it precipitates the substrate (ADP).

The maximum stimulation observed with L-lysine was about ninefold. This is similar to the maximum stimulation of this enzyme preparation by sodium chloride (Fig. 3). It therefore seems probable that the mechanism of stimulation by the low-molecular-weight compounds, whose solutions were adjusted to pH 8 with hydrochloric acid, is similar to that of salt. However, they clearly do stimulate polyA synthesis by *Cl. perfringens* enzyme significantly if present in sufficient amounts.

The optimum sodium chloride concentration (0.6M) for the polynucleotide phosphorylase used in this work is higher than that previously reported (Fitt *et al.* 1968a). This may be related to the different strain of *Cl. perfringens* used, or to the higher purity of the enzyme used in the experiments described in the present paper.

Influence of polybases and Mg^{2+} concentration on the molecular size of polyA formed during Cl. perfringens polynucleotide phosphorylase-catalysed ADP polymerization. To determine if either the presence of polylysine or changes of Mg^{2+} concentration in the reaction mixture could affect the size of polyA formed by polynucleotide phosphorylase, the reaction products were examined by analytical polyacrylamide-gel electrophoresis by the single-gel procedure. The gel concentration (10%, w/v) and pH (8.3) used were those previously found effective by Peacock & Dingman (1967) and Dingman & Peacock (1968) in their electrophoretic studies of RNA. At the end of the reaction, the non-specific nuclease-free protease from *Streptomyces griseus* (Pronase) was used to destroy the polylysines and thus disrupt the insoluble polyA–polylysine complexes (Sober, Schlossman, Yaron, Latt & Rushizky, 1966). The protease was also added to polylysine-free reaction mixtures and found not to affect the electrophoretic mobility of the polyA.

Fig. 4 shows the results of these experiments,

Fig. 2. Comparison of the effect of polylysines and poly-ornithines on the ADP polymerization by *Cl. perfringens* polynucleotide phosphorylase. The experiments were carried out as described in Fig. 1. Polybase used: O, poly-L-lysine, mol.wt. 3000; Δ , poly-DL-ornithine, mol.wt. 3000–6000; $\bullet$, poly-L-ornithine, mol.wt. 21 000.

Table 2. *Effect of L-lysine, D-lysine, putrescine, diethylamine and triethylamine on ADP polymerization by Cl. perfringens polynucleotide phosphorylase*

The experiments were carried out as described in Fig. 1. The pH of the solutions of the activators was adjusted to about 8 before use.

Activator		Stimulation (%)							
Concn. of activator (mg./ml.)	...	2	10	20	40	60	100	200	300
L-Lysine hydrobromide	—	—	75	146	376	812	790	141	—15
D-Lysine hydrobromide	—	—	—	185	370	777	—	—	—
Putrescine	42	86	—91	—98	—	—	—	—	—
Diethylamine	—	28	206	448	—	—	—	—	—
Triethylamine	—	17	244	233	—	—	—	—	—

Fig. 3. Effect of NaCl on ADP polymerization by *Cl. perfringens* polynucleotide phosphorylase. The experiments were carried out as described in Fig. 1. The NaCl concentrations indicated in the reaction mixtures are final concentrations.

from which two main conclusions can be drawn: (1) the mobility of the product depends primarily on the Mg^{2+} concentration used in its formation; (2) the basic activators have some effect on the size of the product provided that the Mg^{2+} concentration is 2 mM or 6 mM, but not when it is raised to 15 mM. At the two lower Mg^{2+} concentrations, the product is smaller and less homogeneous when activators are present.

PolyA formed in the absence of polylysine and with 15 mM- Mg^{2+} was isolated by acid precipitation. It was reprecipitated three times in the presence of 10 mM-EDTA and dissolved in 10 mM-tris-hydrochloric acid buffer, pH 8.1. The solution was dialysed once against 100 vol. of 10 mM-tris-hydrochloric acid buffer, pH 8.1-1 mM-EDTA and once against 100 vol. of EDTA-free buffer. The dialysis tubing was washed thoroughly with 1 mM-EDTA in glass-distilled water before use. The isolated polyA was examined by polyacrylamide-gel electrophoresis in the conditions described in Fig. 4 before and after incubation for 30 min. at 37° with 15 mM- Mg^{2+} . The untreated product was completely heterogeneous and contained no material of mobility less than shown in Fig. 4(e). After incubation with magnesium chloride, the polyA gave an electrophoretic pattern identical with that shown in Fig. 4(g). Thus the product of high mobility formed by the *Cl. perfringens* polynucleotide phosphorylase in the presence of 15 mM- Mg^{2+} could not be converted by removal of the Mg^{2+} into the product of low mobility obtained with 2 mM- Mg^{2+} .

The Mg^{2+} -free polyA described above had no effect on ADP polymerization when added to the standard assay system in the absence of polylysine. This result confirms the observation by Fitt *et al.* (1968a) that the low activity of this enzyme in the absence of activators is not due to product inhibition.

Fig. 4. Effect of polylysine and Mg^{2+} on the product of polynucleotide phosphorylase-catalysed polyA synthesis. The incubation medium contained (final concns.): 100 mM-tris-HCl buffer, pH 8.1; 4 mM-ADP; 1 mM-EDTA; *Cl. perfringens* polynucleotide phosphorylase, 1 μ g./ml.; $MgCl_2$, as indicated; decalysine, polylysine (mol.wt. 3000) or polylysine (mol.wt. 56000), 200 μ g./ml., where appropriate. Incubation was for 24 hr. at 37°. Pronase (4 mg./ml.; 10 μ l./ml. of incubation medium) was added and incubation continued for a further 20 hr. Sufficient 50% (w/v) sucrose was added to give a final sucrose concentration of 10% (w/v) and samples were layered on to the surface of the 10% polyacrylamide gels under the buffer in the upper electrode compartment. Electrophoresis was for 15 min. at 50 v and for 1 hr. at 120 v with 12 gels. The gels were stained with methylene blue and destained with running tap water. (a) 2 mM- $MgCl_2$; no activators; (b) 2 mM- $MgCl_2$; decalysine or polylysine (mol.wt. 3000); (c) 6 mM- $MgCl_2$; no activators; (d) 6 mM- $MgCl_2$; decalysine; (e) 6 mM- $MgCl_2$; polylysine (mol.wt. 3000); (f) 6 mM- $MgCl_2$; polylysine (mol.wt. 56000); (g) 15 mM- $MgCl_2$; with decalysine or polylysine (mol.wt. 3000 or 56000) or without activators. In the diagrams dark bands indicate well-defined, intensely stained regions, bold stippling indicates strong diffuse colour and light stippling indicates weak diffuse colour.

Electrophoretic mobility of polyA in polyacrylamide gels before and after incubation with Mg^{2+} . To determine whether *Cl. perfringens* polynucleotide phosphorylase itself had any significant influence on the results described above and in Fig. 4, the effect of pretreatment of polyA with Mg^{2+} on the mobility of the polymer in 10% (w/v) polyacrylamide gels was studied (Fig. 5). In the experiments described in both Figs. 4 and 5, only the sample contained Mg^{2+} and none was present in the buffers used for either the gels or the electrode baths.

The results in Fig. 5 may be summarized as follows: (1) treatment of polyA with Mg^{2+} causes significant changes in the electrophoretic mobility of the polymer; (2) the effect of Mg^{2+} is pH-dependent and much slower at pH 9 than at pH 8.1;

Fig. 5. Electrophoretic mobility of polyA in polyacrylamide gels before and after incubation with Mg^{2+} . The incubation medium contained (final concns.): 100mM-tris-HCl buffer, pH 8.1 or 9; polyA, 1mg./ml.; 1mM-EDTA; $MgCl_2$, as appropriate. Toluene was added to prevent microbial contamination. Incubation was at 37° for the indicated time. After incubation, samples (100 μ l.) were mixed with 50% (w/v) sucrose (20 μ l.) and layered on to the gels. Electrophoresis and staining of the polyA was carried out as described in Fig. 4. (a) (i) No $MgCl_2$, pH 8.1, 30 min. or 24 hr.; or (ii) 2mM-, 5mM- or 25mM- $MgCl_2$, pH 9, 30 min.; (b) 2mM- $MgCl_2$, pH 8.1, 30 min. or 24 hr.; (c) 5mM- $MgCl_2$, pH 8.1, 30 min. or 24 hr.; (d) 25mM- $MgCl_2$, pH 8.1, 30 min. or 24 hr.; (e) 25mM- $MgCl_2$, pH 8.1, not incubated. At pH 9, the results after 24 hr. at 37° were similar to (b), (c) and (d). In the diagram dark bands indicate well-defined, intensely stained regions, bold stippling indicates strong diffuse colour and light stippling indicates weak diffuse colour.

(3) the effects are dependent on Mg^{2+} concentration; (4) addition to polyA in tris buffer, pH 8.1, at 0° , of Mg^{2+} to a final concentration of 25mM had no effect, but after 30 min. at 37° all the polymer was converted into the more mobile form and prolonged incubation had no observable effect on the results; (5) the mobility of the polyA was unchanged by prolonged incubation at pH 8.1 without Mg^{2+} ; (6) as the Mg^{2+} concentration was raised the product was initially less homogeneous than the original polyA, but after treatment with 25mM- Mg^{2+} it migrated as a single sharp band. No detectable release of acid-soluble products absorbing at 260nm. occurred after incubation of the polyA at pH 8.1 with 25mM- Mg^{2+} for 4 hr. After 26 hr., a maximum of 5% of the polymer was degraded to acid-soluble products.

The effect of Mg^{2+} could not be reversed by addition of a large excess of EDTA to the sample before electrophoresis or by exhaustive dialysis of the treated polyA against 10mM-tris-hydrochloric acid buffer, pH 8.1-1mM-EDTA. Thus a degradation rather than a physical change took place.

Although it is difficult to exclude completely the possibility that a trace contamination of the glassware with an Mg^{2+} -dependent nuclease was responsible for the results, such an explanation is unlikely. Preliminary experiments have shown that Ca^{2+} , La^{3+} , Mn^{2+} and Zn^{2+} can all replace Mg^{2+} in the conditions described; Cu^{2+} is less effective but also catalyses a slow degradation of polyA (P. S. Fitt, unpublished work). The lack of specificity for the metal ion is strong evidence that the degradation is not due to a contaminating nuclease. Further, the enzyme would have to resist rigorous cleaning of the equipment with chromic acid-sulphuric acid, rinsing and subsequent drying at $150-200^\circ$. All solutions were prepared in deionized, glass-distilled water (resistivity $>1M\Omega$) and dialysis tubing was washed thoroughly with 1mM-EDTA in glass-distilled water before use. Another possibility that can be dismissed is that selective precipitation of high-molecular-weight polyA occurred. Although precipitates were formed initially at the higher Mg^{2+} concentrations, they redissolved either during incubation or when the samples were diluted before electrophoresis. Further, the untreated polyA contained no components of high mobility even when Mg^{2+} was added to it just before electrophoresis (Figs. 5a and 5e). Toluene was added to all samples before incubation to prevent microbial contamination.

The simplest explanation of these results is that several metal ions can catalyse a non-enzymic hydrolysis of phosphodiester bonds. In addition, the close similarity between the mobilities of Mg^{2+} -treated polyA and of the products obtained during *Cl. perfringens* polynucleotide phosphorylase-catalysed polyA synthesis suggests that it is the Mg^{2+} concentration that determines the size of product formed during the enzyme-catalysed reaction.

DISCUSSION

The results in this paper show that the mechanism of stimulation of *Cl. perfringens* polynucleotide phosphorylase by basic activators depends to only a limited extent on the nature of the base. Basic poly(amino acids) containing about 20-30 lysyl or ornithyl residues were more effective on a weight-for-weight basis than shorter oligomers. The results suggest that there is an approximately linear increase in efficiency with chain length of the compounds up to about 20-30 residues and that further increases in size have little effect on the ability of the polymers to stimulate polyA synthesis. Polymers of the L and DL series are equally effective on a weight-for-weight basis, so no stereochemical specificity is involved. Since Dolin (1961, 1962) showed that polyvinylamine was an effective

activator of *Cl. perfringens* polynucleotide phosphorylase, the precise nature of the polybase is clearly not important provided that it does not form an insoluble salt with the substrate and is of a certain minimum overall chain length.

Earlier work (Dolin, 1961, 1962) suggested that low-molecular-weight bases, including basic amino acids, were not activators of *Cl. perfringens* polynucleotide phosphorylase. Our results show that this conclusion is incorrect. Provided that a sufficient concentration of the base is used, and that its salt with the substrate is soluble, then many compounds are effective. D-Lysine, L-lysine, diethylamine and triethylamine were all good activators; putrescine was less effective because of the insolubility of its ADP salt. However, the low-molecular-weight compounds were much less effective on a weight-for-weight basis than the polymers, and their optimum concentrations approached that of sodium chloride, which, together with other similar inorganic salts, has been shown to stimulate polyA synthesis by this enzyme almost as much as basic poly(amino acids) (Dolin, 1962; Fitt *et al.* 1968a).

The selective destruction of the polybase-stimulated activity of *Cl. perfringens* polynucleotide phosphorylase by trypsin (Fitt & Wille, 1969) strongly suggests that the effect of basic activators is on the enzyme itself. At the same time, the similarity between the effects of basic polymers and those of salts or low-molecular-weight bases and the simultaneous loss of the salt- and polybase-stimulated activities during proteolysis of the enzyme (Fitt & Wille, 1969) suggest that the mechanism of the stimulation must be similar in both cases. However, it is also necessary to account for the greater effectiveness of the basic polyamino acids with a chain length of over approx. 20 residues. The stimulation by the low-molecular-weight compounds probably involves a generalized ionic effect on the enzyme molecule, causing the latter to adopt a more active configuration. The greater efficiency of the basic polymers might then be due to the comparatively fixed relationship between their multiple basic groups, which could permit a concerted ionic interaction of low specificity with acidic groups on the enzyme surface separated by a distance equivalent to that of a chain of 20–30 amino acid residues. The enzyme would then be held in the active configuration by a dipole–dipole interaction, and removal during proteolysis of a peptide containing some of the acidic groups involved could account for the loss of the polybase-stimulated activity observed by Fitt & Wille (1969).

Our electrophoretic studies show that the size of the polyA formed by *Cl. perfringens* polynucleotide phosphorylase depends primarily on the Mg^{2+} concentration in the reaction medium. When the concentration is low the product is large and vice

versa. At the lower concentrations of Mg^{2+} , the polyA is smaller when polylysine is present, but with 15 mM- Mg^{2+} the product was the same in the absence or in the presence of the activator. The effect of Mg^{2+} is on polyA directly, and is not reversible by removal of the metal ions by dialysis or addition of EDTA.

Incubation of polyA with Mg^{2+} causes a rapid change in the electrophoretic mobility of the polymer. The effect is faster at pH 8.1 than at pH 9 and is therefore not an alkaline hydrolysis. Preliminary experiments have shown that Mg^{2+} may be replaced by several other metal ions. Several workers (Egami & Shimomura, 1948; Shimomura & Egami, 1953; Bamann, Fischler & Trapmann, 1954; Bamann, Trapmann & Fischler, 1954; Miyazaki, 1957) have shown that cerium hydroxide and lanthanum hydroxide catalyse the hydrolysis of RNA in weakly alkaline solution. Shimomura (1954) found that a resistant fraction was formed with lanthanum hydroxide and Miyazaki (1957) showed that it was non-diffusible and had a high purine content. Dimroth, Witzel, Hülsen & Mirbach (1959) showed that the hydroxides of lead, cadmium, zinc, bismuth and aluminium catalyse the hydrolysis of RNA to nucleosides, nucleotides or other small breakdown products, depending on the metal ion. The reactions took place at 100° and pH 7–8. They observed no metal ion-catalysed hydrolysis of RNA at 40°, but their method would not have enabled them to detect a limited degradation of the RNA molecule. Further, Lindahl (1967) showed that the rate of thermal inactivation of transfer RNA at 90° in neutral solution is increased 20-fold by Mg^{2+} . In the presence of the metal ion, chain scission was the most important mechanism of inactivation. It is therefore possible that the effect of Mg^{2+} and other metal ions that we observe may be analogous and involve a metal ion-catalysed hydrolysis of phosphodiester bonds by a mechanism similar to that proposed by Dimroth *et al.* (1959). Further detailed studies will be needed to establish the exact nature of the degradation.

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