Properties of a Human Liver Ribonuclease

INHIBITION BY POLYNUCLEOTIDES AND SPECIFICITY FOR PHOSPHODIESTER BOND CLEAVAGE TO YIELD PURINE NUCLEOSIDES AT THE 5' TERMINI

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A ribonuclease, purified 2500-fold from human liver, was found to be inactive against synthetic homopolynucleotides, whereas synthetic co-polymers containing adenylic acid were rapidly degraded. The specificity of the RNase is unique in that only purine residues, in a 5:4 ratio of guanylic to adenylic acid, are found at the 5' termini of the degradation products of yeast RNA. No specificity was observed at the 3' termini of the fragments. When analyzed by DEAE-cellulose chromatography, approximately 80% of the oligonucleotides were 4 to 11 residues in length.

The hydrolysis of RNA by the liver enzyme, when examined in low ionic strength buffer, could be increased severalfold over control levels by the addition of polyamines. The enzyme was found to exist as two distinct species on sucrose gradients, with molecular weights of 128,000 and 14,000. However, the addition of spermidine to the gradients resulted in the recovery of all the enzyme activity as the smaller species. The polyamines were also shown to reverse the inhibition of the enzyme by the ordered polynucleotides, polyguanylic acid and polyadenylic acid. Inhibition of enzyme activity by the polyadenylic acid segment of various mammalian mRNAs was also demonstrated.

In recent years it has been suggested by a number of authors that variation in the levels of ribonuclease activity in human serum and urine may be indicative of a diseased state (1-3). Since little is actually known of human ribonucleases, this laboratory has begun a systematic attempt at purifying and subsequently studying them in terms of the roles they may play in human physiology. In this respect, an enzyme has been purified from human plasma which exhibits a remarkable proclivity for cleavage of RNA between cytidylic acid residues (4). The enzyme is of interest also because it can exist in several distinct forms, the number and size of which appear to be dependent on the presence of a cation, particularly spermidine. In the presence of the polyamine, the larger species is converted to the smaller one with little loss in enzyme activity. Plasma ribonuclease activity, moreover, is stimulated to a considerable extent by spermine, a characteristic the enzyme has in common with a number of bacterial endonucleases studied in these laboratories (5-7). Finally, enzyme activity can be inhibited completely by the ordered polynucleotide, polyguanylic acid, an inhibition which can be reversed by the addition of spermidine.

In the present paper we should like to report on the purification of a second ribonuclease, one isolated from human liver. The enzyme, purified some 2500-fold, hydrolyzed RNA to yield oligonucleotides containing purines at the 5' termini. Although quite different in this respect from the plasma enzyme, the two ribonucleases did have several characteristics in common. Molecular weight determinations, for example, using sucrose gradients, indicated the presence of both a large and a small species of the enzyme. However, upon addition of spermidine to the gradients, the larger species was converted to the smaller form. In addition, the polyamines were found to stimulate enzyme activity and to reverse the inhibition of the enzyme activity by the polynucleotides, poly(A) and poly(G).

EXPERIMENTAL PROCEDURE

Materials

Samples of human liver were obtained from the Baltimore City Medical Examiner's office and were stored at -20° until needed. The sources of yeast RNA and polynucleotides as well as the methods used in their purification have been described elsewhere (8). Rabbit globin mRNA was purchased from G. D. Searle & Co., Ltd., England.

The following materials were purchased from commercial sources: Sephadex G-150, G-100, G-75, and CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N. J.); poly(A)-agarose (P-L Biochemicals, Milwaukee, Wise.); Escherichia coli alkaline phosphatase and snake venom phosphodiesterase (Worthington Biochemical Corp., Freehold, N. J.); E. coli B polynucleotide kinase and Micrococcus luteus polynucleotide phosphorylase (Miles Laboratories, Inc., Elkhart, Ind.); putrescine, spermidine, and spermine (Calbiochem, San Diego, Ca.); sodium adenosine 5'-[c'32P]diphosphate (ICN, Irvine, Ca.); and triethylammonium adenosine 5'-[y'32P]ribofosphate (New England Nuclear Corp., Boston, Mass).

Methods

Assay of Human Liver Ribonuclease—The standard assay contained in a 1-ml volume, 0.25 mg of yeast RNA, 100 μmol of Tris/HCl buffer.
The rRNA was extracted from the polysomes and polyadenylated equilibrating buffer, the polyadenylated rRNA was eluted with water.

3. Ammonium sulfate fractionation 13.5 536 348,000
2. Acid fractionation 400 1,840 368,000
5. Sephadex gel filtration II 116 57 283,000

The following procedures were carried out at 0–5°C. The purification data are summarized in Table I.

Step 1: Crude Extract—A section of diced human liver (125 g) was suspended in 250 ml of a solution consisting of 0.35 M sucrose; 0.05 M Tris/HCl buffer, pH 7.5; 0.025 M KCl; 0.01 M Mg(OAc)₂; and 0.01 M mercaptoethanol. After homogenization in a Sorvall Omni-mixer for 5 min, cell debris was removed from the crude homogenate by centrifugation at 10,000 x g for 10 min. The supernatant solution was clarified further by an additional centrifugation at 100,000 x g for 2 h, after which a yellow lipid layer was carefully decanted and discarded.

Step 2: Acid Fractionation—The supernatant solution was then brought to 0.25 M by the slow addition of concentrated H₂SO₄ and the precipitate which formed after 16 h was removed by centrifugation. The supernatant solution was set aside and saved. Additional enzyme was extracted from the precipitate by resuspending it in 5 volumes of 0.25 M H₂SO₄ and stirring the cloudy mixture gently for an hour. After centrifugation, the newly extracted enzyme solution was combined with the earlier one and the combined solution was adjusted to pH 5.5 with 1 N NaOH. After an hour, insoluble material was removed by centrifugation and the enzyme solution was adjusted to pH 7.0.

Step 3: Ammonium Sulfate Fractionation—To 400 ml of the enzyme obtained in the previous step, solid ammonium sulfate (100 g) was added slowly with continuous stirring while the pH was maintained at 7.0 by the dropwise addition of 1 N NaOH. After cooling in an ice bath for 20 min, the enzyme solution was clarified by centrifugation and an additional 70 g of ammonium sulfate was added to the supernatant solution. The protein fraction, which precipitated between 36 and 60% of the protein present in the crude extract, was collected by centrifugation and dissolved in a small volume of 0.1 M Tris/HCl buffer, pH 7.5, and 15 mM potassium phosphate (28).

TABLE I

| Step                          | Volume | Protein* | Enzyme activity | Specific activity | Recovery % yield |
|-------------------------------|--------|----------|-----------------|------------------|-----------------|
| 1. Crude extract              | 246    | 16,500   | 590,000         | 36               | 100             |
| 2. Acid fractionation         | 400    | 1,840    | 368,000         | 200              | 62              |
| 3. Ammonium sulfate fractionation | 13.5  | 536      | 348,000         | 649              | 59              |
| 4. Sephadex gel filtration I  | 172    | 115      | 344,000         | 2,990            | 58              |
| 5. Sephadex gel filtration II | 776    | 57       | 283,000         | 5,000            | 48              |
| 6. Affinity chromatography    | 226    | 2.9      | 260,000         | 88,000           | 44              |

* Measured by the method of Lowry et al. (27).
saturation, was collected by centrifugation and redissolved in a solution consisting of 0.05 M Tris/HCl buffer, pH 7.6, to a final volume of 13.5 ml.

Step 4: Sephadex Gel Filtration I—The enzyme solution obtained in the previous step was applied to a column (2.5 x 100 cm) of Sephadex G-150 which had been equilibrated in a buffer consisting of 0.05 M Tris/HCl buffer, pH 7.6. The enzyme was eluted with equilibrating buffer and fractions 4.0 ml in volume were collected at a flow rate of about 15 ml/h. Those enzyme fractions with the highest specific activity which were eluted between 324 and 496 ml were combined and reduced in volume by lyophilization to 60 ml.

Step 5: Sephadex Gel Filtration II—The enzyme was applied in 15-ml aliquots to a column of Sephadex G-100 (2.5 x 100 cm) which had been equilibrated in 0.05 M sodium phosphate buffer, pH 6.2. The enzyme, eluted with equilibrating buffer, was collected in fractions 4.0 ml in volume, and those with the highest specific activity (fractions eluting between 308 and 500 ml) were combined for subsequent purification.

Step 6: Affinity Chromatography—Twenty-five-milliliter aliquots of the enzyme solution were applied separately to a column containing 4 g of CNBr-activated Sepharose 4B (0.9 x 14 cm) to which 22 pmol of poly(G) had been bound covalently (28). After washing the column with 65 ml of 0.05 M sodium phosphate buffer, pH 6.2, to remove all unbound protein, elution of the enzyme was begun with washing buffer containing 1 M KCl. Fractions 1.3 ml in volume were collected at a flow rate of 15 ml/h and those that eluted between 10 and 17 ml were combined and dialyzed against 1 M phosphate buffer, pH 6.2. Unless stated otherwise, this preparation was used for all subsequent studies.

Characteristics of Human Liver Ribonuclease

pH Optima—The hydrolytic activity of the human liver RNase exhibited considerable dependence on the type of buffer used in the reaction mixture. With yeast RNA as substrate, enzyme activity in phosphate buffer was at its highest at pH 6.2. In Tris/HCl buffer, the peak of enzyme activity was not only shifted to pH 7.2, but the RNA was degraded at about 1.5 times the rate seen in phosphate buffer.

Effect of Buffer Concentration and Cations on Enzyme Activity—Hydrolytic activity of the human liver RNase was dependent to a considerable extent on the nature of the buffer as well as its concentration in the reaction medium. Enzyme activity was decreased approximately 4-fold on lowering the buffer concentration from 0.1 to 0.01 M. As has been observed with other ribonucleases, enzyme activity could be restored by the addition of various cations and, in particular, the polyamines (Table II). It should be noted, however, that the effect of cation addition was greater in phosphate buffer than in Tris/HCl. Comparison of the various cations for effectiveness in stimulating enzyme activity in phosphate buffer indicated that both spermine (0.36 mM) and spermidine (0.5 mM) reached their maximum effect at significantly lower concentrations than putrescine or magnesium (5 mM). However, in contrast to earlier studies with microbial enzymes, putrescine gave greater stimulation than did spermidine (5). When the values obtained in Tris/HCl buffer were examined, not only was there less stimulation of enzyme activity by the cations, but spermine was inhibitory. Metal ions other than magnesium were also studied, but only Ca²⁺ was found to stimulate enzyme activity.

Molecular Weight of Enzyme—Estimation of the molecular weight of the human liver ribonuclease by centrifugation in a sucrose gradient (18) gave evidence for the existence of differentially sized forms of the enzyme. One, which constituted about 75% of recoverable enzyme activity, had an estimated molecular weight of 14,200; the second, making up the remaining 25% of enzyme activity, was approximately 9 times the size of the first, having an estimated molecular weight of 128,000. The presence of the larger species appeared to be dependent upon the conditions of the experiment. If, for example, spermidine at 10⁻⁴ M was added to the sucrose/buffer solution (0.05 M Tris/HCl, pH 7.5), the larger enzyme species disappears and all the activity was recovered as the smaller form (14,200 Mₑ). The molecular weight of the ribonuclease was also estimated by gel filtration on a Sephadex G-100 column (19). Approximately 80% of the enzyme activity was recovered in a position corresponding to a molecular weight of 13,900. The higher molecular weight species observed on the sucrose gradients was never detected using the gel filtration technique, unless crude homogenate (prior to acid treatment), was applied to the column. In this case, two distinct species were observed with approximate molecular weights of 190,000 and 15,000, respectively. The two species were found to be immunologically similar, as shown by Neuwell et al. (29).

Hydrolysis of Nucleic Acids

Synthetic Polynucleotides—Comparison of the hydrolysis of the synthetic homopolynucleotides with that of yeast RNA by

| Polynucleotide Inhibition of Human Liver Ribonuclease | 5747 |

Table II

**Effect of cations on hydrolytic activity of human liver ribonuclease**

| Concentration of cation (mM) | Tris/HCl | Sodium phosphate |
|-----------------------------|----------|------------------|
|                             | Putrescine | Sperrmidine | Spermine | Putrescine | Sperrmidine | Spermine |
| 0.125                       | 0.27      | 0.24           | 0.26     | 0.13       | 0.29      | 0.32      |
| 0.5                         | 0.36      | 0.37           | 0.50     | 0.00       | 0.35      | 0.42      |
| 2.5                         | 0.40      | 0.67           | 0.55     | 0.00       | 0.54      | 0.74      |
| 5.0                         | 0.44      | 0.80           | 0.69     | 0.00       | 0.75      | 0.93      |
the human liver RNase showed that the former were poor substrates (Table III). Although the studies described were performed in Tris/HCl buffer, at pH 7.2, similar results were seen when performed in phosphate buffer at pH 6.2. The situation changes completely, however, when the synthetic co-polymers containing adenylic acid were used as substrates. In these studies the rates of hydrolysis of the co-polymers often exceeded that of comparable amounts of yeast RNA (Table III). Other synthetic polynucleotides examined (poly(A,G), poly(G,C), poly(G,U), poly(C,U), poly(C,G,U)) were digested at less than 6% of the rate at which yeast RNA was attacked. Synthetic and naturally occurring polydeoxyribonucleotides were not attacked under any conditions examined.

Hydrolysis Products—In order to determine the mode of action of the enzyme, yeast RNA was subjected to hydrolysis by liver ribonuclease for 16 h and the reaction products separated by DEAE-cellulose chromatography (30) (Fig. 1). Analysis of the size distribution of the oligonucleotides recovered (Table IV) indicated that about 55% averaged between 6 and 11 nucleotides in length, whereas 40% of the fragments were 3 to 5 nucleotides long. Less than 2.5% of the products were mononucleotides which would be the expected product from exonucleolytic attack. The enzyme can therefore be classified as an endonuclease.

Specificity: Identification of \([3'-\text{32P}]\) and \([5'-\text{32P}]\)Phosphoryl Terminal Nucleotides in Digestion Products of Enzyme—Although studies with synthetic homopolymers gave little indication of preferential enzyme activity towards a particular linkage, the presence of adenylic acid in co-polymers containing pyrimidine residues resulted in substantial degradation. To determine whether, in fact, there was preferential enzyme activity towards a particular linkage, the presence of adenylic acid in co-polymers containing pyrimidine residues resulted in substantial degradation. To determine whether, in fact, there was preferential enzyme activity towards a particular linkage, the presence of adenylic acid in co-polymers containing pyrimidine residues resulted in substantial degradation. To determine whether, in fact, there was preferential enzyme activity towards a particular linkage, the presence of adenylic acid in co-polymers containing pyrimidine residues resulted in substantial degradation. To determine whether, in fact, there was preferential enzyme activity towards a particular linkage, the presence of adenylic acid in co-polymers containing pyrimidine residues resulted in substantial degradation. To determine whether, in fact, there was preferential enzyme activity towards a particular linkage, the presence of adenylic acid in co-polymers containing pyrimidine residues resulted in substantial degradation. To determine whether, in fact, there was preferential enzyme activity towards a particular linkage, the presence of adenylic acid in co-polymers containing pyrimidine residues resulted in substantial degradation. To determine whether, in fact, there was preferential enzyme activity towards a particular linkage, the presence of adenylic acid in co-polymers containing pyrimidine residues resulted in substantial degradation. To determine whether, in fact, there was preferential enzyme activity towards a particular linkage, the presence of adenylic acid in co-polymers containing pyrimidine residues resulted in substantial degradation. To determine whether, in fact, there was preferential enzyme activity towards a particular linkage, the presence of adenylic acid in co-polymers containing pyrimidine residues resulted in substantial degradation.

Table III

| Substrate       | Enzyme activity | \(\Delta A_{260} \) |
|-----------------|-----------------|-------------------|
| Poly(A)         | 0.00            |                   |
| Poly(G)         | 0.00            |                   |
| Poly(C)         | 0.02            |                   |
| Poly(U)         | 0.11            |                   |
| Yeast RNA       | 1.6             |                   |
| Poly(A,C,U)     | 3.1             |                   |
| Poly(A,U)       | 3.0             |                   |
| Poly(A,C)       | 2.7             |                   |
| Poly(A,G,U)     | 0.82            |                   |
| Poly(A,G,C)     | 0.24            |                   |

The reaction mixture (1.0 ml) consisted of 1.0 \(\mu\)mol of the substrate indicated, 100 \(\mu\)mol of Tris/HCl buffer, pH 7.2, and 1 unit of enzyme. Enzyme activity was measured as described under “Methods” except that when poly(U) was used as substrate, the reaction was stopped by the addition of 1 ml of 20% perchloric acid containing 20 mM lanthanum nitrate.

Enzyme appears to cleave yeast RNA at purine linkages exclusively, with a slight preference for guanylic acid over adenylic acid. As is indicated in Table V, the actual ratio is about 5:4 of guanylic to adenylic acid, respectively.

**Inhibition of Enzyme Activity by Synthetic Polynucleotides** Polynucleotides polyguanylic acid and other synthetic polynucleotides

**TABLE IV**

Size of oligonucleotide fragments resulting from digestion of yeast RNA by human liver ribonuclease

The reaction conditions are described in Fig. 1. Each combined nucleotide fraction (designated by Roman numeral) was digested with 25 units of human liver ribonuclease in a solution (5 ml) containing 500 \(\mu\)mol of Tris/HCl buffer, pH 7.2. The reaction mixture was incubated at 37°C for 16 h and then heated in a boiling water bath for 10 min. The cloudy mixture was clarified by centrifugation and the supernatant solution (350 \(A_{260}\) units) was applied to a column (2.5 x 60 cm) of DEAE cellulose (chloride form) prepared as described by Tomlinson and Tener (30). The column was washed with water and the oligonucleotides eluted with 3 liters of a linear gradient of elution from 0.0 to 0.2 M NaCl in 7 M urea and 0.0025 M sodium acetate buffer, pH 4.7. Fractions, 12 ml in volume, were collected at a flow rate of about 20 ml/h. The bars at the base of the elution diagram represent the fractions which were combined for phosphorus analysis (31). The Roman numerals at the top of each peak were used to designate the combined fractions.

**Fig. 1.** Chromatography of the human liver ribonuclease digest of yeast RNA. Yeast RNA (35 mg; 415 \(A_{260}\) units) was digested with 25 units of human liver ribonuclease in a solution (5 ml) containing 500 \(\mu\)mol of Tris/HCl buffer, pH 7.2. The reaction mixture was incubated at 37°C for 16 h and then heated in a boiling water bath for 10 min. The cloudy mixture was clarified by centrifugation and the supernatant solution (350 \(A_{260}\) units) was applied to a column (2.5 x 60 cm) of DEAE cellulose (chloride form) prepared as described by Tomlinson and Tener (30). The column was washed with water and the oligonucleotides eluted with 3 liters of a linear gradient of elution from 0.0 to 0.2 M NaCl in 7 M urea and 0.0025 M sodium acetate buffer, pH 4.7. Fractions, 12 ml in volume, were collected at a flow rate of about 20 ml/h. The bars at the base of the elution diagram represent the fractions which were combined for phosphorus analysis (31). The Roman numerals at the top of each peak were used to designate the combined fractions.
having ordered secondary structures are known to inhibit the hydrolytic activity of several endonucleases from microbial and from human sources (4–7, 32, 33). Recently, poly(A) also has been shown to be a competitive inhibitor of a number of ribonucleases (34, 35). With respect to the liver enzyme, both polypurines can cause virtually complete inhibition of enzyme activity (Table VI). Poly(G) is, however, more effective in that at concentrations 10-fold less than poly(A), enzyme activity is inhibited completely. Analysis of the kinetics of the inhibition (36) demonstrated that both poly(G) and poly(A) inhibit the human liver enzyme competitively with inhibitor constants ($K_i$) of $1.2 \times 10^{-4}$ M and $1.46 \times 10^{-4}$ M, respectively. In contrast, neither poly(C) nor poly(U) inhibit enzyme activity.

As shown with other ribonucleases (4–1), polynucleotide inhibition of human liver RNase activity can be reversed by the polyanines (Table VI). In phosphate buffer, spermine is particularly effective, since enzyme activity is completely restored at concentrations about 20% that of spermidine. It is interesting to note that putrescine and Mg$^{2+}$ are able to reverse the inhibition of the enzyme by the more potent agent, poly(G), with greater facility than the inhibition induced by poly(A). Moreover, by raising the concentration of these ions severalfold over the values shown in Table VI, their effectiveness in restoring enzyme activity approaches that of spermine and spermidine. Similar results were obtained in a parallel study using Tris/HCl buffers, except that spermine was completely ineffective.

**Formation of Poly(A)-Enzyme Complex**—The inhibition of enzyme activity by polyadenylic acid and the reversal of this inhibition by the polyamine suggests that a poly(A)-enzyme complex was formed; a phenomenon observed previously between poly(G) and other ribonucleases (4, 5, 7). To visualize the formation of such a poly(A)-enzyme complex, the polynucleotide and enzyme were subjected, independently, to gel filtration. Since the two substances were considerably different in size, there is a substantial separation in their elution positions from the gel (Fig. 2A). If, however, the protein and polynucleotide are incubated for a short period prior to gel filtration, then the elution positions of the two are coincidental (Fig. 2B). Treatment of the poly(A)-enzyme complex with spermidine before gel filtration separated the two substances so that the enzyme no longer migrated with the larger polynucleotide. To compare the efficacy of various substances in releasing enzyme from the poly(A)-enzyme complex, a commercially available agarose which had poly(A) covalently bound to it was used to bind 400 units of the human liver ribonuclease. As shown in Table VII, both spermine and spermidine can effect almost quantitative removal of the enzyme from the poly(A)-agarose column. Mg$^{2+}$ and putrescine, on the other hand, are significantly less effective.

**Inhibition of Human Liver RNase by Naturally Occurring RNA Containing Poly(A)**—Although poly(A) tracts have been known for a number of years to occur at the 3' termini of a variety of mRNAs (37–41), the role of this polynucleotide remains unclear. It has been suggested, however, that poly(A) may stabilize RNA by virtue of the inhibitory effect it has on ribonucleases (34). To examine this view, naturally occurring mRNAs, known to contain poly(A) at their 3' termini, were compared with either yeast RNA or synthetic polynucleotides in their ability to prevent the hydrolysis of another RNA molecule (Escherichia coli 5 S [3H]rRNA) (35).

As the data summarized in Table VIII illustrate, the presence of an equimolar concentration of mRNA and substrate in the reaction mixture can inhibit enzyme activity up to a maximum of 75% of control activity. If the poly(A) segment is removed from the termini of the globin mRNA (line 5, Table VIII), an equimolar amount of the depolyadenylated mRNA is no longer inhibitory. Similarly, the addition of an equimolar quantity yeast RNA, which has no poly(A) segment, results in only a small diminution of enzyme activity. (A strong dilution effect on acid-soluble counts is not observed because the enzyme sites were not saturated under the reaction conditions.) The polynucleotide, poly(A), has the most dramatic effect of all, since at one-third the concentration of substrate in the reaction mixture can inhibit enzyme activity up to a maximum of 75% of control activity. If the poly(A) segment is removed from the termini of the globin mRNA (line 5, Table VIII), an equimolar amount of the depolyadenylated mRNA is no longer inhibitory. Similarly, the addition of an equimolar quantity yeast RNA, which has no poly(A) segment, results in only a small diminution of enzyme activity. (A strong dilution effect on acid-soluble counts is not observed because the enzyme sites were not saturated under the reaction conditions.) The polynucleotide, poly(A), has the most dramatic effect of all, since at one-third the concentration of substrate enzyme activity is reduced by as much as 80%.

Illustrated in this study, as well, is the reversal of poly(A)-induced enzyme inhibition by the polyamine, spermidine. Even in cases where substantial inhibition of hydrolysis was seen, the polyamine brought enzyme activity back to essentially those values found in the absence of inhibitor.

The reversal of poly(A) inhibition by spermidine was used also to show that the poly(A) sequence of mRNA was instrumental in restoring enzyme activity approaches that of spermine and spermidine. Similar results were obtained in a parallel study using Tris/HCl buffers, except that spermine was completely ineffective.

*Table V*

| Condition | Enzyme activity |
|-----------|----------------|
| Standard reaction mixture | Standard reaction mixture |
| +Putrescine | +Putrescine |
| +Spermidine | +Spermidine |
| +Spermine | +Spermine |
| +Mg$^{2+}$ | +Mg$^{2+}$ |
| +Poly(G) | +Poly(G) |
| +Poly(G) + putrescine | +Poly(G) + putrescine |
| +Poly(G) + spermidine | +Poly(G) + spermidine |
| +Poly(G) + spermine | +Poly(G) + spermine |
| +Poly(A) | +Poly(A) |
| +Poly(A) + putrescine | +Poly(A) + putrescine |
| +Poly(A) + spermidine | +Poly(A) + spermidine |
| +Poly(A) + spermine | +Poly(A) + spermine |
| +Poly(A) + Mg$^{2+}$ | +Poly(A) + Mg$^{2+}$ |

Enzyme activity was measured as described under “Methods” using 10 μmol of sodium phosphate buffer, pH 6.2, and 2.5 units of enzyme (see “Methods”). When the effects of inhibiting agents on the reaction were examined, 12.5 nmol of poly(G) or 125 nmol of poly(A) were added to the reaction mixture as shown. When reversal of inhibition was studied either 0.25 μmol of spermidine or 1.25 μmol of the other cations indicated below were added prior to the addition of enzyme.

*Table VI*

| Specific activity | cpm/μmol |
|------------------|----------|
| AMP              | 10,500   |
| GMP              | 20,000   |
| CMP              | 200      |
| UMP              | 300      |

Details of the preparation and purification of the [3'-32P]- and [5'-32P]-phosphoryl terminal mononucleotides from human liver ribonuclease digestion of yeast RNA are illustrated in Table V. When the effects of inhibiting agents on the reaction were examined, 12.5 nmol of poly(G) or 125 nmol of poly(A) were added to the reaction mixture as shown. When reversal of inhibition was studied either 0.25 μmol of spermidine or 1.25 μmol of the other cations indicated below were added prior to the addition of enzyme.
The fact that poly(A) itself is not a substrate for the liver enzyme was shown by the failure to observe hydrolysis of the polynucleotide either in the presence or absence of spermidine. The mRNA was comparable to that observed with yeast RNA. The hydrolysis of products would be small enough to be readily sequenced. In Table VI, the globin mRNA was not degraded. On addition of spermidine to the reaction mixture, however, hydrolysis of the globin mRNA was not degraded. In addition, a second such made; the first being the alteration in size of a human plasma RNase (4). The influences of these ubiquitous substances, the polyamines, are reflected in two additional phenomena, i.e. the increase in enzyme activity and the decrease in the size of a human plasma RNase (4). The influences of these ubiquitous substances, the polyamines, are reflected in two additional phenomena, i.e. the increase in enzyme activity and the decrease in the size of a human plasma RNase (4). The influences of these ubiquitous substances, the polyamines, are reflected in two additional phenomena, i.e. the increase in enzyme activity and the decrease in the size of a human plasma RNase (4). The influences of these ubiquitous substances, the polyamines, are reflected in two additional phenomena, i.e. the increase in enzyme activity and the decrease in the size of a human plasma RNase (4). The influences of these ubiquitous substances, the polyamines, are reflected in two additional phenomena, i.e. the increase in enzyme activity and the decrease in the size of a human plasma RNase (4). The influences of these ubiquitous substances, the polyamines, are reflected in two additional phenomena, i.e. the increase in enzyme activity and the decrease in the size of a human plasma RNase (4). The influences of these ubiquitous substances, the polyamines, are reflected in two additional phenomena, i.e. the increase in enzyme activity and the decrease in the size of a human plasma RNase (4). The influences of these ubiquitous substances, the polyamines, are reflected in two additional phenomena, i.e. the increase in enzyme activity and the decrease in the size of a human plasma RNase (4). The influences of these ubiquitous substances, the polyamines, are reflected in two additional phenomena, i.e. the increase in enzyme activity and the decrease in the size of a human plasma RNase (4). The influences of these ubiquitous substances, the polyamines, are reflected in two additional phenomena, i.e. the increase in enzyme activity and the decrease in the size of a human plasma RNase (4). The influences of these ubiquitous substances, the polyamines, are reflected in two additional phenomena, i.e. the increase in enzyme activity and the decrease in the size of a human plasma RNase (4). The influences of these ubiquitous substances, the polyamines, are reflected in two additional phenomena, i.e. the increase in enzyme activity and the decrease in the size of a human plasma RNase (4). The influences of these ubiquitous substances, the polyamines, are reflected in two additional phenomena, i.e. the increase in enzyme activity and the decrease in the size of a human plasma RNase (4).
reversal of polynucleotide-induced inhibition of enzyme activity by the polyamines (4–7). Spermidine appears to be most effective in the restoration of the hydrolytic activity of the liver enzyme after inhibition by either poly(A) or poly(G). Putrescine and MeG**, however, at significantly higher concentrations, were also able to restore enzyme activity. It was of interest that these agents were more effective in reversing inhibition by poly(G) than poly(A), even though polyguanucleic acid is the stronger inhibitor. The studies with the polyamines, then, lend support to the view that one function of these substances might be to control RNA levels in the cell through regulation of RNase activity (5).

The control of the poly(A)-induced inhibition of the liver RNase by the polyamines was explored further because of the continuing interest in the function of poly(A) segments at the 3’ termini of a number of mRNAs (37–41). In this regard, recent studies have shown poly(A) to be a potent, yet reversible, inhibitor of endonucleases from a variety of sources (34). Moreover, the covalent linking of poly(A) tracts of various lengths to an RNA molecule has been shown to stabilize the molecule in the presence of RNase activity (35).

In the present study it was shown that mRNA isolated from several mammalian sources can act as an inhibitor of the human liver ribonuclease and therefore could protect the general population of RNA molecules from hydrolysis. The inhibition was shown to be localized in the poly(A) segment of the molecule, since removal of the segment from globin mRNA resulted in an almost quantitative loss of inhibitory activity. Clearly, then, the poly(A) segment of the mRNA acts to protect the message portion from hydrolysis. These results suggest that poly(A) may act to maintain the functional stability of mRNA (42–44) by competitively inhibiting ribonuclease activity (34).

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