The Effects of Polyamines on a Residue-specific Human Plasma Ribonuclease

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SUMMARY

A ribonuclease, purified some 2700-fold from human plasma, exhibited a strong predilection for the hydrolysis of internucleotide bonds containing cytidylic acid. Analysis of [3'-32P]- and [5'-32P]-phosphoryl-terminal fragments obtained after enzymic digestion of rabbit liver and yeast RNA indicated that the nucleotide at the 3' terminus of the fragments was invariably cytidylic acid. The nucleotide at the 5' terminus varied between cytidylic and uridylic acids in a ratio of 9:1. When characterized by DEAE-cellulose chromatography, approximately 70% of the digest consisted of oligonucleotides from 4 to 8 nucleotides in length.

Enzyme activity, when measured in low ionic strength buffer, could be increased severalfold above control levels by the addition of either of the polyamines, spermidine or spermine. These substances also restored nucleolytic activity to preparations inhibited by such ordered synthetic polynucleotides as polyguanylic acid. Estimations of the molecular weight of the enzyme, both by Sephadex gel filtration and sucrose density centrifugation, indicate that the weight may vary, depending on the presence or absence of certain cations. Of the cations examined, spermidine and spermine appear to have the greatest effect, causing an alteration in molecular weight from greater than 150,000 to approximately 32,000.

It has been shown that the polyamines can exert considerable effects on the activity of a ribonuclease isolated from a soil-living organism, Citrobacter sp. (1-3). The polyamine, spermidine, was found to enhance enzyme activity, to alter the residue specificity exhibited by the enzyme in its attack on RNA, and to reverse the competitive inhibition of enzyme activity caused by highly ordered synthetic polynucleotides. Inasmuch as these observations suggested that the polyamines may be playing a significantly different role in RNA metabolism than had hitherto been suspected (4, 5), it was of interest to determine whether these effects were limited to the Citrobacter system or were a reflection of a more general phenomenon in which the polyamines regulated RNA concentration within the cell by controlling RNase activity.

To explore this latter view, the effects of polyamines on a number of ribonucleases were examined. In the earlier paper of this series, the enzymes chosen for study were all highly specific, but were of microbial origin. In the present paper, the ribonuclease from human plasma was selected because of a report suggesting specificity for cytidylic acid (6). The enzyme, purified approximately 2700-fold, exhibited a strong predilection for internucleotide bonds of cytidylic acid. Spermidine and spermine were found to cause as much as a 7-fold increase in enzyme activity.

EXPERIMENTAL PROCEDURE

Materials

Human plasma was purchased from a commercial blood bank or was obtained from volunteers. Yeast RNA, purified by the method of Crestfield et al. (7), was prepared in the laboratory. Rabbit liver RNA was purified by the method of Dalilhas and Staehelin (8).

The following materials were obtained from commercial sources: synthetic polynucleotides (Miles Laboratories, Kankakee, Ill., or Schwarz-Mann, Orangeburg, N. Y.); Sephadex G-75, G-100, and CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N. J.); active cellulose phosphate (Reeve Angel, Clifton, N. J.) prepared for use according to the distributor's brochure; Escherichia coli alkaline phosphatase (Worthington Biochemical Corp., Freehold, N. J.) used according to the method of Garen and Levinthal (9); snake venom phosphodiesterase (Sigma Chemical Co., St. Louis, MO.) freed of 5'-nucleotidase and used as described by Koerner and Sinhasheimer (10); E. coli B polynucleotide kinase and Micrococcus lysus polynucleotide phosphorylase (P-L Laboratories, Inc., Milwaukee, Wis.) used according to the procedures of Weiss and Richardson (11) and of Moses and Singer (12), respectively; dialysis tubing (Union Carbide, Chicago, Ill.) freed of impurities by heating at 60° for 30 min in 1 N EDTA; sodium adenosine 5'-[α-32P]diphosphate (ICN, Irvine, Calif.); triethyl ammonium adenosine 5'-[23P]triphosphate (New England Nuclear, Boston, Mass.); fluorosamine for protein assays (Hoffmann-La Roche, Inc., Nutley, N. J.); bovine serum albumin (Armour Pharmaceutical Co., Chicago, Ill., or Schwarz-Mann); and ECTEOLA-cellulose (epichlorohydrin triethanolamine cellulose) thin layer plates (Analtech, Inc., Newark, Del.).

Methods

Assay of Human Plasma Ribonuclease—The standard assay system contained 1.5 μmol of polymer or 1.0 μg of RNA, 100 μmol of buffer (Tris-Cl, pH 7.5, or (Cl), or sodium phosphate, pH 8.0, for RNA), and 0.5 μg of bovine serum albumin and enzyme in 1 ml. After incubation for 15 min at 25°, the reaction was stopped by the addition of 1 ml of 2 N perchloric acid and the reaction vessel chilled for 10 min in an ice bath. The cloudy reaction mixture was clarified by centrifugation and the absorb-
ance of the acid-soluble nucleotides was measured at 260 nm (13). An enzyme unit is defined as that amount of enzyme needed to cause an increase in absorbance of 1.0 under conditions of the assay, when (C), was used as the substrate.

Molecular Weight of Human Plasma Ribonuclease—The molecular weight of human plasma ribonuclease was estimated by the gel filtration method of Andrews (14), using as protein standards bovine serum albumin (67,000) (15), ovalbumin (43,000) (4), chymotrypsinogen A (25,000) (17), and cytochrome c (12,400) (18). The ultracentrifugation method of Martin and Ames (19) was also used to estimate the molecular weight of the enzyme, using yeast alcohol dehydrogenase (130,000) (20) and catalase (230,000) (21) as standards. All standards were prepared from the Sigma Chemical Co.

Preparation of [8-3²P]Phosphoryl-terminal Oligonucleotides—To a solution (2 ml) containing 2 mg of rabbit liver RNA and 0.1 M sodium phosphate buffer, pH 8.0, 40 units of human serum ribonuclease were added. After 16 hours of incubation at 37°C, the digest was subjected to ultrafiltration through a Centriflo membrane cone (CF50, Amicon Corp., Lexington, Mass.) to remove all fragments of molecular weight above 60,000. An aliquot (1.0 ml) then was treated with 0.1 M HCl at 30°C for 1 hour to convert the cyclic phosphodiester bonds to the monoester form followed by dialysis for 5 hours against five changes (15 liters) of 0.1 M Tris-HCl buffer, pH 7.6. Terminal phosphate groups were removed from the dialyzed oligonucleotides by treatment with alkaline phosphatase (9) for 2 hours at 37°C. Mechanical shaking of the reaction mixture with a chloroform-ethyl acetate (1:1) solvent was used to remove all protein from the solution. After centrifugation for 20 min., the aqueous phase was separated, extracted three times with equal volumes of ether, and dialyzed for 2 hours against 4 liters of 0.05 M Tris-HCl buffer, pH 8.6. This fraction (0.8 ml) was added to 0.5 ml of a solution containing 0.05 M Tris-HCl buffer, pH 8.6, 0.01 M MgCl₂, 0.05 M EDTA, 1 mmol of [8-3²P]ADP (specific activity 2 x 10¹⁰ cpm per mmol), and 5 units of polynucleotide phosphorylase (12).

The reaction mixture was incubated for 2 hours at 37°C and extracted with the chloroform-ethyl acetate solvent followed by ether as described above. The [8-3²P]phosphoryl-terminal oligonucleotide fraction was dialyzed for 2 hours against 10 liters of water and digested with 1.0 n NaOH for 16 hours at 37°C. Ten micromoles of each of the four 3'-mononucleotides were added to the digest which was added to a column (0.9 x 6 cm) of Dowex 1-X8 formate. The mononucleotides were eluted with increasing normality of formic acid as described by Hurlbert et al. (22).

Preparation of [5'-3²P]Phosphoryl-terminal Oligonucleotides—Another aliquot (1 ml) was taken from the rabbit liver RNA digest described above and was treated with alkaline phosphatase for 2 hours at 37°C. All protein was removed from the incubation mixture by mechanical shaking with the chloroform-ethyl acetate described above. After removing residual soil by redissolution, precipitation, and dialysis, and to bring the radioactive activity, specific activity was paper electrophoresis (Whatman No. 3MM paper at 3000 volts, 190 ma for 2 hours at 24°C in formic acid) followed by thin layer chromatography on ECTEOLA-cellulose using 0.1 h~(NH₄)OH, and water (66:1:33) as developing solvent was included as the final step.

Radioactivity was measured in a Beckman L-150 liquid scintillation spectrometer.

RESULTS

pH Optima

When the production of acid-soluble nucleotides was used as an index of enzyme activity, considerable variation in pH optima was found, not only between individual substrates, but also between different buffers used in the measurement of hydrolysis of the same substrate. Thus (Fig. 3), at the optimum pH (7.5) in Tris-HCl buffer, hydrolysis of (C)ₙ was approximately 1.5 times as great as it was at the optimum pH (6.5) in phosphate buffer. The hydrolysis of RNA showed the same optimum pH in either Tris-HCl or phosphate buffer (pH 8), but the enzyme activity was lower in the latter buffer. The data are summarized in Table I.

| Step | Volume | Protein* | Activity | Specific activity | Yield |
|------|--------|----------|----------|------------------|-------|
| 1. Plasma | 100 | 5,300 | 40,420 | 7.0 | 100 |
| 2. Ammonium sulfate fractionation | 36 | 2,578 | 35,971 | 14.0 | 89 |
| 3. Phosphocellulose chromatography | 110 | 37.4 | 22,000 | 588.0 | 54 |
| 4. Affinity chromatography | 7 | 0.4 | 8,400 | 21,000.0 | 21 |

* Protein concentration was measured by the method of Lowry et al. (24) or at extremely low concentrations by the fluorescent technique of Böhlen et al. (25).
activity in phosphate buffer was approximately twice that found in Tris-HCl.

Estimation of Molecular Weight of Human Plasma Ribonuclease

Sephadex Gel Filtration—On Sephadex G-100 equilibrated with 0.05 M Tris-HCl buffer, pH 7.5, approximately 10 to 20% of the enzyme eluted within the void volume, indicating a molecular weight in excess of 150,000. It was found that approximately 60% of the enzyme was adsorbed to the column but could be eluted by buffer solution containing 5 mM spermine or spermidine. These latter fractions, or fresh enzyme, when applied to a column equilibrated with 0.5 mM spermine or spermidine in the buffer system were eluted at a position corresponding to a molecular weight of 32,000 (Fig. 4). The aggregation phenomenon was reversible by the addition or removal of polyamine from the equilibrating buffer. Neither Cu²⁺ nor Mg²⁺ at the same concentrations as the polyamines was able to produce these effects.

Sucrose Density Centrifugation—The molecular weight of the plasma ribonuclease also was determined by ultracentrifugation in a sucrose density gradient, both in the presence and absence of spermidine. As seen in Fig. 5, the peak of enzyme activity, in the absence of spermidine, was found at the bottom of the gradient with a gradual reduction in activity as the less dense sucrose concentrations were approached. The pattern seemed to indicate a spectrum of molecular species ranging in weight from well over 150,000 downward. In the presence of the polyamine two sharp peaks of enzyme activity, with estimated molecular weights of 45,120 and 28,050, respectively, were readily differentiated. Centrifugation in the presence of spermine produced similar results, except considerably greater recovery of enzyme was attained. Although Ca²⁺ and Mg²⁺ at the same concentration produced an apparent shift in the
FIG. 4. Gel filtration chromatography of human plasma ribonuclease on a Sephadex G-100 column (2 × 42 cm). The figure above illustrates results obtained by chromatography under three different sets of conditions. In each case, 700 units of enzyme were applied to a column. In the first experiment (— — ), the column was equilibrated and eluted with 0.05 M Tris-HCl buffer, pH 7.5. The other curves show the elution patterns when the column was equilibrated and eluted with the same buffer containing 0.5 mM spermine (- - -) or spermidine (— — ). Enzyme activity was measured against (C)n in the standard system and is shown in terms of increase in absorption at 260 nm.

FIG. 5. Sucrose density gradient centrifugation of human plasma ribonuclease: the effect of spermidine. Ultracentrifugation of 0.1 ml of enzyme (200 units) was performed as described by Martin and Ames (19) both in the presence (— ) and absence (— — ) of 0.5 mM spermidine. Enzyme activity was measured as described in Fig. 4.

Table II

| Addition | ΔAbs nm | RNA | (C)n |
|---------|---------|-----|------|
| 0       | 0.262   | 0.516|
| Spermidine | 1.826   | 1.519|
| Putrescine | 0.693   | 0.801|
| Spermine   | 1.515   | 1.093|
| Ca*+      | 0.492   | 0.566|
| Mg*+      | 0.626   | 0.560|

Effect of Cations on Enzyme Activity—The hydrolytic activity of human plasma ribonuclease is decreased significantly when the buffer concentration in the reaction medium is reduced from 0.1 M to 0.01 M. Activity against RNA could be restored by the addition of various cations to the dilute buffer medium (Table II). The greatest stimulations occurred when either of the polyamines, spermidine or spermine, was added at an optimal concentration of 1 mM. At the latter concentration, enhancement of activity by Ca*+ or putrescine was considerably less. Raising the concentration of Mg*+ or putrescine to 5 mM resulted in an enhancement of activity which approached that seen with spermidine and spermine. Other cations did not affect activity, with the exception of Zn*+ and Ni*+ (not shown in Table), which acted as strong inhibitors.

The diminished hydrolysis of (C)n in the dilute buffer system was similarly increased by various cations (Table II). At a concentration of 0.5 mM, spermine and spermidine caused 2- and 3-fold stimulations of activity against (C)n, respectively, whereas Ca*+, Mg*+, and putrescine had lesser or no effect. At a 10-fold greater concentration, all of the cations save spermine produced further enhancement of hydrolysis. Spermine inhibited above 0.5 mM, possibly because of precipitation of the substrate.

It should be noted that the conditions under which the hydrolysis of the synthetic polymer and that of RNA were studied are quite different. More enzyme was required, as well as a longer incubation time for the digestion of the latter substance than for polycytidylic acid. The enzyme has a considerably greater affinity for the synthetic polymer than for RNA.

Inhibition of Nuclease Activity by Synthetic Polynucleotides—A number of synthetic polynucleotides having ordered secondary structure have been reported to inhibit microbial nucleolytic enzyme activity (1, 27). Although some of these same compounds also inhibit human plasma ribonuclease, the degree of inhibition appears to be dependent on the substrate under study (Table III). The amount of ordered polynucleotide needed to produce 50% inhibition of RNA hydrolysis can be as molecular weight, the recovery of enzyme activity was approximately 40% of that added to the gradient originally.

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Activity was measured in the standard assay system using either 1.5 μmol of (C)ₙ or 1.0 mg of yeast RNA as substrate. The inhibitor concentrations shown are those which caused 50% inhibition of hydrolytic activity. Enzyme activity was assayed as described under "Methods,"

| Substrate | Inhibitor |
|-----------|-----------|
| (G)ₙ      | (A)ₙ      | (A, G)ₙ | (G, U)ₙ | (A, G, U)ₙ |
| (C)ₙ      | 0.5       | 0.1     | 5.0     | 1.0     | 2.5     |
| RNA       | 100       | 50      | 500     | 50      | 100     |

**Table IV**

Effect of addition of cations on inhibition of hydrolysis of (C)ₙ

Reaction conditions were as described in Table II, except that the reaction mixtures were incubated for 7/₈ min at 25°. When the effects of inhibiting agents and the cations on the reaction were studied, 0.5 μmol of cation and inhibitor (at the concentration indicated below) was added. Hydrolytic activity, measured as described under "Methods,"

| Additions          | Hydrolytic activity in inhibitors |
|--------------------|----------------------------------|
|                    | (G)ₙ (3 μM) | (A)ₙ (0.5 μM) | (A, G)ₙ (5 μM) | (G, U)ₙ (50 μM) | (A, G, U)ₙ (50 μM) |
| -                  | 14.9       | 6.8       | 27.4       | 8.6       | 10.8       |
| Inhibitor + spermidine | 112.5      | 109.8     | 98.2       | 37.3      | 134.0      |
| Inhibitor + spermine    | 94.2       | 120.2     | 119.0      | 81.3      | 110.0      |
| Inhibitor + Ca²⁺       | 38.5       | 47.7      | 41.7       | 16.9      | 29.9       |
| Inhibitor + Mg²⁺       | 33.5       | 26.0      | 44.3       | 12.1      | 21.3       |

| Additions          | % control |
|--------------------|-----------|
| -                  | 100       |
| Inhibitor + spermidine | 100.0     |
| Inhibitor + spermine    | 100.0     |
| Inhibitor + Ca²⁺       | 100.0     |
| Inhibitor + Mg²⁺       | 100.0     |

**Table V**

Effect of cations on inhibition of enzymic hydrolysis of RNA

Experimental conditions were as described in Table II, except that 1.0 mg of yeast RNA was used as substrate in each reaction mixture and incubation time was 15 min.

| Additions          | Hydrolytic activity in inhibitors |
|--------------------|----------------------------------|
|                    | (G)ₙ (200 μM) | (A)ₙ (200 μM) | (A, G)ₙ (500 μM) | (G, U)ₙ (300 μM) |
| -                  | 30.0               | 19.9               | 23.3               | 28.3               |
| Inhibitor + spermidine | 43.7               | 36.5               | 27.0               | 28.1               |
| Inhibitor + spermine    | 63.2               | 85.9               | 45.4               | 19.5               |
| Inhibitor + Ca²⁺       | 35.1               | 33.9               | 25.9               | 29.3               |
| Inhibitor + Mg²⁺       | 31.3               | 18.5               | 19.5               | 19.0               |

Although the synthetic polynucleotides are not as strong inhibitors of RNA hydrolysis, reversal of this inhibition is less readily accomplished (Table V). This may be related to the fact that much higher inhibitor levels are used. Only spermine appeared to be consistently effective, and it induced only a partial restoration of activity.

**Table VI**

Hydrolysis of synthetic polynucleotides by purified human plasma ribonuclease

Four units of enzyme were added to a reaction mixture containing 1.5 μmol of substrate and 100 μmol of Tris-HCl buffer, pH 7.5. After incubation at 25° for 7½ min, the reaction was stopped by the addition of 1 ml of 20 mm lanthanum nitrate in 2 n perchloric acid. Enzyme activity was measured as described under "Methods,"

| Substrate | ΔMm am |
|-----------|--------|
| (C)ₙ      | 1.936  |
| (A, C, U)ₙ | 1.441  |
| (A, G)ₙ    | 1.108  |
| (C, G)ₙ    | 1.018  |
| (U)ₙ       | 0.490  |
| (A, U)ₙ    | 0.267  |
| (A, C, G, U)ₙ | 0.132  |
| (A, G, U)ₙ | 0.067  |

**Hydrolysis of Nucleic Acids**

Analysis of Digestion Products of RNA—No evidence of hydrolysis of either single- or double-stranded DNA was found. When yeast RNA (2.5 mg) was incubated in 0.1 m phosphate buffer, pH 8, with 20 units of enzyme at 37°, the analysis of the digestion products (28) after 16 hours revealed the presence of only one mononucleotide, cyclic 2'-3'-CMP. Analysis of the 3' and 5' termini of the digestion products, large and small, was carried out. Rabbit liver RNA was chosen as a substrate, primarily because it is mammalian in origin and, secondly, because it can be purified in relatively large quantities. Following a 16-hour hydrolysis of this RNA by human plasma ribonuclease, ³²P was introduced enzymatically into the 3' and 5' termini of the digestion fragments, and their terminal (i.e., radio active) nucleotides were isolated and characterized. Approximately 90% of the total radioactivity at the 5' terminus was in cytidylic acid, whereas 10% was found in uridylic acid (Table VII). Almost 100% of the radioactivity at the 3' terminus was in cytidylic acid (Table VII). Clearly the predominant cleavage of phosphodiester bonds in rabbit liver RNA was between cytidylic acid moieties. Identical results were obtained in the digestion of yeast RNA. In studies in which undigested rabbit liver RNA was used, incorporation of ³²P was less than 2% of that occurring in the enzyme-degraded RNA. Thus, corrections for pre-existing end groups would not significantly change the data presented.

**Size of Oligonucleotide Fragments**—To establish the average

much as 200 times greater than the amount needed to produce the same degree of inhibition of the hydrolysis of polycytidylic acid (Table III).

An earlier study (1, 2) showed that this type of inhibition often can be completely reversed by polyamines. Complete restoration of the enzyme activity against (C)ₙ can be induced by 0.5 mm spermine or spermidine, except in the case of (G, U)ₙ inhibition (Table IV). The same concentration of Ca²⁺ or Mg²⁺ is significantly less effective in overcoming inhibition.
length of oligonucleotides produced within a given period, use was made of the procedure of Tomlinson and Tener (29) in which the products of hydrolysis were separated on a DEAE-cellulose column using 7 M urea as the eluting solvent. The hydrolytic products were separated on the basis of the length of the nucleotide chain (Fig. 6). The degree of polymerization within each combined fraction, designated by Roman numerals, was established by determining the ratio of total phosphorus to terminal phosphorus in each fraction. The results of this analysis, as well as the percentage of the distribution of the oligonucleotides, are summarized in Table VIII. From these data approximately 70% of the recovered products of rabbit RNA hydrolysis consisted of oligonucleotides equal to or greater than 5 nucleotides in length. Essentially the same type of results was obtained with yeast RNA.

**Table VII**

| Specific activity | CMP | UMP | AMP | GMP |
|------------------|-----|-----|-----|-----|
|                  | cpm/mol |
| 5' Terminus      | 41,000 | 6,100 | 55 | 60 |
| 3' Terminus      | 63,000 | 300 | 60 | 60 |

**Table VIII**

| Combined fractions | Volume | Absorbance units (260 mμ) | Per cent of recovered absorbance units | Ratio of total phosphorus to terminal phosphorus | Average No. of nucleotides |
|--------------------|--------|---------------------------|----------------------------------------|-----------------------------------------------|----------------------------|
|                    | ml     |                           |                                        |                                               |                            |
| I                  | 162    | 2.9                       | 0.7                                   | 0.9                                           | 1                          |
| II                 | 270    | 24.2                      | 6.2                                   | 1.8                                           | 2                          |
| III                | 315    | 44.1                      | 11.3                                  | 3.2                                           | 3                          |
| IV                 | 190    | 51.3                      | 15.2                                  | 4.1                                           | 4                          |
| V                  | 500    | 135.0                     | 34.8                                  | 4.9                                           | 5                          |
| VI                 | 287    | 95.7                      | 24.6                                  | 6.8                                           | 7                          |
| VII                | 90     | 34.8                      | 9.0                                   | 8.2                                           | 8                          |

**Discussion**

The liberation of C-cyclic-1' during the course of hydrolysis of polyuridylic acid suggests that the human plasma ribonuclease functions as other ribonucleases in that it cleaves the phosphodiester bond between a nucleoside 3'-phosphate and the 5'-hydroxyl group of the adjacent nucleotide (31). That this method of hydrolytic attack is probably the same when RNA is the substrate is indicated by the finding in RNA digests of C-cyclic-1' and oligonucleotides terminating in cyclic phosphates as digestion products. Aside from revealing that the human plasma ribonuclease possesses endonucleolytic activity, analysis of the fragments present in the RNA digest is of considerable interest in another sense as well, primarily because of the apparent high specificity exhibited by the enzyme for ribonucleotide bonds containing cytidylic acid residues. Initially, a strong suggestion of this specificity came from the rather poor hydrolytic activity against synthetic polynucleotides that did not contain cytidylic acid. After the introduction, by enzyme means, of 32P into the 3' and 5' termini of oligonucleotide fragments present in the RNA digest, it was possible to show that the enzyme cleaved exclusively between cytidylic acid residues at the 3' terminus and exhibited a preferential specificity for cytidylic over uridylic acid residues in an approximate ratio of 9:1 at the 5' terminus. The high specificity for cytidylic acid residues is of particular moment in view of the recent report of a large tract of cytidylic acid residues within the RNA of an echovirus (32). This latter observation suggests the possibility that other RNAs with long tracts of cytidylic residues may exist and that it is these substances which are the natural substrates of the human enzyme.

Another aspect of the enzyme's characteristics which is particularly striking is its response to the polyamines, especially spermidine and spermine. The effects of these compounds on
enzyme activity are in many respects similar to those seen with a microbial enzyme, *Citrobacter* nuclease (1, 2). Enzyme activity, for example, in the presence of these compounds is enhanced severalfold over control levels. The inhibition of hydrolytic activity observed with the ordered polynucleotides can, with some exceptions, be reversed by spermidine and spermine. Other cations which can enhance enzyme activity weren’t as effective in this area.

The most dramatic effect of the polyamines on the human enzyme is their apparent ability to control the degree of aggregation of the enzyme molecule. It was found, for example, that the apparent molecular weight of the enzyme, when determined by gel filtration on Sephadex columns, is decreased from approximately 150,000 to 32,000 in the presence of the polyamines, spermidine and spermine. These compounds similarly cause a change in the position of the enzyme in a sucrose gradient after centrifugation, indicating a shift in molecular weight from well over 150,000 to approximately 29,000. These studies suggest that the enzyme undergoes aggregation-disaggregation reactions under the influence of the polyamines. The previously expressed view (1, 2) that polyamines may represent control factors in the metabolism of nucleic acids thus is further supported by the effects noted upon human plasma ribonuclease.

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