CHANGE OF SUBSTRATE SPECIFICITY BY POLYAMINES OF RIBONUCLEASES WHICH HYDROLYZE RIBONUCLEIC ACID AT LINKAGES ATTACHED TO PYRIMIDINE NUCLEOTIDES

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SUMMARY: The activities of ribonucleases (RNase HS and RNase A), which hydrolyze ribonucleic acid at linkages attached to pyrimidine nucleotides were stimulated by polyamines, while the activities of ribonucleases (RNase T_1 and RNase M), which attack ribonucleic acid at linkages attached to purine nucleotides were not influenced by polyamines. In the presence of polyamines, the cleavage of C_5'-O-P linkages adjacent to cytosine nucleotide was stimulated, while the cleavage of C5'-O-P linkages adjacent to uracil nucleotides was inhibited slightly. The effect of polyamines on the activities of ribonucleases occured through the binding of the polyamines to nucleic acid.

Polyamines have been implicated in numerous growth processes (1). Therefore, the effect of polyamines on DNA replication (2), RNA synthesis (3-5) and protein synthesis (6-11) has been studied extensively. However, there are few reports on the effect of polyamines on DNA and RNA degradation. Recently Levy et al. (12,13) have reported that spermidine can stimulate the activity and alter the base specificity of a ribonuclease from Citrobacter species.

In this communication, we have studied the effect of polyamines on the activities of ribonucleases which have different base specificity.

Abbreviations: C-3'-P and U-3'-P, 3'-phosphates of cytidine and uridine; C-cyclic-P and U-cyclic-P, 2',3'-cyclic phosphates of cytidine and uridine.

MATERIALS AND METHODS

Materials - A ribonuclease from horse submaxillary gland (RNase HS) was prepared as described previously (14). RNase M from Aspergillus saitoi (15) was a kind gift from Dr. M. Irie of Hoshi College of Pharmacy. Bovine pancreatic RNase A (Type I-A), RNase T_1 from Aspergillus oryzae and polyamines (HCl salt) were purchased from Sigma Chemical Co. Yeast RNA (Nutritional Biochemicals Co.) was used after purification by acid-precipitation and dialysis against distilled water. Poly (U) and poly (C) were purchased from Boehringer Mannheim GmbH.

Assay of RNase - The standard assay system (0.5 ml) contained either 1.25 mg of yeast RNA or 250 µg of synthetic polynuceotide, 50 µg of bovine serum albumin, 25 µmoles of Tris-HCl buffer (pH 8.3) and enzyme. In the assay of RNase M, a substitution of 25 µmoles of sodium acetate-acetic acid buffer (pH5.5) for the Tris-HCl buffer was made. After incubation at 37° for 15 min, the reaction was terminated by the addition of 0.5 ml of 5% perchloric acid containing 0.25% uranyl acetate. After being cooled in an ice bath for 30 min, the mixture was centrifuged and the resulting supernatant was diluted with 4 volumes of water. The acid-soluble nucleotides therein were measured at 260 nm.

Measurement of pyrimidine 3'-phosphates and pyrimidine 2',3'-cyclic phosphates - The reaction mixture (10 ml) contained 100 mg of yeast RNA, 1 mg of bovine serum albumin, 0.5 mmoles of Tris-HCl buffer (pH 8.3) and 0.15 µg of RNase HS. After incubation at 37° for 15 min, the reaction was terminated by the addition of 2 ml of 12.5% perchloric acid containing 0.625% uranyl acetate. Upon being cooled in an ice bath for 30 min, the mixture was centrifuged and the resulting supernatant was neutralized with 1.6 N KOH. After the precipitate was removed by centrifugation, the supernatnat was concentrated to 0.4 ml by rotary vacuum evaporator at 37°. Forty µl were spotted on Toyo Roshi NO. 51A filter paper and ascending paper chromatography was performed with a solvent containing 41.2% ammonium sulfate, 2-propanol, and 0.2 M sodium acetate buffer, pH 5.0 (90.5 : 2.5 : 7.0 by volume). The spots of pyrimidine 3'-phosphates and pyrimidine cyclic phosphate were cut off from the paper, and eluted overnight with 0.1 N HCl at room temperature. Under our eluting conditions, pyrimidine cyclic phosphates were converted to pyrimidine 3'-(or 2'-) phosphates. The molecular extinction coefficients of U-3'-(or 2'-)P at 260 and 280 nm were calculated to be 9878 and 3117, respectively, at pH 1.0 from pH 7.0 data (16). Those of C-3'-(or 2'-)P at 260 and 280 nm were similarly calculated to be 6632 and 12796, respectively, at pH 1.0. The contents of U-3'-(or 2'-)P and C-3'-(or 2'-)P in the eluate were calculated from the absorbances of each eluate at 260 and 280 nm using the simultaneous equations.

Binding of [^{14}C]spermine to yeast RNA or RNases by Sephadex G-50 gel filtration - The reaction mixture (0.5 ml) used to study the interaction of spermine with yeast RNA or RNases contained 0.1 M Tris-HCl buffer (pH 8.3), 0.5 mM [^{14}C]spermine (specific activity 1.24 µCi/µmole, New England Nuclear Co.) and 500 µg of yeast RNA or RNase. After incubation at 37° for 15 min, the reaction mixture was subjected to gel filtration on Sephadex G-50 (1 x 23 cm) previously equilibrated with 0.1 M Tris-HCl buffer (pH 8.3). The column was eluted with the same buffer. A 0.1 ml aliquot of each 1 ml fraction was placed on a paper disc (25 mm diameter) and radioactivity was counted. Absorbance at 260 or 280 nm was measured on samples diluted 5-fold.

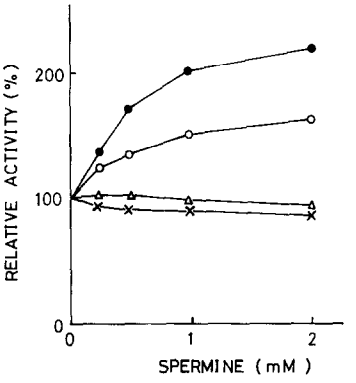

Fig. 1. Effect of cations on the activities of various RNases. The assays were carried out under standard conditions except that spermine was added to the reaction mixture as specified in the figure. The enzymes used in this experiment and absorbances at 260 nm without cations were as follows: RNase HS (●), 6 ng and 0.121; RNase A (o), 4.5 ng and 0.114; RNase T_1 (x), 10 ng and 0.132; RNase M (Δ), 100 ng and 0.142. Relative activities were calculated from the above values being considered as 100 per cent.

RESULTS

Effect of polyamines on the activities of various RNases - The effect of polyamines on the activities of various RNases was studied using yeast RNA as substrate. As shown in Fig. 1, the activities of RNases which hydrolyze RNA at linkages attached to pyrimidine nucleotides (RNase A and RNase HS) were stimulated by spermine, while the activities of RNases which hydrolyze RNA at linkages attached to purine nucleotides (RNase T_1 and RNase M) were not influenced. Other polyamines (spermidine and putrescine) had the same effect as spermine on the RNase HS activity (Fig. 2), although the effective concentrations were different.

Content of pyrimidine nucleotides in digestion products by RNase HS in the presence or absence of spermine - In order to examine whether base specificity of RNase HS was changed by spermine or not, the content of pyrimidine nucleotides in the digestion products was measured. As shown in Table I, the amounts of cytosine nucleotides (C-3'-P plus C-cyclic-P) increased markedly in the

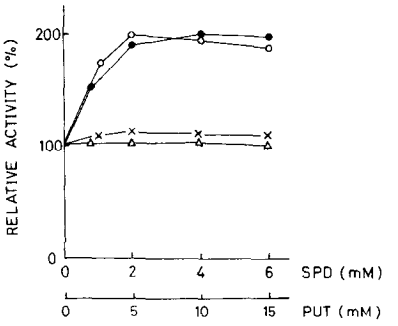

Fig. 2. Effect of polyamines on the activities of RNase HS and RNase T_1. The assays were carried out under standard conditions except that polyamines were added to the reaction mixture as specified in the figure. Absorbances at 260 nm without cations were as follows: RNase HS (6 ng), 0.114; RNase T_1 (10 ng), 0.124. ●, RNase HS, spermidine; o, RNase HS, putrescine; Δ, RNase T_1, spermidine; x, RNase T_1, putrescine.

Table I. Digestion products of yeast RNA hydrolyzed by RNase HS in the presence or absence of spermine.

Addition	Product (μmoles/100 mg RNA)			
	C-3'-P	C-cyclic-P	U-3'-P	U-cyclic-P
none	0.36	0.19	0.41	0.26
2 mM spermine	0.68	0.35	0.46	0.33

The amounts of pyrimidine 3'-phosphates and pyrimidine 2',3'-cyclic phosphates were measured as described under " Materials and Methods ".

presence of spermine, while the increase of uracil nucleotides (U-3'-P plus U-cyclic-P) was very slight. No significant amount of purine nucleotides was observed even in the presence of spermine. These results suggest that the stimulation of RNase HS activity by spermine was due mainly to an increase of cytosine nucleotides.

Activity of RNase HS toward synthetic polynucleotides in the presence of polyamines - To gain further support for the change

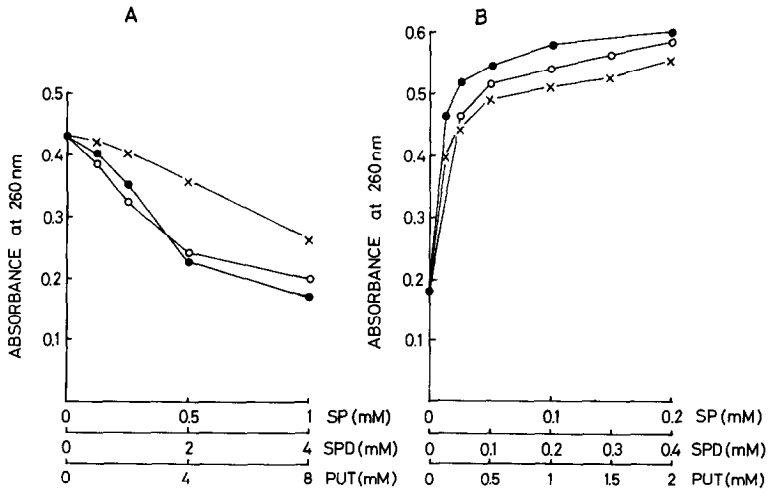

Fig. 3. Activity of RNase HS toward synthetic polynucleotides in the presence of polyamines. The assays were carried out under standard conditions except that polyamines were added to the reaction mixture as specified in the figure. The content of RNase HS used was 80 ng. Poly (U) and poly (C) were used as substrate in (A) and (B), respectively. ●, spermine; o, spermidine; x, putrescine.

of base specificity of RNase HS by spermine, the effect of polyamines on RNase HS activity was studied using synthetic polynucleotides as substrate. As shown in Fig. 3A, the degradation of poly (U) by RNase HS was inhibited by the addition of polyamines, while the degradation of poly (C) was stimulated by the addition of polyamines. These data confirm that the base specificity of RNase HS was altered by polyamines. Similar results were obtained with RNase A.

Relationship of spermine and RNA content on the stimulation of RNase HS activity - As shown in Fig. 4B, the optimal concentration of spermine for stimulation increased as RNA content increased. However, the optimal stimulatory concentration of spermine was not altered by an increase of enzyme concentration (Fig. 4A). These results suggest that the effect of spermine on enzyme activity was induced through the binding of spermine to ribonucleic acid.

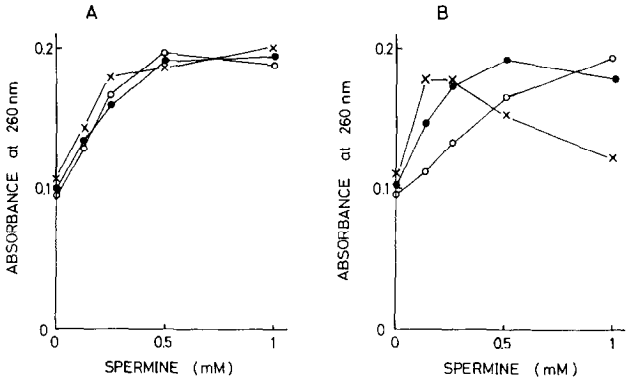

Fig. 4. Effect of RNA and enzyme concentration on the stimulation of RNase HS activity by spermine. The standard assay conditions were used except as specified. A. 500 µg of yeast RNA were used as substrate. o, 1.5 ng of RNase HS, 30 min incubation; •, 3 ng of RNase HS, 15 min incubation; x, 6 ng of RNase HS, 7.5 min incubation. B. The content of RNase HS used was 3 ng. o, 1 mg of yeast RNA; •, 500 µg of yeast RNA; x, 250 µg of yeast RNA.

Table II. Interaction of [^{14}C]spermine with yeast RNA or RNases.

Addition	Bound [^{14}C]spermine (cpm/100 µg of substrate)
RNase HS	14
RNase A	15
Yeast RNA	5416

The radioactivy bound to yeast RNA or RNases was measured as described under " Materials and Methods ".

This suggestion also was supported by the data of Table II, which shows that [^{14}C]spermine is bound to RNA but not to RNases.

DISCUSSION

The data presented show that the substrate specificity of RNases which hydrolyze RNA at linkages attached to pyrimidine nucleotides is altered by polyamines. RNase A and RNase HS stimulate the production of cytosine nucleotides in the presence of

polyamines, but the production of uracil nucleotides is inhibited by polyamines when a uracil homopolymer is used as substrate. Two possible explanations for the slight increase of uracil nucleotides in digestion products prepared from yeast RNA hydrolyzed by RNase HS in the presence of spermine (Table I) are proposed. Since the stimulatory effect of spermine depends upon RNA concentration (Fig. 4), the large amounts of yeast RNA used in the experiment of Table I may prevent the inhibitory effect of polyamines on the production of uracil nucleotides. Secondly, in situations where the base of C_5,-O-P linkages adjacent to uracil nucleotides is cytosine, the production of uracil nucleotides may be stimulated by spermine.

If a similar change in substrate specificity by polyamines occurs in RNases which control mRNA degradation in cells, polyamines may play an important role in regulating the existence of various kinds of mRNA during the various phases of the growth cycle because the polyamine content is higher in rapidly growing cells (1,17). Therefore, we are now studying the effect of polyamines on ribonucleases concerned with mRNA degradation in cells. We have recently reported that polyamines are necessary for maximum polypeptide synthesis in E. coli and rat liver cell-free systems (11) and that the stimulation of polypeptide synthesis by spermidine depends on the uracil content of mRNA (18). This observation is of interest in connection with the change of substrate specificity of RNases by polyamines.

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