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EFFECT OF POLYAMINES ON RIBONUCLEASE ACTIVITY OF RICE (*ORYZA SATIVA* L.)

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Key Word Index—*Oryza sativa*; Gramineae; rice; RNase; putrescine; spermidine; spermine.

Abstract—The RNA content and RNase activity were determined during maturation and germination of rice seeds. The RNA content reached a maximum after the 16th day from anthesis but RNase activity steadily increased up to the last stage of maturation. During germination RNA content was greatest after 24 hr and associated with a very low level of RNase activity. Maximum RNase activity was observed at 72 hr from germination and it afterwards gradually declined. During germination, exogenous application of polyamines decreased the level of RNase activity. RNase was partially purified (448-fold) from 72 hr germinated embryonic axis. Effects of polyamines and other divalent cations were observed on the purified enzyme.

INTRODUCTION

The presence of significant amount of polyamines was reported from various plant sources [1–6]. A high titer of polyamines is associated [7, 8] with a high rate of cellular activity. Application of polyamines inhibited the senescence of radish leaves [9], oat leaf protoplasts [10, 11] and storage tissues of various plants [12], by decreasing RNase and protease activity. On the other hand application of polyamines enhanced ^{14}C-uridine incorporation [10], and also stimulated DNA-dependent RNA polymerase activity in *Micrococcus* [13]. Polyamines appear to be implicated in virtually every step of RNA synthesis and their involvement in cellular activity may be mediated via interaction with macromolecules [14]. As the presence of significant amount of polyamines had already been reported [6] from rice, it was of interest to observe whether polyamines can inhibit RNase activity. The RNA content and RNase activity were also estimated during development and germination of rice seeds.

RESULTS AND DISCUSSION

During development, RNA content was relatively low at the initial stage which reached the greater level (1610 μg/g dry wt) after the 16th day of anthesis, and at the last stage of maturation it declined to 680 μg/g dry wt. RNase activity steadily increased and at the final stage of maturation the activity became 15 times higher than that of anthesis (Fig. 1a).

During germination, RNA content was maximal at 24 hr with very low RNase activity. RNase activity reached the peak value at 72 hr and then declined (Fig. 1b). The results indicate that the higher level of RNA is generally associated with the low RNase activity. During ageing of rice seeds, total and ribosomal RNA contents also dropped, along with the increment of RNase activity [unpublished].

When decoated rice seeds were allowed to germinate in the presence of polyamines, RNase activity was inhibited depending on the concentration and on the structure of the polyamines. Spermine was the most effective inhibitor of RNase, while spermidine was less effective. Putrescine showed virtually no inhibition at the tested concentrations. Maximum inhibition of RNase was found in the presence of 1 mM spermidine and spermine (15% and 24% inhibition respectively). Greater concentrations of the polyamines significantly retarded the rate of germination (Table 1).

For *in vitro* experiments, the enzyme was purified from 72 hr germinated embryonic axis. Table 2 shows the steps of purification. At the final stage of purification the specific activity of the enzyme was increased *ca* 450-fold and the recovery was 45%. The pH optimum of the enzyme was 4.8 and temperature optimum was 37°. *In vitro* treatment with polyamines showed more pronounced inhibition of RNase. The spermidine and spermine at 10 mM gave 31 and 40% inhibition respectively. Putrescine showed only 8% and 10% decrease of activity at 1 mM and 10 mM respectively (Fig. 2). The above results of both *in vivo* and *in vitro* experiments revealed the trend of inhibition from polyamine to diamine, i.e. spermine > spermidine > putrescine. Such type of charge dependent inhibition was also reported by other workers [12].

At physiological pH, polyamines being polyvalent cations remain strongly bound to the acid group of nucleic acids [15, 16]. *In vitro* inhibition probably results from neutralization of negative sites on the substrate by the positively charged polyamines, thereby rendering nucleic acid less accessible to the enzyme. Effects of various inorganic divalent cations on purified enzyme were also compared to study their inhibition efficiency (Table 3). Except copper sulphate no inorganic cation was more effective than polyamines at that concentration. Very high level of inhibition by copper sulphate was also reported by other workers [17] and it is highly probable that Cu^{2+} ion binds strongly to the enzyme.

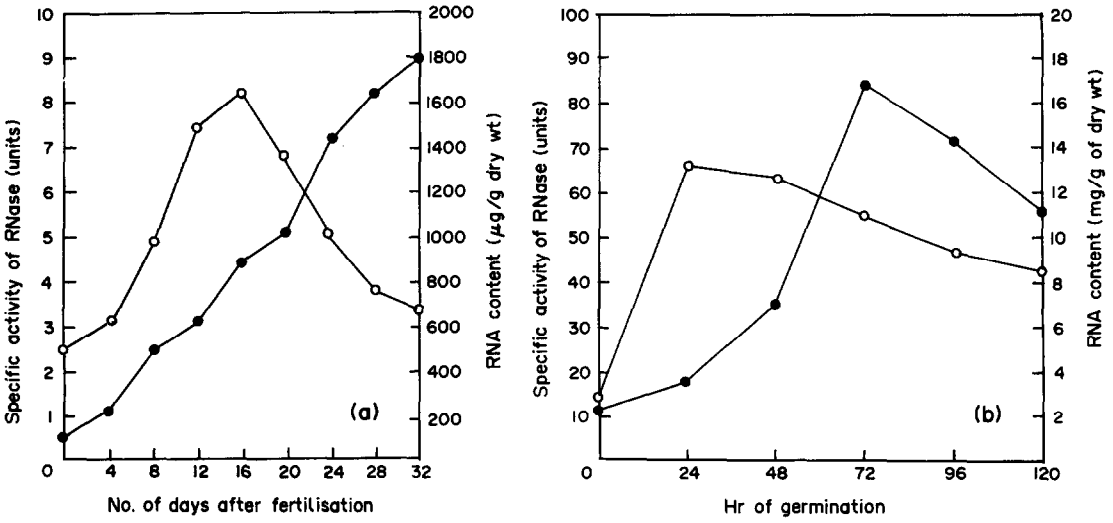

Fig. 1. (a) Changes in RNA content (O—O) and RNase activity (●—●) during development of rice seeds. (b) Changes in RNA content (O—O) and RNase activity (●—●) during germination of rice seeds.

Table 1. Effect of polyamines (*in vivo*) on RNase in 72 hr-germinated rice seedling embryonic axis

Concn (mM)	% of inhibition Spermidine	Spermine
10^{-4}	5	7
10^{-3}	7	10
10^{-2}	9	15
10^{-1}	12	19
1	15	24

EXPERIMENTAL

Chemicals. Putrescine, spermidine, spermine, BSA, *E. coli* RNA, DEAE-cellulose and CM-cellulose were purchased from Sigma.

Seeds were collected and germinated according to ref. [6] To study the *in vivo* effect of the polyamines, 50 decoated seeds were allowed to germinate in presence of various concns of the polyamines. Seeds germinated in H_2O served as control.

Purification of RNase. 50 g of 72 hr germinated embryonic axis were extracted with 4 vol of 20 mM NaPi buffer (pH 7) in a prechilled mortar and pestle. The homogenate was filtered with cheese cloth and the brei was centrifuged at 10 000 *g* for 30 min. The supernatant (crude enzyme) was then adjusted to pH 5 by 1% HOAc and left overnight. It was again centrifuged at 10 000 *g* for 15 min and the ppt was discarded. This step of acidification was essential because omission caused a significant loss of activity during later steps [17, 18]. Protein of the resulting supernatant was concd by 40–85% $(NH_4)_2SO_4$, the ppt dissolved in 20 mM NaPi buffer (pH 6) and dialysed against the same buffer. The protein was applied to DEAE-cellulose column (1.5 × 30 cm), pre-equilibrated with 20 mM NaPi buffer (pH 6). The protein fraction was eluted by the same buffer containing a linear gradient of NaCl (0.05–0.5 M) at a flow rate of 0.5 ml/min and 7 ml fractions were collected. A small peak of RNase activity was found between 35 and 70 ml and the second major peak was obtained between 70 and 168 ml of eluate. The major fraction was collected, concd and dialysed against the equilibrating buffer. This protein was again loaded on a CM-cellulose column (1.5 × 25 cm) previously equilibrated with 50 mM NaOAc buffer (pH 4.5). Protein was eluted by NaCl in a linear gradient of 0.1–1 M of NaCl at a flow rate of 0.6 ml/min and 5 ml was collected in each tube. Within 49–63 ml a sharp peak of RNase was eluted.

Table 2. Purification steps of RNase from 72 hr germinated embryonic axis

Fraction	Specific activity (units)*	Total protein (mg)	Total activity	Degree of purification	Yield (%)
Crude	73	251	18 300	1	100
Acidification	180	93	16 800	2	92
40–85% $(NH_4)_2SO_4$	829	20	16 600	11.2	91
DEAE-Cellulose	2890	5.1	14 600	40	80
CM-Cellulose	32 690	0.25	8160	448	45

*1 enzyme unit is defined which increase *A* 0.001 at 260 nm per mg of protein per min under the assay conditions.

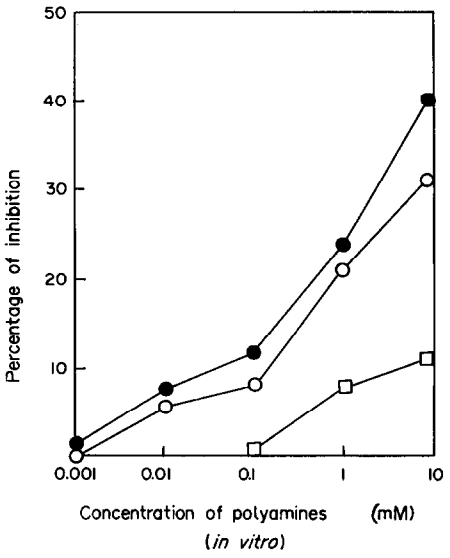

Fig. 2. *In vitro* effect of different polyamines on RNase activity. Putrescine (□—□), spermidine (○—○) and spermine (●—●).

Table 3. Effect of various metal ions (at 1 mM) on RNase activity (*in vitro*)

Concn	% inhibition
Control	0
$FeCl_2$	3
$CaCl_2$	9
$MgCl_2$	16
$BaCl_2$	16
$ZnCl_2$	29
$CuSO_4$	81

This was again dialysed against 20 mM NaPi buffer (pH 6) and the dialysed protein was considered as purified enzyme.

Enzyme assay. The assay mixture consisted of 1 mg highly polymerized yeast RNA, 0.2 ml of enzyme and made 1 ml with 0.1 M NaOAc buffer (pH 4.8) with or without adding polyamines or other reagents, and was incubated at 37° for 30 min. The reaction was terminated by adding 1 ml of PCA-uranyl acetate reagent (0.25% uranyl acetate in 10% PCA). Correction was made for a zero time blank in each set. After keeping at 0° for 30 min, the tubes were centrifuged at 10000 *g* for 10 min. The supernatant was diluted to 20 ml with H_2O and *A* was measured at 260 nm.

RNA and protein were estimated by orcinol reagent [19] and Lowry's [20] method respectively.

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