Dual Mechanisms in Polyamine-mediated Control of Ribonuclease Activity in Oat Leaf Protoplasts

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ABSTRACT

Dibasic amino acids and polyamines added to oat (Avena sativa L.) leaf protoplast isolation media decrease the RNase activity of extracted protoplasts relative to controls. This effect, which is manifested even when the added polyamine is removed by exhaustive dialysis prior to assay, is due to a prevention of the rise in RNase activity which usually follows protoplast isolation. Polyamines, but not dibasic amino acids, also decrease RNase activity in vitro. This in vitro effect seems to result from electrovalent attachment of the polyamine to the RNA, because the greater the net positive charge on the polyamine, the greater is its inhibitory effect in vitro. The activity of dibasic amino acids when added during protoplast isolation probably results from their conversion to polyamines.

In previous investigations (1, 9, 11), we reported that the RNase activity of oat leaf protoplasts increases sharply during the several hr of their incubation. This rise in RNase is accompanied by decreased net incorporation of uridine into RNA, as well as by general deterioration and ultimate lysis of the protoplasts. We suggested that this increased RNase activity in protoplasts and leaves (24, 25) may be in part responsible for the limited survival and only rare cell division activity of cereal protoplasts in culture (5, 19).

The rise in RNase activity is drastically inhibited if protoplasts are incubated in the presence of L-arginine or if they are isolated from leaves pretreated with L-arginine, L-lysine, cadaverine, putrescine, cycloheximide, or kinetin (1, 11, 13). Protoplasts so treated are more stable to lysis and show higher RNA-synthetic activity (1, 11, 13, 14) than controls. It appeared that an understanding of the mechanism by which polyamines inhibit the rise in RNase activity might help in obtaining more stable protoplasts which could be useful in cell culture experiments aimed at improving cereal crops.

Although polyamines are known to affect the synthesis of DNA, RNA, and protein (2, 4, 6, 20), there are only a few reports, limited to bacterial and mammalian systems, dealing with the effect of polyamines on DNA and RNA degradation by nucleases (3, 17, 23). The main objective of this investigation was to determine the effects of L-arginine and related diamines and polyamines on the degradation of RNA by RNase from oat leaf protoplasts.

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RESULTS AND DISCUSSION

Reduction in the RNase activity of protoplasts from oat leaves had been observed previously when leaves were pretreated with diamines prior to protoplasting (11, 13) or when protoplasts from untreated leaves were isolated and incubated in the presence of L-arginine (1). We now report that protoplast RNase activity is dramatically decreased when the diamines or polyamines are present only during the 2-hr incubation in Cellulysin needed for preparation of protoplasts. For example, when compared with control treatments, 10 mM L-arginine or 1 mM spermine in the protoplast isolation medium reduced RNase activity by 72 and 80%, respectively. Concentrations of spermine higher than 1 mM caused protoplasts to aggregate, while higher arginine levels had no further effects. If the protoplasts are incubated for an additional 3 hr, the RNase activity in controls rises by more than 40%, but remains constant in the polyamine-treated series (Table I). Since the crude RNase from the protoplasts was exhaustively dialyzed to remove excess polyamines prior to addition of the RNA substrate, this effect must be due to differences in enzyme activity.

Compounds containing multiple amine groups, when added to the enzymic assays solutions in vitro along with yeast RNA, had various effects. With L-arginine and L-lysine no decrease in RNase activity was observed, with the diamines cadaverine and putrescine activity was reduced slightly, while with the polyamines spermidine and spermine marked decreases in the activity of protoplastic RNase occurred (Table II). Spermine is more effective than spermidine in decreasing the activity of protoplastic RNase and this effect becomes more pronounced with increasing concentrations of the polyamine (Table III). Increase in incubation time to 1 hr did not decrease RNase activity further, indicating that the action of polyamines does not involve further biological transformations, but is most likely the result of a direct reaction between amine and some reactant in the assay. Similar results were obtained in vivo with pancreatic RNase, indicating that the effect is not specific for plant or for oat leaf protoplast RNase.

Polyamines, which are positively charged in the range of cellular pH values, are known to bind strongly to the acidic phosphate groups of RNA and DNA in bacteria, animals, and plants (2, 4, 6, 22). This electrovalent bonding makes the nucleic acids more compact spatially and hence less available to nucleases (18, 23). The in vitro reduction in the activity of protoplastic RNase by polyamines is probably the result of such binding. Depending on the type of RNase involved, the presence of spermidine and spermine may render natural RNA and synthetic polyadenylic acid less susceptible to degradation by endonucleases (10, 15). The

Table III. Effect of various concentrations of compounds containing amine groups on in vitro activity of oat leaf protoplastic RNase

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Relative RNase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(A at 260 nm)</td>
</tr>
<tr>
<td>0 hr</td>
<td>Absolute</td>
</tr>
<tr>
<td>3 hr</td>
<td>Absolute</td>
</tr>
<tr>
<td>Control</td>
<td>0.1</td>
</tr>
<tr>
<td>Spermine, 1mM</td>
<td>0.01</td>
</tr>
<tr>
<td>L-arginine</td>
<td>0.02</td>
</tr>
<tr>
<td>L-lysine</td>
<td>0.02</td>
</tr>
<tr>
<td>Putrescine</td>
<td>0.02</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>0.02</td>
</tr>
<tr>
<td>Spermidine</td>
<td>0.02</td>
</tr>
<tr>
<td>Spermine</td>
<td>0.02</td>
</tr>
</tbody>
</table>

RNase of oat leaves is an endoribonuclease, specific for linkages involving purines (24, 25) and its action is probably affected in this way by polyamines. Recently (7, 12, 16) polyamines have been shown to promote RNase activity in bacteria and animals. But these RNase's have specificity for bonds involving pyrimidines rather than purines. No such effects have yet been noted for plant ribonuclease.

Thus, both dibasic amino acids and polyamines decrease the RNase activity of oat leaf protoplasts relative to controls when added in vivo during protoplasting. This effect is due to an interference with RNA synthesis, since it involves the prevention of the rise in RNase activity seen during protoplasting and aging. In vitro, only polyamines are effective in decreasing RNase activity, probably through direct combination with substrate. Since the dibasic amino acids are known to be rapidly metabolized to polyamines in plant tissues (21), it is probably the polyamines which are primarily effective in reducing RNase activity in the in vivo experiments as well.

LITERATURE CITED

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