# RESISTANCE OF S-RNA TO RIBONUCLEASES IN THE PRESENCE OF MAGNESIUM ION

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It is well known that bovine pancreatic ribonuclease and RNase from Bacillus subtilis are not markedly inhibited by divalent cations such as magnesium ion, when enzymic activity is determined by measuring an increase in acid-soluble material using high molecular weight yeast RNA as substrate (Dickman et al., 1956; Nishimura, 1960). The present communication deals with a marked resistance of Escherichia coli S-RNA to hydrolysis by RNase coupled with a striking shift of the melting curve to higher temperatures when S-RNA is mixed with magnesium ion.

S-RNA was prepared from E. coli B stationary cells by the procedure of Berg and Ofengand (1961), except that a DEAE cellulose column was used instead of an ECTEOLA-cellulose column. Yeast RNA (insoluble in 1 M NaCl) was prepared as described by Crestfield et al. (1955). Fig. 1 shows the increase in acid-soluble, UV-absorbing material when S-RNA is incubated with different amounts of the two RNases in the presence and the absence of 0.01 M $Mg^{++}$. Enzymic digestion of S-RNA by both RNases was markedly inhibited by 0.01 M $Mg^{++}$, whereas only 50% inhibition was observed with high molecular weight yeast RNA as substrate. Other divalent cations, such as manganese and calcium, gave similar results.

When loss of amino acid acceptor function of S-RNA is taken as a measure of hydrolysis, the inhibition of B. subtilis RNase by $Mg^{++}$ is more striking. As shown in Table I, about 100 times as much B. subtilis

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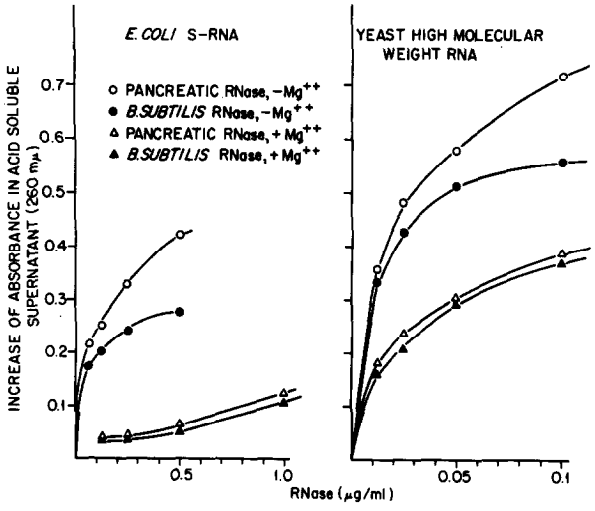


Figure 1. Inhibition of enzymic hydrolysis of E. coli S-RNA by magnesium cation. E. coli S-RNA or yeast RNA (3.7 OD units at 260 mμ in 0.05 M NaOH), 25 μmoles tris-HCl buffer (pH 7.2), and 2.5 μmoles magnesium acetate where indicated were incubated with different amounts of RNases at 37° C for 15 min. After the incubation, magnesium acetate was added into the -$Mg^{++}$ reaction tubes. Total volume was 0.25 ml. The reaction mixture was cooled and 0.25 ml 2 N HCl was added. 0.3 ml of supernatant was diluted to 3 ml with water in order to measure UV absorption. The data were corrected for acid-soluble UV absorption in absence of enzymes (0.015 OD units for E. coli S-RNA, 0.115 OD units for yeast RNA.

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RNase was required to obtain the same loss of leucine acceptor activity when $Mg^{++}$ was present. The same results were obtained with RNase $T_1$ from Takadiastase, which produces only oligonucleotides terminated by 3' guanylic acid. However, with bovine pancreatic RNase, magnesium failed to protect the leucine acceptor activity. This finding suggests that the terminal nucleotide sequence

adenylyl-5' ⟶ 3'-cytidylyl-5' ⟶ 3'-cytidylyl

at the amino acid acceptor end, does not participate in helix formation and is therefore easily hydrolyzed by pancreatic RNase. This would result in loss of acceptor activity even though the rest of the molecule were resistant to hydrolysis. The B. subtilis RNase, on the other hand, produces mainly 3'-purine nucleotide end products and thus cannot attack the terminal nucleotide sequence.

TABLE I. Inhibition of inactivation of leucine acceptor activity by magnesium cation

| Enzyme | Concentration of enzyme (μg/ml) | Condition | Acceptor activity (%) |
|---|---|---|---|
| B. subtilis RNase | 1 | + $Mg^{++}$ | 93 |
| | 5 | | 49 |
| | 20 | | 4.4 |
| | 0.03 | - $Mg^{++}$ | 37 |
| | 0.1 | | 5.8 |
| | 1 | | 0.5 |
| | 5 | | 0 |
| Bovine pancreatic RNase | 0.001 | + $Mg^{++}$ | 47 |
| | 0.01 | | 1.1 |
| | 0.1 | | 0.1 |
| | 0.001 | - $Mg^{++}$ | 40 |
| | 0.01 | | 1.1 |
| | 0.1 | | 0.1 |

Each reaction mixture contained 250 μg of S-RNA (7.5 OD units at 260 mμ in 0.05 M NaOH), 50 μmoles cacodylate-Na buffer (pH 7.0), 5 μmoles magnesium acetate where indicated, and the amounts of RNase specified. The mixture was incubated for 20 min at 37°C. Added after the first incubation were: (1) 0.05 ml of crude amino acid charging enzyme preparation (150 μg of protein), (2) 0.1 ml ATP and $C^{14}$-leucine mixture which contained 0.5 μmoles ATP, and 0.2 μc $C^{14}$ uniformly labeled leucine (45.6 μc/μmoles), and (3) 5 μmoles magnesium acetate if it was not contained during the first incubation. The final volume was 0.5 ml. Of the reaction mixture, 0.1 ml was taken out at 5 min and put on filter paper disks. The filter paper disks were dipped into cold TCA, washed twice with cold TCA, ether-alcohol mixture (1:1), and ether successively. The radioactivity was measured as described previously (Mans and Novelli, 1961).

E. coli extract, which was obtained by using a French pressure cell, was treated with 1% streptomycin. The supernatant was fractionated by ammonium sulfate to collect the precipitate between 0.5 and 0.65 saturation.

Since the inhibition of hydrolysis by the two RNases was most pronounced with S-RNA as substrate in the presence of $Mg^{++}$, a possible explanation could be that $Mg^{++}$ causes a change in the physical configuration of S-RNA. In this connection it should be pointed out that complementary RNA becomes resistant to pancreatic RNase after it assumes a double-stranded structure (Geiduschek et al., 1962) and

enzymic degradation of S-RNA is slower than that of yeast RNA even in the absence of $Mg^{++}$ (Cantoni et al., 1962; Asano et al., 1961). Cantoni et al. (1962) suggested that this may be a consequence of a partially ordered secondary structure of S-RNA. Fig. 2 shows the striking shift of the melting curve of S-RNA to higher temperatures when heated in the presence of 0.01 M magnesium. It is of interest that the melting curve obtained in the presence of $Mg^{++}$ is not parallel to the original curve as that obtained by increasing salt concentration (Tissières, 1959). A shift in the melting curve by $Mg^{++}$ was also observed in the case of high molecular weight RNA, but in this case the new curve was parallel to the original curve. It is possible that $Mg^{++}$ may affect the physical state of S-RNA, causing it to assume a more rigid secondary structure and thereby rendering it more resistant to attack by RNases. While this work was in progress, Giacomoni and Spiegelman (1963) described a similar melting curve for S-RNA in the presence of 0.001 M $Mg^{++}$. In their case the $T_m$ was 70°C, whereas with 0.01 $Mg^{++}$ we obtained a value of about 75°C. Apparently the extent of the shift in melting curve is a function of the $Mg^{++}$ concentration.

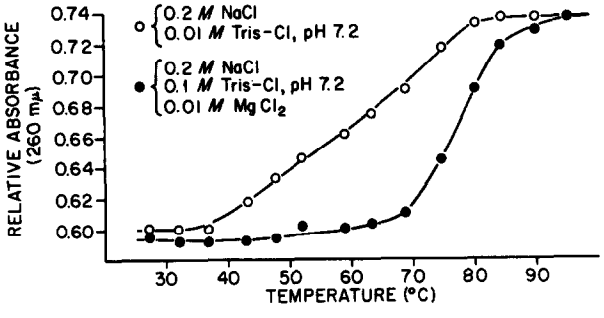


Figure 2. Dependence of thermal denaturation of E. coli S-RNA on the presence of magnesium cation.

We have also studied the loss of amino acid acceptor function of S-RNA for other amino acids such as arginine, aspartic acid, glutamic acid, serine, threonine, and tryptophan by B. subtilis RNase treatment. Even in the presence of $Mg^{++}$, S-RNA loses its acceptor function for

these amino acids more readily than for leucine, showing that resistance in the presence of $Mg^{++}$ varies with different amino acid S-RNA's.

Resistant leucine S-RNA molecules are indistinguishable from untreated leucine S-RNA by sucrose density gradient centrifugation, DEAE cellulose chromatography and gel filtration. The only difference thus far observed is a slight shift in the chromatographic profile on methylated albumin. This suggests that relatively small alterations can be made in leucine S-RNA without complete loss of acceptor activity.

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