Inhibition of Ribonuclease II of *Escherichia coli* by Sodium Ions, Adenosine-5’-Triphosphate, and Transfer Ribonucleic Acid

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Ribonuclease II action on polyuridylate is competitively inhibited by transfer ribonucleic acid and noncompetitively inhibited by sodium ions. At low substrate levels, adenosine-5’-triphosphate is also inhibitory.

Ribonuclease II is a highly active potassium-dependent exonuclease of *Escherichia coli* (12–14). In studies of ribonuclease II function, or of the synthesis of protein in extracts, the enzyme can be inactivated by using mutants with temperature-sensitive lesions in ribonuclease II (7); however, the heat treatment in extracts also tends to inactivate other components, e.g., initiation factor F3 (8). Here several auxiliary ways are reported to inhibit ribonuclease II. They have been useful in studies of the transition of precursor ribosomal ribonucleic acid (RNA) to 16S ribosomal RNA (1).

The source of ribonuclease II activity was *E. coli* strain D10 (5). Enzyme purified by the method of Spahr (fraction IV, reference 13) and unfractonated soluble enzymes (S100, reference 10) have been used. In addition, kinetic parameters were measured for the inhibitors under two conditions of possible usefulness: (i) low substrate to enzyme ratios and (ii) saturating levels of substrate.

In Fig. 1, the inhibition of ribonuclease II by stripped transfer RNA (tRNA) (General Biochemicals Corp., Chagrin Falls, Ohio) is reported by using either S100 freed of tRNA (6) and diluted to 0.17 µg of protein/ml or an equivalent amount of purified enzyme. Saturating levels of polyuridylate[poly (U)] (12) were used to give an assay which was linear for at least 15 min. A K, of about 750 µg/ml (about 3 x 10^{-4} M) was obtained. The inhibition is competitive, which is understandable, since ribonuclease II works in the 3’ to 5’ sense (11) and can remove the first several 5'-mononucleotides from the 3’-end of tRNA (reference 11 and E. Bruns and G. Phillips, personal communication). The K, for periodate-oxidized tRNA (prepared by the method described in reference 15) was lower than that of tRNA itself.

When undiluted S100 and low, nonsaturating concentrations of poly (U) were used and the ratio of enzyme to substrate was high, the assay was no longer linear with time, but comparable extents of inhibition were observed with tRNA and periodate-oxidized tRNA.

Noncompetitive inhibition by sodium ions of purified enzyme or enzyme in dilute S100 is shown in Fig. 2. A K, of about 0.03 M was found. In undiluted S100, with the attendant nonlinear assay conditions, inhibition by sodium ions was also observed.

Strong inhibition by adenosine-5’-triphosphate (ATP) was observed with purified ribonuclease II, dilute S100, or undiluted S100 when low substrate-enzyme ratios were employed, with either poly (U) or polyadenylate as substrate (Table 1). However, if the total poly (U) in a series of assay tubes was successively 0.025, 7, 14, and 29 µg/0.05 ml of assay, the corresponding inhibitions (Table 1) by 5 x 10^{-3} M ATP were 91, 49, -0.8, and -16%. The inhibition by ATP is therefore probably intrinsically competitive, in that it is overcome by high substrate levels, but is too weak to be effective in the presence of high substrate concentrations.

The enzyme that sediments with ribosomes in crude cell extracts (13), the same enzyme washed off ribosomes with 1 M NH₄Cl solutions, or purified ribonuclease II are all inhibited to comparable extents by ATP and sodium. However, in three trials, concentrations of tRNA sufficient to give 75% inhibition of soluble enzyme instead showed a 5 to 20% activation of the ribonuclease II activity bound to ribosomes. Thus, the enzyme may be in a different form in the presence of ribosomes.

It is not known whether inhibition of enzyme occurs in intact cells. Since the intracellular concentration of sodium ions is very low, inhibition...
NOTES

Fig. 1. Inhibition of ribonuclease II by tRNA or periodate-oxidized tRNA. The reaction mixture contained (in a final volume of 0.05 ml): 0.5 mM MgCl₂, 0.1 M KCl; 0.1 M tris(hydroxymethyl)aminomethane buffer, pH 7.5; 3H-labeled poly (U) (Miles Laboratories, 0.05 µg, specific activity 78.1 cpm/mmol P); unlabeled poly (U) (Miles Laboratories, in concentration as indicated below); different amounts of tRNA or periodate-oxidized tRNA; and the enzyme (2 units, reference 1). After incubation at 37°C for 15 min, the reaction was stopped by addition of 0.15 ml of cold ethanol and 0.01 ml of carrier albumin solution (15 mg/ml). The resulting suspension was mixed on a mechanical agitator and then kept on ice for 15 min. It was then centrifuged at 3,000 x g for 15 min; 0.1 ml of the clear supernatant fluid was counted in 10 ml of Bray’s solution. The Kᵣ was determined by the method of Dixon (2). (Panel A) S100 (soluble enzymes from an alumina-ground cell extract) without tRNA, prepared by the method described in reference 6, was used as a source of ribonuclease II activity (with 8 µg of protein per 50 µl of reaction mixture). Top curves, inhibition with tRNA in presence of 15 µg (●●●), or 10 µg (△△△) of unlabeled poly (U) in incubation mixtures. Bottom curves, inhibition with periodate-oxidized tRNA in presence of 15 µg (○○○) or 10 µg (●●●) of unlabeled poly (U). (Panel B) Purified ribonuclease II (3) was used at a level comparable to S100; inhibition with periodate-oxidized tRNA in presence of 10 µg (△△△) or 20 µg (○○○) of unlabeled poly (U) in each 0.05-ml reaction.

Fig. 2. Inhibition of ribonuclease II by sodium ions. Assay conditions as in Fig. 1, but, instead of adding tRNA, sodium was incubated at the indicated molarities. Purified ribonuclease II was used; inhibition in presence of 10 µg (●●●) or 20 µg (○○○) of unlabeled poly (U).

Table 1. Effect of adenosine-5′-triphosphate (ATP) concentration on ribonuclease II activity

<table>
<thead>
<tr>
<th>Source of activity</th>
<th>Percentage of inhibition</th>
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<tr>
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* Assay conditions as in Fig. 1, but, instead of transfer ribonucleic acid, ATP was included at the indicated molarities. Percentage of inhibition is given, compared to the activity of 2 units of ribonuclease II (purified enzyme or S100 soluble enzymes; reference 1) in 15-min incubations in the presence of 1 µg of polyuridylicate [poly (U)] per ml (“low substrate level”) or 150 µg of poly (U) per ml (“high substrate level”).

* In this series, the substrate was 3H-polyadenylate of the same specific activity and concentration as the poly (U). Identical inhibitions were observed with 10 mM Mg⁺² instead of 0.5 mM, so that the inhibition by ATP is not attributable to chelation of Mg⁺².
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LITERATURE CITED


