Purification and Characterization of the *Escherichia coli* Exoribonuclease RNase R

**COMPARISON WITH RNase II**

Zhuan-Fen Cheng and Murray P. Deutscher†‡

*From the Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, Florida 33101*

*Escherichia coli* RNase R, a 3′ → 5′ exoribonuclease homologous to RNase II, was overexpressed and purified to near homogeneity in its native untaged form by a rapid procedure. The purified enzyme was free of nucleic acid. It migrated upon gel filtration chromatography as a monomer with an apparent molecular mass of ~95 kDa, in close agreement with its expected size based on the sequence of the *rnr* gene. RNase R was most active at pH 7.5–9.5 in the presence of 0.1–0.5 mM Mg²⁺ and 50–300 mM KCl. The enzyme shares many catalytic properties with RNase IL. Both enzymes are nonspecific processive ribonucleases that release 5′-nucleotide monophosphates and leave a short undigested oligonucleotide core. However, whereas RNase R shortens RNA processively to di- and trinucleotides, RNase II becomes more distributive when the length of the substrate reaches ~10 nucleotides, and it leaves an undigested core of 3–5 nucleotides. Both enzymes work on substrates with a 3′-phosphate group. RNase R and RNase II are most active on synthetic homopolymers such as poly(A), but their substrate specificities differ. RNase II is more active on poly(A), whereas RNase R is much more active on RNAs. Neither RNase R nor RNase II can degrade a complete RNA-RNA or DNA-RNA hybrid or one with a 4-nucleotide 3′-RNA overhang. RNase R differs from RNase II in that it cannot digest DNA oligomers and is not inhibited by such molecules, suggesting that it does not bind DNA. Although the *in vivo* function of RNase R is not known, its ability to digest certain natural RNAs may explain why it is maintained in *E. coli* together with RNase II.

*Escherichia coli* contains eight distinct exoribonucleases that play important roles in every aspect of RNA metabolism (1, 2). One of these enzymes, now termed RNase R, was originally identified and partially purified and characterized as a nonspecific residual exoribonuclease present in strains lacking RNase II (3, 4). It was subsequently rediscovered in our laboratory as a nuclease active against rRNA and given the name RNase R (5, 6). The enzyme accounts for ~2% of poly(A)-degrading activity in crude extracts of cells lacking RNase I and RNase II (7). Partially purified RNase R is a 3′ → 5′ exoribonuclease that releases 5′-nucleoside monophosphates, and catalytically, it resembles RNase II (3, 4).

RNase R is encoded by the *rnr* gene located at 95 min on the *E. coli* chromosome (7). This gene was originally termed *vacB*; and in *Shigella* and in enteroinvasive *E. coli*, it is necessary for expression of virulence (8). In laboratory strains of *E. coli*, RNase R is dispensable for cell viability (7). Moreover, multiple mutant strains lacking RNases R and T, RNases R and PH, and RNases R, II, D, and BN grow essentially normally on rich media (7). On the other hand, a double mutant strain devoid of RNase R and polynucleotide phosphorylase is inviable (7). This observation suggests that RNase R and polynucleotide phosphorylase serve some overlapping essential function(s) in *E. coli* that cannot be satisfied by any of the other cellular exoribonucleases. Preliminary data indicate that at least one of these functions is an RNA quality control process that eliminates defective rRNA.

RNase R, together with RNase II, is a member of the RNR superfamily of exoribonucleases (1). As might be expected from their similarity in catalytic properties, RNase R and RNase II also share structural properties, including ~60% sequence homology. Interestingly, RNase R is even more widespread among eubacteria than is RNase II (1). In fact, RNase R is the only one of the eight known bacterial exoribonucleases to be present in *Mycoplasma* (1).

As part of our continuing efforts to determine the physiological role of RNase R, we have developed a simple procedure to purify the enzyme to near homogeneity. In this study, we describe the purification of RNase R and provide a detailed characterization of its structure, catalytic properties, and substrate specificity. In view of its high degree of similarity to RNase II, for many experiments, we have directly compared the properties of RNase R with those of a homogeneous preparation of RNase II (9).

**EXPERIMENTAL PROCEDURES**

*Materials—* Restriction endonucleases, T4 DNA ligase, T4 polynucleotide kinase, and the Klenow fragment of DNA polymerase I were obtained from New England Biolabs Inc. Calf intestine alkaline phosphatase was from Promega. The QIAEX II gel extraction kit was purchased from Qiagen Inc. [α-32P]ATP (6,000 Ci/mmol) were obtained from PerkinElmer Life Sciences. Polyvinylidene difluoride membranes were a product of Pall Corp. [3H]Poly(A) and Ultragel ACA44 were obtained from Amersham Biosciences. Poly(A), DNase I, and bacterial alkaline phosphatase (from *E. coli*) and its substrate *p*-nitrophenyl phosphate were from Sigma. Affi-Gel blue agarose (100–200 mesh) and protein size markers were obtained from Bio-Rad. SequaGel, used to make urea-polyacrylamide gels, was purchased from National Diagnostics, Inc. All other chemicals were reagent-grade. RNA oligomers were synthesized by Dharmacon Research.

1 Z.-F. Cheng and M. P. Deutscher, unpublished data.
Inc., and DNA oligomers were synthesized by the DNA Core Facility of our department. The RNA oligomers used for substrate specificity studies were 5'-CCCCACCCACCAUCACUU-3' (17-mer), its 17-mer complement, and the homolog-oligomers \( A_6, A_7, A_{10}, \) and \( A_{17} \). The DNA oligomers used were 5'-GATGGTGTTGAAAGG-3' (13-mer) and 5'-AAGTGATGGTGTTGAAAGG-3' (17-mer).

**Bacterial Strains and Plasmids**—The E. coli K12 strain CMA201 (\( \lambda \)resB01::tet) was a kind gift from Dr. C. Arriano (Centro de Tecnologia Química e Biológica, University of Lisbon, Lisbon, Portugal) (10). It was used to prepare the P1\( \mu r \) lysate for conversion of strain BL21(DE3)/pETR (Novagen) to an RNase II\( ^- \) strain by P1-mediated transduction. The resulting strain, BL21II (DE3)/pETR, was the host for plasmid pETR-dependent overexpression of RNase R. The RNase II\( ^- \) strain was employed to avoid problems in the assay of RNase R during purification due to the greater activity of RNase II on poly(A). Strain CA244-4 was used to prepare ribosomes and rRNAs.

Plasmid pETR was generated by insertion of a NaeI-Bcl fragment containing the \( rnr \) gene and a portion of the upstream yjeB gene at the NcoI (blunted)-BamHI site of plasmid pET15b (Novagen) (Fig. 1). During the cloning procedure, the His tag coding region of pET15b was removed, and the recombinant RNase R protein was not tagged.

**Overexpression of RNase R**—Strain BL21II (DE3)/pETR harboring pETR was grown to an \( A_60 = 1 \) with vigorous shaking at 37 °C in 4 liters of yeast-tryptone medium supplemented with 100 \( \mu \)g/ml ampicillin plus 34 \( \mu \)g/ml chloramphenicol to maintain the pETR plasmid. Isopropyl-\( \beta \)-D-thiogalactopyranoside was then added to a final concentration of 1 \( \mu \)M to induce RNase R expression, and 100 \( \mu \)g/ml chloramphenicol was added to maintain pETR. One h after adding isopropyl-\( \beta \)-D-thiogalactopyranoside, the culture was quickly cooled on ice, and cells were harvested by centrifugation. The RNA oligomers used for substrate specificity studies were 5'-CCCCACCCACCAUCACUU-3' (17-mer), its 17-mer complement, and the homolog-oligomers \( A_6, A_7, A_{10}, \) and \( A_{17} \). The DNA oligomers used were 5'-GATGGTGTTGAAAGG-3' (13-mer) and 5'-AAGTGATGGTGTTGAAAGG-3' (17-mer).

**Preparation of Cell Extracts**—To determine the amount of RNase R expressed from plasmid pETR, cells from the 5-ml portions of culture were resuspended in 30 ml of buffer containing 10 \( \mu \)g/ml Tris-Cl (pH 7.6), 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride. Extracts were prepared by sonication using two 15-s pulses. For RNase R purification, 12 \( \mu \)g of wet cells overexpressing RNase R were suspended in 60 ml of buffer A (10 mM Tris-Cl (pH 7.6), 1 mM dithiothreitol, 10 mM MgCl\(_2\), 0.1 mM phenylmethylsulfonyl fluoride, and 15 units/ml DNase I). Cells were ruptured by two passes through an Amino French press at 12,000 p.s.i. The suspension was centrifuged at 30,000 \( \times \)g for 2 h. The pellet obtained was resuspended in 30 ml of buffer B500 (10 mM Tris-Cl (pH 7.6), 1 mM dithiothreitol, 500 mM KCl, 0.5 mM EDTA (potassium salt; pH 7.4), 10% glycerol, and 0.1 mM phenylmethylsulfonyl fluoride). The sample was centrifuged at 30,000 \( \times \)g for 45 min and then at 150,000 \( \times \)g for 2 h to obtain an S150 supernatant fraction. All steps of the purification were carried out at 4 °C or below.

**SDS-PAGE of RNase R purification fractions.** Ten \( \mu \)g of protein from each fraction was denatured and loaded on an 8% SDS-polyacrylamide gel. After electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250. Lane 1, sonicated cell extract before induction; lane 2, sonicated cell extract after induction; lane 3, no-salt soluble fraction; lane 4, S150; lane 5, pooled Affi-Gel blue (AGB) peak fractions. The migration positions of molecular mass standards are shown on the left (in kDa). The position of RNase R is indicated by the arrow.
The cells (Table I).

Nine- (Fig. 2, lane 1; lane 2 with lane 1). Quantitatively, with poly(A) as substrate, an extract of cells collected after induction with 2 volumes of RNA loading buffer. The upper panel (marked R) presents the results of RNase R digestion, and the lower panel (marked II) presents the results of RNase II digestion.

Preparation of a Substrate with a 3'-Phosphate—An oligonucleotide substrate containing a 3'-phosphate terminus (A3P) was generated by periodate oxidation of A3.

RESULTS

Overexpression and Purification of RNase R—Upon isopropyl-β-d-thiogalactopyranoside induction of strain BL21II (DE3)/pLys harboring plasmid pETR, RNase R was overexpressed to an extent that it was the most abundant protein in the sonicated extract (Fig. 2, compare lane 2 with lane 1). Quantitatively, with poly(A) as substrate, an extract of cells collected after induction had a specific activity of 1,550 nmol/min/mg of protein, which was ~100-fold higher than that of an extract from uninduced cells.

RNase R was rapidly purified from the induced cell extract by taking advantage of its insolubility in low salt (4). Thus, most of the proteins in the extract were soluble in buffer A (no KCl), whereas RNase R was not detectable in this fraction (Fig. 2, lane 3). However, resuspension of the pellet in buffer B500 (containing 500 mM KCl) and recentrifugation revealed that RNase R was the major protein in the S150 supernatant fraction (Fig. 2, lane 4). These precipitation steps resulted in an ~5-fold purification of RNase R (Table I). A portion of the S150 fraction was further purified on an Affi-Gel blue column, leading to an additional ~2–3-fold purification (Table I). SDS-PAGE of the purified RNase R indicated that it was at least 95% pure (Fig. 2, lane 5). Based on this simple procedure, 7.5 mg of highly purified RNase R was obtained from the equivalent of 3 g of wet cells (Table I).

Spectral Analysis—Spectral analysis of purified RNase R in the range of 200–700 nm revealed no unusual peaks (data not shown). The A260/A280 ratio was 1.87, indicating that purified RNase R does not contain nucleic acid and therefore that RNase R does not require nucleic acid for activity.

Molecular Mass of RNase R—When gel filtration of purified RNase R was performed in buffer B500 (containing 500 mM KCl), RNase R eluted as a globular protein of ~95 kDa, in close agreement with its predicted size of 92 kDa based on the rnr gene sequence. This observation supports our previous suggestion that RNase R is a monomer (7). In contrast, when gel filtration was carried out in buffer A (lacking KCl), RNase R eluted in the void volume, indicating that it aggregates at low ionic strength, as originally observed by Kasai et al. (4) with partially purified enzyme. The fact that activity could be detected when the protein was aggregated suggests either that aggregation does not abolish RNase R activity or that the protein dissociates in the assay mixture.

N-terminal Sequencing of RNase R—To confirm that RNase R is encoded by the rnr gene and also to conclusively determine whether the two potential translation start sites is used, purified RNase R was subjected to N-terminal sequencing. The enzyme was resolved by 8% SDS-PAGE, followed by electroblotting onto a polyvinylidene difluoride membrane. Subsequent N-terminal analysis revealed the sequence SQDPFQE, in agreement with that predicted in the Swiss Protein Database, which is MSQDPFQE. These data indicate that 1) the rnr gene encodes RNase R; 2) the open reading frame predicted in the Swiss Protein Database is correct, but that the N-terminal formyl-Met residue is removed; and 3) the downstream AUG codon is the initiation site for RNase R synthesis.

Heat Stability of RNase R—To determine the response of RNase R to heat, its activity was measured at various temperatures. RNase II activity was measured at the same temperatures for comparison. Both RNase R and RNase II displayed similar temperature activity profiles, being most active at ~50 °C in a 5-min assay. RNase R stability at 50 °C was further examined by preincubating at that temperature for varying lengths of time and then assaying at 37 °C. The enzyme was relatively stable, losing ~25% of its activity in 15 min and ~80% over a 30-min period (data not shown).

Optimal Reaction Conditions for RNase R Activity on Poly(A)—Purified RNase R was assayed on poly(A) under a variety of conditions to assess its requirements for optimal activity. RNase II was assayed in parallel for comparison. Both RNase R and RNase II displayed a broad optimal pH range between 7.5 and 9.5. Both enzymes required a divalent cation, preferably Mg2+, for activity. In the presence of 1 mM EDTA, the activity of each enzyme was abolished. However, the optimal Mg2+ concentrations for the enzymes were quite different. RNase R was most active at 0.1–0.5 mM Mg2+, whereas RNase II was most active at 10 mM Mg2+. Both RNase R and RNase II were stimulated by the presence of a monovalent cation. RNase R was stimulated ~2-fold by 50–500 mM K+ and 40% more by Rb+. For RNase II, K+ was the most stimulatory of the monovalent cations tested, increasing activity ~5-fold over a similarly wide range of concentrations (data not shown).

Mechanism of Action of RNase R—Earlier work with partially purified enzyme indicated that RNase R is an exoribonuclease releasing 5'-nucleoside monophosphates (4). To en-

TABLE I

Summary of purification of RNase R

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Relative purification</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induced extract</td>
<td>mg</td>
<td>nmol/min</td>
<td>nmol/min/mg</td>
<td>fold</td>
<td>%</td>
</tr>
<tr>
<td>S150 fraction</td>
<td>220</td>
<td>346,060</td>
<td>1,550</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Affi-Gel blue peak fractions 7.5</td>
<td>35</td>
<td>276,850</td>
<td>7,910</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>147,900</td>
<td>19,720</td>
<td>13</td>
<td>40</td>
</tr>
</tbody>
</table>

Values based on assay of induced extract prepared by sonication.
TABLE II
Products of RNase R action

In Experiment 1, \[^{3}H\]poly(A) was digested with RNase R (0.15 \(\mu g\)) in a 50-\(\mu l\) reaction for 30 min as described under “Experimental Procedures.” The reaction mixture was heated at 70° C for 10 min to inactivate RNase R, followed by addition of 6 \(\mu l\) of 10× alkaline phosphatase buffer and 4 \(\mu l\) of 1 unit/\(\mu l\) calf intestine alkaline phosphatase. After incubation at 37° C for 30 min, the acid-soluble fraction was extracted four times with ether to remove trichloroacetic acid. The pH of the sample was adjusted to 7.0, and a portion of the sample was loaded onto a 1.2-ml Dowex AG 1-X2 column. Nucleosides were eluted with 6.2 ml of water (in seven fractions), and the nucleotide material (including any charged oligonucleotides) was eluted with 6.2 ml of 0.01 M HCl (also seven fractions). In Experiment 2, \(E.\ coli\) rRNA (40 \(\mu g\)) was treated with either 0.15 or 3.0 \(\mu g\) of RNase R for 10 min at 37° C in 50-\(\mu l\) reaction mixtures containing 20 mM glycine/KOH (pH 8.8), 100 mM KCl, and 0.25 mM MgCl\(_2\). The reaction mixture was heated at 70° C for 10 min, followed by addition of 60-\(\mu l\) tRNA nucleotidyltransferase reaction mixtures containing 80 mM glycine/KOH (pH 8.8), 10 mM MgCl\(_2\), 1 mM \[^{3}H\]ATP, and excess tRNA nucleotidyltransferase. After an additional incubation at 37° C for 20 min, acid-precipitable radioactivity was determined.

<table>
<thead>
<tr>
<th>After treatment with phosphatase</th>
<th>[^{3}H]AMP incorporation into tRNA</th>
<th>Amount due to RNase R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-soluble products</td>
<td>90%</td>
<td>10 pmol</td>
</tr>
<tr>
<td>Exp. 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-RNase R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+RNase R (0.15 (\mu g))</td>
<td>653</td>
<td>40 pmol</td>
</tr>
<tr>
<td>+RNase R (3 (\mu g))</td>
<td>1232</td>
<td>578 pmol</td>
</tr>
</tbody>
</table>

FIG. 4. Digestion of 16 S and 23 S rRNAs by RNase R and RNase II. Fifty \(\mu g\) of total RNA (purified from isolated ribosomes) was treated with 0.15 \(\mu g\) of RNase R or RNase II in 50-\(\mu l\) reactions under the conditions described under “Experimental Procedures,” except that KCl was present at 100 mM. Nine-\(\mu l\) aliquots were taken at the times indicated, and reaction products were resolved on a 1.1% agarose gel. The oligomer used to detect 16 S RNA was 5′-CTCTTGATCGTTGACTG-3′, and the oligomer used to detect 23 S RNA was 5′-CTTGTATCGCCTTGACTGCCA-3′. Quantitation was done on a PhosphorImager (Molecular Dynamics, Inc.).

Sure that the product released was not due to a secondary reaction by a contaminating activity, we re-examined the mechanism of action of RNase R using the purified enzyme. Thus, when 5′-\[^{32}P\]P-labeled RNA substrates (of varying length from oligomer to polymer) were digested by purified RNase R, intermediates with sizes between the starting material and final product were not observed to accumulate (Fig. 3 and data not shown). This observation is consistent with the conclusion that RNase R is a processive exoribonuclease. To confirm this point, the products of the reaction catalyzed by highly purified RNase R were examined.

If RNase R were an exoribonuclease, the major acid-soluble product with \[^{3}H\]poly(A) as substrate would be expected to be AMP. Upon treatment with alkaline phosphatase, this would be converted to the uncharged molecule, \[^{3}H\]adenosine, and would elute with the nucleoside fraction from an anion-exchange column (Dowex AG 1-X2). If, on the other hand, RNase R were an endoribonuclease, the acid-soluble oligonucleotides produced would remain charged after phosphatase treatment and would elute with the “nucleotide” fraction. As shown in Table II, 90% of the acid-soluble radioactivity was eluted from the anion-exchange column with water, consistent with its being the nucleoside, adenosine.

A second test of the mechanism of RNase R was its action on tRNA. Because tRNA is a relatively poor substrate of RNase R, we reasoned that RNase R might act on it in a distributive fashion such that tRNA-CCA might be generated due to removal of 1 AMP residue from tRNA to generate tRNA-CC, supporting the conclusion that RNase R is an exoribonuclease.

Substrate Specificity of RNase R—Important clues to the physiological role of RNase R may come from an analysis of its substrate specificity. Earlier work suggested that RNase R is a nonspecific enzyme able to act on homopolymers, rRNA, and mRNA (4, 7). Inasmuch as the previous studies of RNase R were carried out with only partially purified preparations, we felt that it was important to examine the specificity of the...

![Image](https://via.placeholder.com/150)
purified enzyme. We have used a greatly expanded catalogue of substrates and also compared its specificity with that of its homolog, RNase II.

The relative activities of RNase R and RNase II on poly(A) and the major classes of RNA are presented in Table III. RNase II was ~4-fold more active on poly(A) compared with RNase R, whereas RNase R was much more active on the natural RNAs. Both enzymes worked best on poly(A). The $K_m$ values for poly(A) in the RNase R reaction was ~30 nM. Among the natural RNAs, RNase R worked well on 23 S and 16 S RNAs, but relatively poorly on 5 S RNA and tRNA. Nevertheless, 5 S RNA and tRNA were potent inhibitors of RNase R action on poly(A) (data not shown). Fig. 4 shows that the acid-soluble material released from the 16 S and 23 S rRNA preparations actually led to the disappearance of the RNA bands and thus could not be due to degradation of a contaminating RNA. In separate experiments using gel analysis rather than acid solubility, degradation of homopolymers by RNase R was in the order poly(A) > poly(U) > poly(C) > poly(G) (data not shown). The latter polymer was essentially inactive as a substrate. The limit products of digestion of the poly(A), poly(U), and poly(C) homopolymers were di- and trinucleotides in each instance.

The activities of RNase R and RNase II on A oligonucleotides 6–17 nucleotides in length were also examined. Both RNase R and RNase II could hydrolyze RNAs as short as a 6-mer (A 6); the end products of digestion of the poly(A), poly(U), and poly(C) homopolymers by RNase R was in the order poly(A) > poly(U) > poly(C) > poly(G) (data not shown). The latter polymer was essentially inactive as a substrate. The limit products of digestion of the poly(A), poly(U), and poly(C) homopolymers were di- and trinucleotides in each instance.

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described by Kasai et al. (4). Moreover, the RNase R described here displayed a native molecular mass of ~95 kDa, as expected based on the sequence of the rrr gene (7). This contrasts with a mass of ~40–50 kDa reported by Kasai et al. (4) based on a comparison of the size of RNase R with that of RNase II. In some preparations of RNase R, we have also found a smaller active form of RNase R that consisted of the central region and the C-terminal S1 RNA-binding domain of the protein (1). Inasmuch as this form of the enzyme could be eliminated by inclusion of EDTA in the buffers during purification, the data are consistent with the idea that the C-terminal S1 RNA-binding domain of the protein (1). We thank Dr. Cecilia Arraiano for providing a plasmid.

Both RNase R and RNase II are members of the RNR superfamily of exoribonucleases (1). Structurally, both are large monomeric enzymes. Likewise, RNase R and RNase II have more than half of their residues in the central core. We have found that this core is somewhat larger with RNase R (di- and trinucleotides) than is observed with RNase II (tri- to pentanucleotides) (this work and Refs. 17 and 18). The inability of these enzymes as well as polynucleotide phosphodiesterase to act on small oligoribonucleotides is the apparent reason for the existence of an additional RNase in most cells (19). Another similarity in the properties of RNase R and RNase II is that both enzymes can act on 3′-phosphate-terminated RNA molecules, indicating that neither of them requires a free 3′-hydroxyl group to initiate degradation.

On the other hand, there are a number of significant differences in the actions of RNase R and RNase II. For example, the latter enzyme works relatively efficiently with DNA, and its action on RNA is strongly inhibited by DNA oligomers. In contrast, RNase R acts very poorly on DNA, and its activity is scarcely affected by the presence of a DNA oligomer. Based on this information, it is likely that RNase R binds relatively weakly to DNA. A second major difference between the two RNases is their substrate specificity. Although both enzymes work most effectively on synthetic homopolymers such as poly(A), RNase R also can degrade rRNAs quite well. However, RNase II is essentially inactive against such natural RNA substrates. Considering that rRNA molecules contain extensive secondary structure, these observations suggest that RNase R may be much more effective than RNase II in digesting through such structured regions. It is already known that RNase II slows down as it approaches within 10 nucleotides of a double-stranded RNA structure (20). The data presented here show that RNase R cannot degrade a completely double-stranded short RNA molecule or one with a 4-nucleotide 3′-overhang. Nevertheless, the fact that RNase R can digest both 16 S and 23 S rRNA molecules indicates that it is able to work through secondary structure in the context of the rRNA. How this is accomplished remains to be determined.

The in vivo role of RNase R is not yet known, but the data presented here indicate that the enzyme would be capable of acting on a variety of natural RNA molecules. Perhaps, its ability to act on structured RNAs is the reason why E. coli maintains this enzyme in addition to RNase II.

Acknowledgment—We thank Dr. Cecilia Arraiano for providing a strain.

REFERENCES