Substrate Recognition and Catalysis by the Exoribonuclease RNase R*

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RNase R is a processive, 3′ to 5′ hydrolytic exoribonuclease that together with polynucleotide phosphorylase plays an important role in the degradation of structured RNAs. However, RNase R differs from other exoribonucleases in that it can by itself degrade RNAs with extensive secondary structure provided that a single-stranded 3′ overhang is present. Using a variety of specifically designed substrates, we show here that a 3′ overhang of at least 7 nucleotides is required for tight binding and activity, whereas optimal binding and activity are achieved when the overhang is 10 or more nucleotides in length. In contrast, duplex RNAs with no overhang or with a 4-nucleotide overhang bind extremely poorly to RNase R and are inactive as substrates. A duplex RNA with a 10-nucleotide 5′ overhang also is not a substrate. Interestingly, this molecule is bound only weakly, indicating that RNase R does not simply recognize single-stranded RNA, but the RNA must thread into the enzyme with 3′ to 5′ polarity. We also show that ribose moieties are required for recognition of the substrate as a whole since RNase R is unable to bind or degrade single-stranded DNA. However, RNA molecules with deoxyribose or dideoxyribose residues at their 3′ termini can be bound and degraded. Based on these data and a homology model of RNase R, derived from the structure of the closely related enzyme, RNase II, we present a model for how RNase R interacts with its substrates and degrades RNA.

The action of ribonucleases (RNases) is central to RNA metabolic processes such as the maturation of RNA precursors, the end-turnover of RNAs, and the degradation of RNAs that are either defective or are no longer required by the cell. Complete degradation of an RNA typically requires endoribonucleolytic cleavages followed by the action of a nonspecific 3′ to 5′ processive exoribonuclease (1). In Escherichia coli there are three such exoribonucleases: RNase II, RNase R, and polynucleotide phosphorylase (PNPase). RNase II and PNPase were originally thought to be responsible for mRNA decay (2). However, recent work has shown that mRNAs with extensive secondary structure, such as those containing repetitive extragenic palindromic sequences, are degraded by PNPase or by RNase R (3). Likewise, PNPase and/or RNase R is required for the degradation of rRNA (4) and tRNA (5), both of which are highly structured molecules. These data suggest that PNPase and RNase R are the universal degraders of structured RNAs in vivo.

Despite its role in the degradation of structured RNAs, purified PNPase is unable to digest through extensive secondary structure and stalls 6−8 nucleotides (nt) before a stable RNA duplex (3). However, in vivo PNPase associates with an RNA helicase, RhlB, in the degradosome, which also contains the endoribonuclease, RNase E, and the glycolytic enzyme, enolase (6). A direct interaction with RhlB in the form of an αβ complex has also been reported (7, 8). It is likely to be the association with this helicase that allows PNPase to degrade through structured RNAs.

In contrast, purified RNase R readily degrades structured RNAs, such as rRNA (9). Intermediate degradation products are not detected for such substrates, indicating that RNase R action is highly processive even on molecules with extensive secondary structure (9). Moreover, a GC-rich 17-bp duplex with an extended single-stranded 3′ overhang of 17 nt is also completely degraded, resulting in limit products of di- and trinucleotides (3). RNase R is the only known exoribonuclease able to degrade through such extensive duplex RNA without the aid of a helicase activity. For this reason its mechanism of action is of great interest. Furthermore, RNase R from Bacillus subtilis is also able to degrade structured RNAs (10), suggesting that this function may be conserved among all RNase R homologues.

A first step in elucidating the mechanism of RNase R is to understand how it recognizes its substrates. In this study we define the substrate specificity of RNase R and provide an explanation for how it interacts with and degrades its substrates. Because the physiological role of RNase R appears to be the degradation of RNAs with significant secondary structure (3−5), our studies focused on such substrates. We show that a single-stranded overhang, which must be 3′ to the duplex, is required for tight binding and for subsequent degradation by RNase R. The poor binding and lack of activity on a molecule with a 5′ overhang indicates that the substrate must thread into the enzyme in a directional manner. We also demonstrate that recognition of the substrate as a whole requires ribose moieties, because RNase R is unable to bind or degrade a DNA substrate efficiently. Interestingly, RNAs that are 3′-phosphorylated or that contain terminal 3′ deoxyribose or dideoxyribose residues

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The abbreviations used are: RNase, ribonuclease; PNPase, polynucleotide phosphorylase; nt, nucleotide(s); Pcf, cytidine 3′,5′-bis(phosphate); DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride.

5 S. Chebolu, C. Kim, and M. P. Deutscher, unpublished observations.
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are effectively bound and degraded by RNase R, although such modifications cause a reduction in the rate of degradation. Although the structure of the 3′ terminal nucleotide can influence the initiation of degradation, cleavage nevertheless occurs exclusively at the 3′ terminal phosphodiester bond. The data obtained from these studies suggest that substrate binding plays a critical role in RNase R action and that tight binding and catalysis requires that the substrate interact with a site at the base of a channel accessible only to single-stranded RNA.

EXPERIMENTAL PROCEDURES

Materials—RNA oligonucleotides were synthesized by Dharmacon, Inc. The sequences of the oligoribonucleotides used are presented in Table 1. T4 polynucleotide kinase, T4 RNA ligase, and DNase I were purchased from New England Biolabs, Inc. [γ-32P]ATP and 5′-[32P]pppCp were from PerkinElmer Life Sciences. SequaGel for denaturing urea-polyacrylamide gels was from National Diagnostics. Nitrocellulose and Biodyne Plus nylon membranes were obtained from Pall Corp, and DE81 column from Amersham Biosciences, and Affi-Gel Blue-agarose (100–200 mesh) was purchased from Bio-Rad. All other chemicals were reagent grade.

Overexpression of RNase R—Strain BL21(DE3) II R− harboring the plasmids pLySs and pET44R (which has the full-length RNase R gene inserted into the NdeI and BamHI sites) was grown at 37 °C to an A600 of 0.6 in 1 liter of yeast-Tryptone medium supplemented with 100 μg/ml ampicillin, 34 μg/ml chloramphenicol, 25 μg/ml kanamycin, and 10 μg/ml tetracycline. Expression was induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside, and the culture was incubated for an additional 3 h at 37 °C. Cells were harvested by centrifugation at 10,000 × g for 10 min at 4 °C, and the resulting cell pellet was stored at −80 °C.

Purification of RNase R—The frozen cell pellet was thawed on ice and resuspended in 3 ml of 20 mM HEPES (pH 7.5), 500 mM KCl, 0.5 mM EDTA, 1 mM DTT, 0.1 mM PMSF, and 5 units of DNase I/g wet weight of cells. Cells were disrupted by three passes through an Amnicco French press at 20,000 p.s.i. The lysate was centrifuged at 150,000 × g for 2 h at 4 °C. The resulting S150 fraction was applied to an Affi-Gel Blue column, and the column was washed with two column volumes of 20 mM HEPES (pH 7.5), 500 mM KCl, 0.5 mM EDTA, 1 mM DTT, and 0.1 mM PMSF. RNase R was eluted from the column with 20 mM HEPES (pH 7.5), 1 mM KCl, 0.5 mM EDTA, 1 mM DTT, and 0.1 mM PMSF. The fractions containing RNase R were diluted to 300 mM KCl with 20 mM HEPES (pH 7.5), 0.5 mM EDTA, 1 mM DTT, and 0.1 mM PMSF and applied to a Mono S HR 10/10 column using an AKTA fast protein liquid chromatography system (Amersham Biosciences). RNase R was eluted at 450–500 mM KCl upon application of a linear gradient from 300 mM to 1 M KCl in a solution containing 20 mM HEPES (pH 7.5), 0.5 mM EDTA, 1 mM DTT, 0.1 mM PMSF, and 10% glycerol. Fractions containing RNase R were pooled, separated into aliquots, and stored frozen at −80 °C.

Substrate Preparation—Oligoribonucleotide substrates were deprotected according to the manufacturer’s instructions. Unless stated otherwise, single-stranded oligoribonucleotide substrates were 5′-labeled with 32P using T4 polynucleotide kinase and [γ-32P]ATP. Substrates containing a duplex were prepared by mixing a 5′-32P-labeled oligoribonucleotide with the nonradioactive complementary oligoribonucleotide in a 1:1.2 molar ratio in the presence of 10 mM Tris-HCl (pH 8.0) and 20 mM KCl and heating the mixture in a boiling water bath for 5 min and then allowing the solution to cool slowly to room temperature.

RNase R Activity Assays—Assays were typically carried out in 50-μl reaction mixtures containing 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.25 mM MgCl2, and 1 mM DTT. Purified RNase R and substrate concentrations were as indicated. Reaction mixtures were incubated at 37 °C for the indicated times, and reactions were terminated with 2 volumes of gel loading buffer (95% formamide, 20 mM EDTA, 0.025% bromphenol blue, and 0.025% xylene cyanol). Reaction products were resolved on denaturing 7.5 M urea, 20% polyacrylamide gels and visualized using a PhosphorImager (GE Healthcare). Quantification was carried out using ImageJ software (NIH).

Filter Binding Assays—The double-filter nucleic acid binding assay developed by Wong and Lohman (11) and adapted by Tanaka and Schwer (12) was used. Briefly, nitrocellulose membranes were presoaked in 0.5 M KOH for 10 min and then rinsed in H2O until the pH returned to neutral. Biodyne Plus nylon membranes were washed once in 0.1 M EDTA (pH 8.8) for 10 min, 3 times in 1 M KCl for 10 min each followed by rinsing in 0.5 M KOH for 1 min, and finally rinsed with H2O until the pH returned to neutral. Nitrocellulose and nylon membranes were equilibrated in binding buffer (20 mM Tris-HCl (pH 8.0), 100 mM KCl, 1 mM EDTA, 10 mM DTT, and 10% glycerol) at 4 °C for at least 1 h before use. Fifty-μl reaction mixtures containing 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 1 mM EDTA, 10 mM DTT, 10% glycerol, 200 pm 32P-labeled RNA substrate, and varying amounts of RNase R were incubated on ice for 30 min. No degradation of the RNA occurs in the absence of Mg2+. A 96-well dot-blot apparatus (Bio-Rad) was assembled so that the nylon membrane was placed underneath the nitrocellulose membrane. Each well was washed with 100 μl of ice-cold binding buffer just before loading the sample and immediately after sample application. The apparatus was disassembled, and the membranes were allowed to air dry and were visualized using a PhosphorImager (GE Healthcare). Quantification was carried out using ImageJ software (NIH), and the Kd was determined using nonlinear regression analysis in GraFit 4 software (Eratistics software).

Paper Chromatography—Samples were applied to DE81 ion-exchange chromatography paper and resolved using 0.6 M ammonium formate (pH 3.1). The paper was allowed to air dry and was visualized using a PhosphorImager (GE Healthcare).

RESULTS

Length Requirement of 3′ Overhang—Earlier work indicated that the physiological role of RNase R is the degradation of RNAs that contain significant secondary structure (3–5). Consistent with such a role, RNase R is able to degrade through a 17-bp duplex with a 17-nt single-stranded extension 3′ to the duplex (3, 9). However, the same duplex, lacking a 3′ overhang or with only a 4-nt overhang, was inactive as a substrate (3, 9). To better understand the role of the 3′ overhang and to deter-
mine what structural RNA properties are necessary for RNase R to degrade through duplex RNA, a variety of molecules were prepared and examined as substrates (Table 1).

Activity assays in which reaction products are analyzed by denaturing PAGE were used to compare the action of RNase R on a 17-bp duplex with no overhang or with 4-, 7-, 10-, 13-, or 17-nt overhang (see ss 17 sequence). All the substrates, with the exception of the homopolymers and the complementary ss 17, share a common 17-nucleotide region (see ss 17 sequence) corresponding to the 3′ terminus of tRNA 1/11032,[3]/H11032. This sequence was chosen to enable comparison with previous studies. The complementary ss 17 is the reverse complement of this sequence. Substrates containing a duplex region were created by annealing one of the single strands containing this 17-nucleotide sequence with the complementary ss 17 as described under "Experimental Procedures." dU is 2′ deoxyuridine; ddC is 2′,3′ dideoxycytidine.

**TABLE 1**

**Synthetic oligoribonucleotide substrates used in this study**

All the substrates, with the exception of the homopolymers and the complementary single strand (ss) 17, share a common 17-nucleotide region (see ss 17 sequence) corresponding to the 3′ terminus of tRNA 1/11032,[3]/H11032. This sequence was chosen to enable comparison with previous studies. The complementary ss 17 is the reverse complement of this sequence. Substrates containing a duplex region were created by annealing one of the single strands containing this 17-nucleotide sequence with the complementary ss 17 as described under "Experimental Procedures." dU is 2′ deoxyuridine; ddC is 2′,3′ dideoxycytidine.

| Single-stranded substrates | 5′-CCCCACCACCAUCAUCCC-3′ |
|----------------------------|-----------------------------|
| ss 17                      | 5′-CCCCACCACCAUCAUCCC-3′    |
| ss 17-A4                   | 5′-CCCCACCACCAUCAUAAAAAAAA-3′ |
| ss 17-A7                   | 5′-CCCCACCACCAUCAUAAAAAAAAA-3′ |
| ss 17-A10                  | 5′-CCCCACCACCAUCAUAAAAAAAAAA-3′ |
| ss 17-A13                  | 5′-CCCCACCACCAUCAUAAAAAAAAAAA-3′ |
| ss 17-A17                  | 5′-CCCCACCACCAUCAUAAAAAAAAAAA-3′ |
| Complementary ss 17        | 5′-AAGUAGUAGUAGUAGUAGUAG-3′ |
| ss A17                     | 5′-AAAAAAAAAAAAAAAAAAAAAA-3′ |
| ss G17                     | 5′-CCCCCCCCCCCCCCCCCCCCC-3′ |
| ss U12                     | 5′-UUUUUUUUUUUUUUUUUUUU-3′ |
| ss U17                     | 5′-UUUUUUUUUUUUUUUUUUUU-3′ |
| ss A17-C10                 | 5′-CCCCACCAUCUCAUCCCCCCCCCCCCCCC-3′ |
| ss U17-2′                  | 5′-CCCCACCAUCUCAUCCCCCCCCCCCCC-3′ |
| 5′ A10 ss 17               | 5′-AAAAAAAAAAAAAAAAAAAAAAA-3′ |
| ss 16-dU                   | 5′-CCCCACCACCAUCAUCAUCC(dU) 3′ |
| ss 17-ddC                  | 5′-CCCCACCACCAUCAUCC(3′dC) 3′ |

| Duplex-containing substrates | 3′-GUCGCGCGCGGUGGUAGUGAA-5′ |
|-----------------------------|-------------------------------|
| ds 17                       | 3′-GUCGCGCGCGGUGGUAGUGAA-5′ |
| ds 17-A4                    | 3′-GUCGCGCGCGGUGGUAGUGAA-5′ |
| ds 17-A7                    | 3′-GUCGCGCGCGGUGGUAGUGAA-5′ |
| ds 17-A10                   | 3′-GUCGCGCGCGGUGGUAGUGAA-5′ |
| ds 17-A13                   | 3′-GUCGCGCGCGGUGGUAGUGAA-5′ |
| ds 17-A17                   | 3′-GUCGCGCGCGGUGGUAGUGAA-5′ |
| ds 17-C10                   | 3′-GUCGCGCGCGGUGGUAGUGAA-5′ |
| ds 17-U10                   | 3′-GUCGCGCGCGGUGGUAGUGAA-5′ |
| 5′ A10 ds 17                | 3′-GUCGCGCGCGGUGGUAGUGAA-5′ |

**FIGURE 1.** **Length of overhang required for RNase R Action.** Reactions were carried out as described in "Experimental Procedures" with the addition of 1.3 μg of RNase R and 10 μM 32P-labeled duplex substrate with different lengths of 3′ overhang as shown. Aliquots of 3 μl were taken at 0, 5, 15, 30, 60, and 120 min, as indicated. The weaker bands that migrate more slowly than the substrate band represent a small amount of duplex RNA substrate that was not denatured by formamide in the gel-loading buffer or by the heat treatment. The small amount of product that accumulates for the substrates with either no overhang or with a 4-nt overhang likely originates from the smaller contaminants in the starting material and not from the substrate being tested.
by the addition of a complementary 17-mer behaved differently. Duplex RNAs with no overhang or with only a 4-nt 3’ overhang bound with a $K_d$ greater than 5 $\mu$M (Table 2), indicating that RNase R binds very poorly to these molecules and explaining why they are inactive as substrates. However, increasing the length of the 3’ overhang from 4 to 7 nt resulted in a dramatic decrease in $K_d$ value (>200-fold). These data suggest that the additional 3 nt simply enable RNase R to make more contacts with the substrate or, alternatively, that they enable the substrate to reach a tight binding region within the active site of RNase R that cannot be accessed by molecules with shorter overhangs. Increasing the length of the overhang further from 7 to 10, 13, or 17 nt resulted in only a minor additional decrease in $K_d$, indicating that 7 nt are sufficient for nearly maximal binding. These data show that a single-stranded 3’ overhang of at least 7 nt is required for RNase R to bind to a substrate and to subsequently degrade it.

It should be noted that substrates with a 3’ overhang 10 nt or more in length bound with $K_d$ values that were apparently 4–5-fold higher than their completely single-stranded counterparts. This was likely due to the small excess of nonlabeled, complementary strand that was added to ensure that all of the substrate was present in duplex form. The excess complementary strand would be expected to act as a competitor, raising the apparent $K_d$ of the duplex substrate.

Sequence Composition of the 3’ Overhang—Although RNase R is a nonspecific exoribonuclease, earlier work indicated that it degrades different homopolymers at somewhat different rates (9). To determine whether RNase R also displays sequence specificity for the 3’ overhang, 17-bp duplexes with 10-nt overhangs of different nucleotide composition were tested as substrates. Overhangs contained either poly(A), poly(C), or poly(U). A poly(G) sequence was not tested because, as noted earlier, it is an extremely poor substrate (9). It is clear from Fig. 2 that a duplex carrying the A10 sequence was preferred, having a degradation rate of 14 pmol min$^{-1}$ $\mu$g$^{-1}$ RNase R compared with 7 pmol min$^{-1}$ $\mu$g$^{-1}$ for U10 and 4 pmol min$^{-1}$ $\mu$g$^{-1}$ for C10. These data follow the order observed for single-stranded homopolymers (9) and indicate that the presence of an attached duplex does not alter the sequence preference of the enzyme.

$K_d$ values of the single-stranded homopolymers and of molecules with homopolymer 3’ overhangs were also determined using the filter binding assay. As can be seen from Table 3, A17 and U17 are bound very tightly by RNase R, with $K_d$ values of ~1 nM, whereas C17 and G17 are bound somewhat more weakly with $K_d$ values in the range of ~15 nM. A similar trend was observed for the longer single-stranded substrates and for the substrates containing a 17-bp duplex with a 10-nt 3’ overhang of poly(A), poly(U), or poly(C). Based on these data it appears that the sequence of an RNA molecule does affect the intrinsic affinity with which RNase R is able to bind to it. The basis of these differences is not yet understood. It should be noted that the binding data do not explain the differences in degradation rates because activity assays were performed at substrate concentrations of 10 $\mu$M, much higher than the $K_d$ values for each of the substrates tested. Thus, the observed differences in activity reflect actual differences in degradation of homopolymers by RNase R.

Position Requirements of the Overhang—Inasmuch as RNase R is a 3’ to 5’ processive exoribonuclease, it would be expected that a single-stranded overhang would only be degraded if it
was located 3’ to a duplex. However, the possibility remained that a 5’ overhang could serve as a binding site for RNase R. Once bound, RNase R could loop around to the 3’ terminus of the same strand and initiate degradation. Alternatively, binding to a 5’ overhang might place RNase R near the 3’ end of the complementary strand of the duplex and thereby allow it to initiate degradation on that strand. To test these possibilities a 17-bp duplex with a 10-nt 5’ overhang was used as a substrate. Each strand of the substrate was separately 5’-32P-labeled, and degradation of each strand was monitored. However, neither strand served as a substrate for RNase R (data not shown). Thus, RNase R can act only on single-stranded overhangs at the 3’ terminus of a duplex RNA.

Most importantly, RNase R also binds very poorly to a molecule with a 5’ overhang. As shown in Table 4, the observed $K_d$ for such a molecule is greater than $1 \mu M$ compared with less than 10 nM for the corresponding molecule with a 3’ overhang. There was no difference in binding of the corresponding single-stranded substrate. These data indicate that RNase R does not simply bind to any region of single-stranded RNA. Rather, there is directionality to the process, implying that the 3’ overhang actually threads into the enzyme. Such threading cannot be accomplished with the opposite polarity of the 5’ overhang. These data also indicate that the dramatically enhanced binding of a molecule with a 7-nt 3’ overhang compared with one with a 4-nt overhang is likely due to its ability to access a tight binding site deep within the enzyme that cannot be reached by the molecule with a shorter overhang (see the model in Fig. 4).

**Requirement for Ribose Moieties**—Previous work showed that RNase R is essentially inactive on a DNA molecule (dT17) and acts only distributively when present in amounts 20-fold greater than those needed to degrade the corresponding RNA (9). Its distributive action suggests that RNase R is unable to bind sufficiently tightly to DNA. To directly examine this point, filter binding was used to determine the $K_d$ for single-stranded DNA. As shown in Table 5, the $K_d$ for DNA was found to be more than 5 $\mu M$, at least 103-fold greater than for the corresponding RNA. These data show that RNase R binds to DNA extremely poorly. The poor binding also explains why DNA is a poor substrate (Table 5 and Ref. 9). The inability to bind DNA suggests that ribose 2’ hydroxyl groups are an important recognition element for RNA binding to RNase R.

However, when DNA was incorporated as the complementary strand in a substrate with a 17-bp DNA-RNA heteroduplex and a 17-nt 3’ overhang, the RNA strand was degraded as rapidly as for an RNA-RNA duplex (data not shown). This indicates that the complementary strand does not play a role in substrate specificity.

**DISCUSSION**

This work provides important new information regarding substrate binding and catalysis by the exoribonuclease RNase R. Using a variety of specifically designed substrates, we have gained insights into how RNase R interacts with its substrates...
and to the determinants important for the recognition of those substrates. Thus, we found that a single-stranded 3’ overhang of 7 nt is sufficient for RNase R to bind and degrade through duplex RNA, whereas an overhang of 10 nt or more results in maximal rates of degradation. In addition, the dramatic increase in binding and activity observed when the overhang length was increased from 4 to 7 nt together with the finding that binding occurs only with 3’ overhangs revealed that RNase R needs to bind tightly to an RNA molecule to degrade it and that this tight binding site apparently lies deep within the active site of the enzyme, where it is inaccessible to a molecule with only a 4-nt overhang.

The crystal structure of RNase II, a paralogue of RNase R (14), recently was solved, and based on this structure, homology modeling was used to predict a structure for RNase R. In this structure a funnel-like RNA binding region formed by the N-terminal cold shock domains, and the C-terminal S1 domain sits above a narrow channel that has the catalytic center at its base. A, a substrate with a 4-nt single-stranded 3’ overhang is unable to reach the tight binding site or the catalytic center. B, a substrate with a 7-nt 3’ overhang is able to access the base of the channel where it is tightly bound and the terminal phosphodiester bond is positioned at the catalytic center. Cleaved nucleotides are thought to exit through an exit channel located to the side of the catalytic center.

As might be expected for a 3’ to 5’ processive exoribonuclease, RNase R is inactive on molecules with a single-stranded overhang 5’ to a duplex. Most importantly, however, is the finding that RNase R also binds poorly to such molecules, as the \( K_d \) for a 5’ overhang is several orders of magnitude higher than that for a 3’ overhang. This observation indicates that RNase R does not simply “sit down” on single-stranded RNA but, rather, that the RNA must thread into the enzyme in a specific direction, 3’ to 5’. Based on the modeled structure of RNase R (Fig. 4), we propose that this threading is through the funnel-like structure and into the active site channel. Inasmuch as duplex RNA cannot enter the channel, for catalysis and tight binding to occur, the 3’ single-stranded overhang must be of sufficient length, \( \geq 7 \) nt, to reach the catalytic center where phosphodiester bond cleavage takes place (Fig. 4).

In addition to sufficient length, a second important structural determinant for tight binding and catalysis is that the nucleic acid within the channel be RNA. DNA binds poorly and is an extremely poor substrate. Although it may access the

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channel, it presumably does not remain there long enough for catalysis to occur. These data suggest that amino acid residues within the channel directly contact the 2’ hydroxyl groups of the RNA to help bind and orient it for catalysis to occur. Interestingly, the 3’ terminal residue itself appears to be less important as substrates that are 3’-phosphorylated or have a 3’ deoxyribose or 3’ dideoxyribose are bound and readily degraded by RNase R, albeit at somewhat slower rates. Similar results have also been observed for RNase II with substrates containing different 3’ termini (15). However, contacts between a PNPase homologue and the 2’ and 3’ hydroxyl groups of the terminal nucleotide have been reported in the structure of the archaeal exosome (16). Specifically, the contact to the 2’ hydroxyl has been proposed to be critical for the discrimination between RNA and DNA substrates (16). Thus, for RNase R, although interaction with ribose 2’ hydroxyl groups is important, interaction with the 3’ terminal sugar appears not to occur. Nevertheless, there must be correct anchoring of the 3’-terminal nucleotide as RNase R initiates degradation solely at the 3’-terminal phosphodiester bond. Perhaps the penultimate nucleotide residue also plays a role.

RNase R can be distinguished from other exoribonucleases by its ability to degrade structured RNAs without the aid of a helicase activity. The work presented here provides a necessary first step in understanding how RNase R accomplishes this task. In particular, the specificity of this unusual enzyme with regard to binding and catalysis coupled with the structural model that explains these data afford important insights into the RNase R mechanism of action. We anticipate that further studies with this interesting enzyme will explain its unique catalytic properties.

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