Traynor-Kaplan, A. E., Harris, A. L., Thompson, B. L., Taylor, P., & Sklar, L. A. (1988) *Nature 334*, 353–356.
Traynor-Kaplan, A. E., Thompson, B. L., Harris, A. L., Taylor, P., Omann, G. M., & Sklar, L. A. (1989) *J. Biol. Chem. 264*, 15668–15673.
Urumow, T., & Wieland, O. H. (1986) *FEBS Lett. 207*, 253–257.
Van Dongen, C., Zwiers, H., & Gispen, W. (1984) *Biochem. J. 223*, 197–203.
Varticovski, L., Druker, B., Morrison, P., Cantley, L., & Roberts, T. (1989) *Nature 342*, 699–702.
Vogel, S., & Hoppe, J. (1986) *Eur. J. Biochem. 154*, 253–257.
Walker, D., & Pike, L. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7513–7517.
Walker, D., Dougherty, N., & Pike, L. J. (1988) *Biochemistry 27*, 6504–6511.
Whitman, M., Kaplan, D. R., Schaffhausen, B., Cantley, L., & Roberts, T. M. (1985) *Nature 315*, 239–242.
Whitman, M., Kaplan, D., Roberts, T., & Cantley, L. (1987) *Biochem. J. 247*, 165–174.
Whitman, M., Downes, C. P., Keeler, M., Keller, t., & Cantley, L. (1988) *Nature 332*, 644–646.
Yamakawa, A., & Takenawa, T. (1988) *J. Biol. Chem. 263*, 17555–17560.
Yamamoto, K., & Lapetina, E. (1990) *Biochem. Biophys. Res. Commun. 168*, 466–472.
Yamamoto, K., Graziani, A., Carpenter, C., Cantley, L., & Lapetina, E. (1990) *J. Biol. Chem.* (in press).

Accelerated Publications

Mixed DNA/RNA Polymers Are Cleaved by the Hammerhead Ribozyme†

Jing-hua Yang,‡ Jean-Pierre Perreault,‡ Damian Labuda,§ Nassim Usman,∥ and Robert Cedergren*,‡

Département de biochimie and Centre de recherche de l'Hôpital Ste-Justine, Université de Montréal, Montréal, Québec H3C 3J7, Canada, and Biology Department, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received September 6, 1990; Revised Manuscript Received October 23, 1990

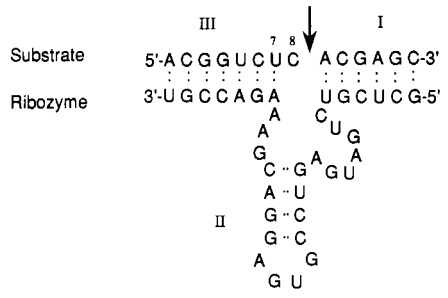
ABSTRACT: A series of chemically synthesized oligodeoxyribonucleotides containing one or two ribonucleotides (DNA/RNA mixed polymers) at and/or adjacent to the cleavage site of the substrate can be cleaved by the "hammerhead" ribozyme. In comparison with the all-RNA substrate, the predominantly deoxyribonucleotide substrates have (1) lower optimal temperatures of cleavage, (2) approximately 6-fold higher K_m's and 7-fold lower k_{cat}'s at 30 °C, and (3) 15-fold higher K_m's and 8-fold lower k_{cat}'s at 37 °C. The extent to which the RNA substrate cleavage is inhibited in the presence of an all-DNA (K_I = 13 μM) and an RNA substrate analogue with a dC at the cleavage site (K_I = 0.96 μM) supports the contention that the formation of the ribozyme–substrate complex with the predominantly deoxyribonucleotide substrates (D substrates) is impaired. The weaker binding of D substrates was confirmed by thermal denaturation and determination of the T_m of the complex. Analysis of the kinetic data also suggests that the conformation of the catalytic core of the ribozyme–substrate complex differs from that of the all-RNA complex, a change that results from the presence of a DNA/RNA heteroduplex in the complex.

Self-cleavage of RNA has been demonstrated to play an important biological role in the processing of some RNAs, i.e., in plant virus satellite, viroid, virusoid, hepatitis δ virus, and newt satellite RNAs (Forster et al., 1988; Talbot & Bruening, 1988; Epstein & Gall, 1987). Unlike self-splicing and RNase P catalysis, RNA self-cleavage leads to 2′,3′-cyclic phosphate and 5′-hydroxyl products and, with the exception of the hepatitis virus in the above list, is associated with a consensus secondary structure and 13 consensus nucleotides called the hammerhead domain (Uhlenbeck, 1987; Gerlach & Haseloff, 1988; Koizumi et al., 1988; Forster et al., 1988; Jeffries & Symons, 1989). This structural domain can be separated into two RNA fragments: an enzymatic fragment (the ribozyme) and a substrate fragment as shown in Figure 1. The structural model consists of three RNA double helices which delimit the consensus nucleotides CUGAXGA (X can be any nucleotide) and GAAAC in the ribozyme and GUC in the substrate, many of which have been the object of nucleotide replacement studies in order to obtain information on their role in the cleavage mechanism (Koizumi et al., 1988; Sheldon & Symons, 1989a; Sampson et al., 1987).

We have recently introduced the use of chemically synthesized DNA/RNA mixed polymers to evaluate the significance of various 2′-hydroxyls in hammerhead catalysis (Perreault et al., 1990). These molecules may also give some clue to the reason why nucleic acid catalysis seems to be restricted to RNA. This work showed that 2′-OHs in the region of U^8GAUGA^{13} of the ribozyme and the 2′-hydroxyl adjacent to the scissile phosphate bond are important factors in cleavage. Now, we describe the application of the mixed polymer analogues of Figure 1 to analyze the structural requirements of the substrate in this reaction. Our results show that predominantly DNA substrates can be cleaved, demonstrating that the helical regions I and III of the hammerhead

† This work was funded by the Medical Research Council of Canada. J.-P.P. was supported by a FCAR (Québec) predoctoral fellowship. R.C. is a Fellow of the Canadian Institute of Advanced Research. N.U. holds a NASA and an NIH Fogarty International Research Fellowship.
* Corresponding author.
‡ Département de biochimie, Université de Montréal.
§ Centre de recherche de l'Hôpital Ste-Justine, Université de Montréal.
∥ Biology Department, Massachusetts Institute of Technology.

0006-2960/90/0429-11156$02.50/0



Substrate analogs:

DSrC8:	ACGGTCTCACGAGC
DSrU8:	ACGGTCTUACGAGC
DSrA8:	ACGGTCTAACGAGC
DSrU7rC8:	ACGGTCUCACGAGC
DS:	ACGGTCTCACGAGC
RSdC8:	ACGGUCUCACGAGC
RSdT7:	ACGGUCTCACGAGC

FIGURE 1: Structure of the hammerhead and the DNA/RNA substrate analogues. The ribozyme sequence was derived from the consensus structure as modified in Perreault et al. (1990). The arrow indicates the cleavage site. The bottom of the figure shows the mixed DNA/RNA substrate analogues used in this study; the positions of ribonucleotides are underlined. Substrate and ribozyme analogues are abbreviated as follows: the first letter designates the predominant nucleotide used in the synthesis, R for oligoribonucleotides and D for oligodeoxyribonucleotides, and following letters denote substitution by either deoxy- or ribonucleotides at the indicated position. Note that the sequences used here differ from the consensus sequence in that the G6-C30 base has been inverted; this modification does not affect catalytic efficiency (Koizumi et al., 1988).

model can be replaced by DNA/RNA heteroduplexes, but not without affecting both the K_m and the k_{cat} of the reaction.

Materials and Methods

Synthesis of DNA/RNA Mixed Polymers. The syntheses of the all-RNA ribozyme and all-RNA and RSdC8[1] substrates were published previously (Perreault et al., 1990). The mixed oligonucleotides reported here were made with a Pharmacia DNA synthesizer using a modified 02-METHX.MTD program where the coupling time was increased from 3 to 5 min. The support was taken out of the cassette and treated with 0.5 mL of a thiophenol solution (thiophenol–triethylamine–1,4-dioxane, 1:2:2) at room temperature for 1 h and washed with methanol. The beads were collected and incubated in Eppendorf tubes with 200 μL of concentrated ammonia/95% ethanol (3:1) at 55 °C for 10 h to remove protecting groups. The supernatant was lyophilized in a Speed-Vac concentrator and incubated with 1 M tetrabutylammonium fluoride in THF at room temperature for 6 h to remove 2′-protecting groups. The reaction mixture was passed through a 10-mL Sephadex G-25 column and eluted with sterile water. Fractions were collected, the appropriate tubes were lyophilized in a Speed-Vac, and the crude product was resuspended in sterile water and stored at −20 °C. All analogues were purified by 20% PAGE and characterized by specific RNase digestions.

Purification and Labeling of DNA/RNA Mixed Polymers. The 5′-^{32}P labeling of crude hybrid oligomer was performed as for DNA with the addition of 0.5 μL of RNAguard, Pharmacia (Ogilvie et al., 1988). After separation on 20% PAGE in 7 M urea, the major bands detected by autoradiography were cut out and extracted twice with sterile water at 4 °C for 10 h. The supernatant was concentrated to 200 μL in a Speed-Vac and then passed through a 5-mL Sephadex G-25 column. After lyophilization, the ^{32}P-labeled oligomers were resuspended in 500 μL of sterile water. The concentration of each fraction was determined by their absorption at 260 nm.

The Cleavage Reaction. Cleavage reactions were carried out under the conditions described by Uhlenbeck (1987). Unlabeled ribozyme in Tris-HCl (pH 7.5) was heated to 65 °C for 0.5 min and then mixed with labeled substrate. After snap cooling, magnesium chloride was added to start the reaction. Final concentrations in a typical reaction were 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 0.1 μM substrate, and 0.05 μM ribozyme. In kinetic experiments, 0.1–8 μM DNA/RNA mixed substrate or 0.5–6 μM all-RNA substrate and 0.05 μM ribozyme were incubated in 5- or 10-μL total volume for 0–5 h. The experiments involving inhibition kinetics were performed with 1.15 μM RSdC8 analogue (ribozyme 0.05 μM) or 0.58 μM all-DNA analogue (ribozyme 0.025 μM) and 0.13–6 μM RNA substrate at 37 °C. To prevent evaporation of such small volumes, 0.5-mL Eppendorf tubes were fully submerged in the water bath during incubation. In this way, volume reduction was not detected after 24 h of incubation. The reactions were stopped by the addition of an equal volume of loading buffer (80% formamide, 0.1% xylene cyanol, 0.1% bromophenol blue) containing 100 mM EDTA and loaded on a 20% PAGE in 7 M urea. After electrophoresis and autoradiography, the intact substrate and product were located and excised. Percentage of cleavage was determined by scintillation counting of the gel slices and by direct scanning of the autoradiogram.

Thermal Denaturation. The thermal denaturation of the ribozyme and ribozyme–substrate complex was performed with a CARY 2300 spectrophotometer and a jacketed cuvette. The melting curves were obtained for the all-RNA ribozyme at 0.35 μM and equimolar mixtures (0.35 μM each) of the all-RNA ribozyme and RSdC8 and the ribozyme with the all-DNA substrate in 100 mM NaCl and 10 mM sodium cacodylate buffer at pH 6.8, in both the presence and absence of 10 mM $MgCl_2$. The samples were preheated to 65 °C for 0.5 min and snap cooled as above. The absorption of the solutions was determined continuously as the temperature varied from 10 to 80 °C, at the rate of 1 °C/min.

Results

Cleavage of DNA/RNA Mixed Substrates by the RNA Ribozyme. We have shown that a mixed DNA/RNA oligomer substrate composed of ribonucleotides and one deoxyribonucleotide at the cleavage site cannot be cleaved by the ribozyme (Perreault et al., 1990). This result left open the possibility that predominantly DNA substrates (D substrates) could be cleaved if they were to contain at least one ribonucleotide at the cleavage site and if the hybrid duplex (region I and III) created upon binding of the substrate would not greatly affect the conformation at the catalytic site (Jeffries & Symons, 1989; Chou et al., 1989; Uhlenbeck, 1987). We, thus, proceeded to the synthesis of several substrate analogues shown in Figure 1. Cleavage was studied under a variety of conditions with a chemically synthesized 35-mer ribozyme. Figure 2 shows that all of the mixed oligonucleotides DSrC8, DSrA8, DSrU8, and DSrU7rC8 can be cleaved by the ribozyme, although longer incubation times are necessary than for

[1] Abbreviations: The first letter of the analogues designates the predominant nucleotide used in the synthesis, R for oligoribonucleotides and D for oligodeoxyribonucleotides. The second letter indicates that the oligomer is a (S) substrate. The nature and the position of variant nucleotides are indicated by rA, rC, rG, and rU for ribonucleotide substitution followed by the number of the position from the 5′ terminus and by dA, dC, dG, and dT and the position for deoxyribonucleotide substitution. D and R used before the words substrate, analogue, and ribozyme indicate the predominant nucleotide in the molecule. PAGE is polyacrylamide gel electrophoresis. A_{260} is the absorbancy of a solution at 260 nm in a 1-cm light path.

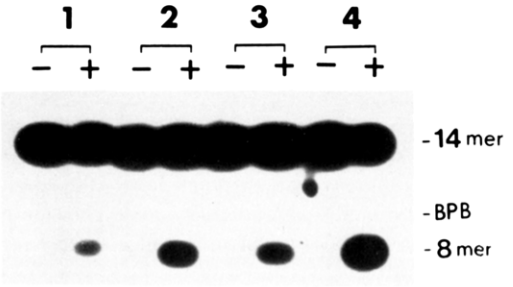


FIGURE 2: Cleavage of DNA/RNA substrate analogues by the ribozyme. Reactions were performed with (+) or without (−) 0.05 μM unlabeled ribozyme and 0.05 μM 5′-labeled substrates as described under Materials and Methods: DSrA8 (lane 1), DSrC8 (lane 2), DSrU8 (lane 3), an DSrU7rC8 (lane 4), at 32 °C for 2.5 h. (+) and (−) indicate the presence and absence of the ribozyme. Products were analyzed on 20% PAGE with 7 M urea. The 14-mer indicates the position of the substrate, and 8-mer indicates the position of the 5′ product fragment.

the all-RNA substrate. The order of efficiency is DSrU7rC8 > DSrC8 > DSrU8 > DSrA8. The analogue containing a rG at the cleavage site was not synthesized, since substrates with rG[8] have low activities or are not cleaved (Koizumi et al., 1988; Sheldon & Symons, 1989a). Under normal cleavage conditions no degradation could be detected at 32 °C without added ribozyme, although the use of temperatures greater than 50 °C for long incubation times did lead to some degradation.

The reported optimal temperature for the hammerhead ribozyme activity is 55 °C (Jeffries & Symons, 1989; Gerlach & Haseloff, 1988; Koizumi et al. 1988; Uhlenbeck, 1987) which compares favorably with 51 °C under the conditions reported here. In contrast, the temperature optima for both the rC[8] deoxyribonucleotide (DSrC8) and the rU[7],rC[8] deoxyribonucleotide (DSrU7rC8) substrates were some 20 °C lower, i.e., 32 °C. This temperature effect suggested that the thermal stability of the complex with D substrates was less than in the case of R substrates. We, thus, determined the melting curves for the ribozyme and the ribozyme–substrate complex. Since any cleavable substrate would not be appropriate for this determination, the RNA substrate analogue containing dC[8] and the all-DNA substrate were used to simulate the melting of the complex in the presence of the corresponding cleavable substrates. The melting curves in Figure 3 show that the T_m values in the presence of Mg^{2+} are 51 °C for the ribozyme with the R substrate and 32 °C for the ribozyme with the D substrate. The presence of Mg^{2+} ion has only a slight effect on both the ΔA_{260} and the T_m, although melting is more cooperative.

Ribozyme catalysis is dependent on the presence of divalent metal ions, and the efficiency of these ions has been shown to follow the order $Mn^{2+} > Mg^{2+} > Ca^{2+}$ (Uhlenbeck, 1987). The cleavage rate of the D substrates in the presence of Ca^{2+} was expectedly 3 times slower than in the presence of Mg^{2+}. Unexpectedly however, the D substrates were cleaved at an even lower rate (10 times) in the presence of Mn^{2+}, in contrast to the reaction with RNA substrates which was stimulated in the presence of Mn^{2+}.

Kinetics of Substrate Analogues Cleaved by the Ribozyme. Two reasons may be invoked to explain the lower reaction rate with D substrates: (1) the binding of the substrate and Mg^{2+} to give the ternary complex with hybrid duplexes is less favored for the analogue substrates, and/or (2) the active conformation of the ribozyme–substrate complex is altered in some way. We have attempted to resolve this question partially by a kinetic description of the reactions. First, the cleavage rate was found to be first order with respect to ribozyme concentration over

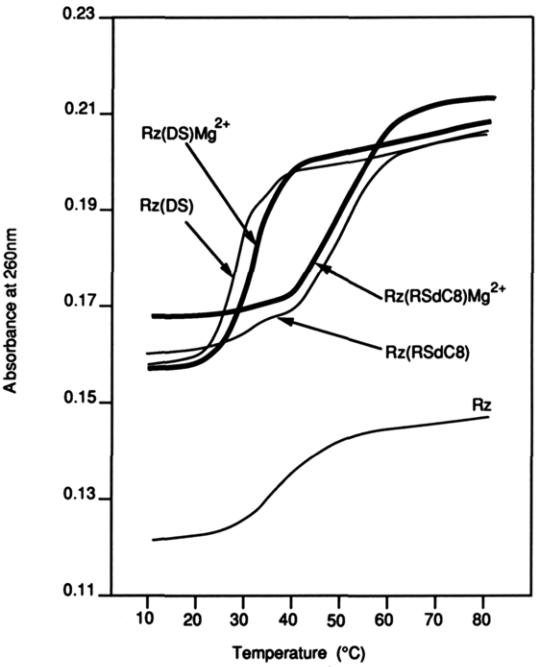


FIGURE 3: Melting curves. The absorbancy of solutions containing ribozymes with or without substrates was determined as a function of temperature. Curves in bold lines represent experiments carried out on the full ternary complex, i.e., ribozyme, substrate, and Mg^{2+}.

Table I: Kinetic Parameters of Substrate Analogues

substrate	temp (°C)	K_M (μM)	k_{cat} (min^{-1})	k_{cat}/K_M ($\mu M^{-1}\cdot min^{-1}$)
DSrC8	37	14	0.25	0.018
DSrU7rC8	37	13	0.31	0.024
RSdT7	37	7.4	0.12	0.016
all RNA	37	0.9	2.6	2.9
DSrU7rC8	30	4.5	0.17	0.038
all RNA	30	0.7	1.2	1.7
DNA/RNA ratio[a]	37	16	1/8	1/120
	30	6	1/7	1/45

[a] The ratio between parameters of DSrU7rC8 and all-RNA substrate.

the range of 0.005–0.2 μM (data not shown), indicating that the active form of this ribozyme is monomeric (Sheldon & Symons, 1989b; Ruffner et al., 1989). The cleavage reaction of DSrC8 and DSrU7rC8 was studied in detail with 5′-labeled substrate and unlabeled ribozyme. All initial rates were calculated from the linear regression of product vs time within the range of 10% cleavage (five to nine data points). The substrate/ribozyme ratio varied from 2 to 120 for DSrC8 and from 2 to 160 for DSrU7rC8. The relationship between initial concentration of substrate and initial cleavage rate was analyzed according to the Michaelis–Menten model with the ENZFITTER program (Elsevier-Biosoft). The results at 30 °C are shown in Table I, and examples of the dependence of initial velocities on substrate concentrations are given in Figure 4.

Comparison of the cleavage of the all-RNA substrate and the D substrates shows that changing the RNA/RNA helices to DNA/RNA helices in the ribozyme–substrate complex increases the apparent K_m by a factor of approximately 15 and lowers the k_{cat} by a factor of 8 at 37 °C. At 30 °C, the apparent K_m differs only by a factor of 6, i.e., half the factor found at 37 °C. The k_{cat} of the two substrates differs by a factor of 7, the same factor as obtained at 37 °C. The values of k_{cat} for both D substrates and the RNA substrates show a normal doubling effect in going from 30 to 37 °C, thereby implying that there is no unexpected temperature effect on

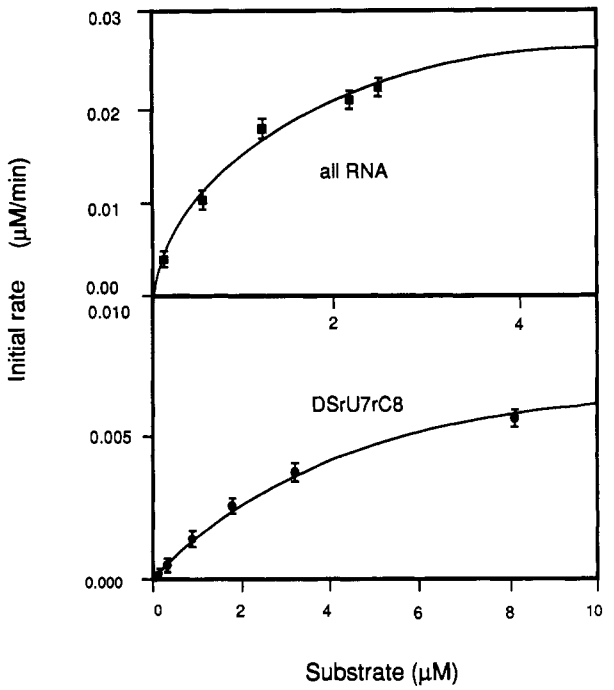


FIGURE 4: Kinetic analysis of catalytic cleavages. Initial rates of cleavage (v) at 30 °C were determined by plotting cleavage percentage versus time. The data were analyzed according to the Michaelis–Menten equation, and the derived parameters are compiled in Table I.

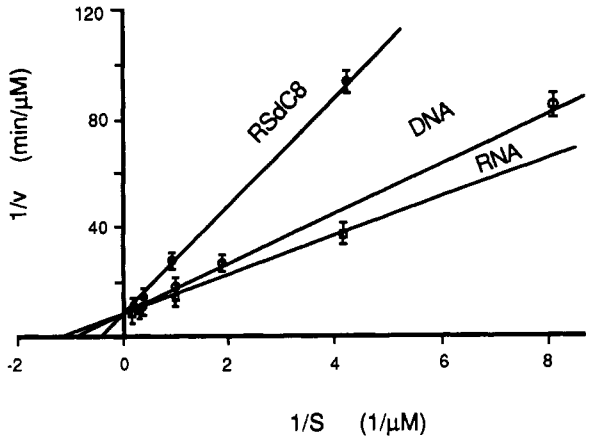


FIGURE 5: Kinetics of inhibition. Inhibition of RNA substrate cleavage was performed with 0.58 μM DS and 1.15 μM RSdC8. Concentrations of the all-RNA substrate vary from 0.13 to 6 μM at a constant ribozyme concentration of 0.05 μM for RSdC8 and 0.025 μM for DS determinations. The Lineweaver–Burk plots show the data for the RNA substrate alone (□), the RNA substrate in the presence of DS after normalization to 0.05 μM ribozyme (○), and the R substrate in the presence of the RSdC8 analogue (●).

the k_{cat}'s of either substrate type.

The availability of the all-oligodeoxyribonucleotide (DS) and the dC[8] oligoribonucleotide (RSdC8) substrate analogues (Figure 1) allowed us to study their inhibitory effect on the reaction. As expected, both cases yield Lineweaver–Burk plots characteristic of competitive inhibition (Figure 5); V_{max} is unchanged whereas the apparent K_m is increased. The DS analogue has a K_I of 13 μM, whereas RSdC8 has a K_I of 0.96 μM at 37 °C. Both values compare very well with the K_m values of 13 and 14 μM for SDrU7rC8 and SDrC8, respectively, and 0.9 μM for the all-RNA substrate. The effect of inhibition is also consistent with the T_m values determined above for the D and R substrates.

Discussion

Although the interpretation of the kinetic parameters of this ribozyme-catalyzed process would require a detailed examination of all the pertinent constants of the reaction, including cation binding, the kinetic data here do allow analysis of the relative importance of some constants implicated in the composite K_m and k_{cat} values. First, the K_m is seen to be more temperature sensitive for D substrates than the all-RNA substrate. Assuming that the apparent K_m is strongly affected by the dissociation constant of the substrate, we have determined melting curves with the D- and RSdC8 substrates. These demonstrate the expected effect that RNA/RNA duplexes are more stable than DNA/RNA duplexes (Walker, 1988). The optimal temperature of D substrate cleavage (32 °C) is, thus, understandably lower than that of RNA cleavage (51 °C). Since the reactions of the D substrates at 37 °C were carried out slightly above the melting temperature of the complex, the amount of D substrate bound to the ribozyme and, thus, the K_m would vary more in this temperature range than in the RNA substrate case. The virtually identical values of K_I of the all-DNA inhibitor which cannot undergo cleavage and the K_m of the D substrate as well as the K_I of the RNA analogue containing dC[8] and the K_m of the all-RNA substrate strengthen our conclusion that the K_m is strongly affected by the stability constant of the reactive complex. Previous data showing that the K_m's of various ribozyme analogues substituted in single-stranded regions were all on the order of 0.9 μM, whereas k_{cat} varied by factors of 20–200 (Perreault et al., 1990), further support the notion that the stability of the reactive complex must play an important role in determining the value of K_m. The implication of these data is that the catalytic step is rate limiting, since the use of D substrates, which do not bind as well to the ribozyme as R substrates, should increase the catalytic rate, not decrease it as was found, if product inhibition was a factor.

The difference between the k_{cat}'s of the D substrates compared to the RNA substrate implies either that the geometry of the active site has changed making the transition to product more difficult and/or that the Mg^{2+} binding is impaired in these complexes. For example, it could be that the modified C2′-endo conformation of the deoxyribose moiety of the DNA strand imparts a torsional strain on the hybrid helix, resulting in a modified catalytic site (Chou et al., 1989). However, previously we have argued that in the case of analogue ribozymes the lack of specific 2′-OH's in the *single-strand* region may affect Mg^{2+} binding but not the conformation of the reactive complex in any important way. It was thus of interest to examine a substrate where only the U7 was substituted with a deoxyribonucleotide (analogue RSdT7), even though no major difference was observed between the K_m and k_{cat} values of DSrC8 and DSrU7rC8. Unexpectedly, both K_m and even more significantly k_{cat} are modified (Table I). The K_m of RSdT7 is 8 times higher than that of the all-RNA substrate. On the basis of the arguments above, this factor would represent lower complex stability; however, the magnitude of this change is likely too high to be accounted for solely on the basis of base pairing. Therefore, either an element of Mg^{2+} binding is a component of the K_m of deoxyribonucleotide-containing substrates, or a hydrogen-bonding contact between the 2′-OH of U7 with another part of the molecule is missing.

The fact that the k_{cat} of the RSdT7 substrate is even lower than that for the D substrates clearly indicates that the differences in the behavior of RNA and D substrates is greater than a simple effect of substrate and/or Mg^{2+} binding. The simplest, although not the only, model to reconcile these results is the following: in the case of RNA substrates, the presence

of the 2′-hydroxyl of U^7 is important for the binding of Mg^{2+} as predicted by the computational model (Mei et al., 1989), whereas for D substrates there is a change in the conformation of the active catalytic site such that cation binding is not influenced by the absence of the 2′-OH. A modified conformation for D substrates is also supported by the fact that Mn^{2+} is less effective than Mg^{2+} in stimulating the reaction, the opposite of R substrates, and that the k_{cat} values for the D and R substrates differ by a factor of 7. Other more complicated interpretations due to the complexity of the interplay between Mg^{2+} binding and substrate binding related to conformational changes in the catalytic core cannot be ruled out for the moment.

The catalytic cleavage of the ribophosphate bond presupposes an appropriate substrate conformation and a suitable nucleophile. In the case of ribozyme catalysis the conformation is dictated by forces which exist between nucleotides. We have argued that deoxyribonucleotides could be expected to mimic ribonucleotide conformations in single-stranded regions, since the nitrogen base moieties of both are identical (Perreault et al., 1990; Paquette et al., 1990). It is therefore surprising, but not entirely unexpected, that the D analogues are cleaved by the R ribozyme. The finding that an intron-derived ribozyme is able to cleave deoxyribonucleotides is less surprising since the length of the presumed base-pairing region between ribozyme and substrate is shorter than for the hammerhead domain (Herschlag & Cech, 1990; Robertson & Joyce, 1990). And more importantly, the nucleophile in the intron ribozyme is disjoint; i.e., it is not adjacent to the scissile bond. This cleavage can be thought of as a more general, nucleic acid type catalysis than that of the hammerhead ribozyme where the nucleophile is conjoint, i.e., adjacent to the scissile bond (Cedergren et al., 1987). For this reason, the presence of a ribonucleotide at the cleavage site is facultative for the intron ribozyme and RNase P catalysis (Forster & Altman, 1990), whereas it is obligatory in the case of the hammerhead ribozyme.

Acknowledgments

We express our thanks to Dr. Taifeng Wu for providing ribonucleotide intermediates for the synthesis as well as Dr. Gabriel Gingras and Dr. Ted Mar for assistance in the thermal denaturation study.

References

Cedergren, R., Lang, B. F., & Gravel, D. (1987) *FEBS Lett. 226*, 64–66.
Chou, S. H., Flynn, P., & Reid, B. (1989) *Biochemistry 28*, 2435–2443.
Epstein, L. M., & Gall, J. G. (1987) *Cell 49*, 211–220.
Forster, A. C., & Altman, S. (1990) *Science 249*, 783–786.
Forster, A. C., Davies, C., Sheldon, C. C., Jeffries, A. C., & Symons, R. H. (1988) *Nature 334*, 365–267.
Gerlach, W. L., & Haseloff, J. (1988) *Nature 334*, 585–591.
Herschlag, D., & Cech, T. R. (1990) *Nature 344*, 405–409.
Jeffries, A. C., & Symons, R. H. (1989) *Nucleic Acids Res. 17*, 1371–1377.
Koizumi, M., Iwai, S., & Ohtsuka, E. (1988) *FEBS Lett. 228*, 228–230.
Mei, H.-Y., Kaaret, T. W., & Bruice, T. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 9727–9731.
Ogilvie, K. K., Usman, N., Nicoghosian, K., & Cedergren, R. J. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 5764–5768.
Paquette, J., Nicoghosian, K., Qi, G. R., Beauchemin, N., & Cedergren, R. (1990) *Eur. J. Biochem. 189*, 259–265.
Perreault, J.-P., Wu, T.-F., Cousineau, B., Ogilvie, K. K., & Cedergren, R. (1990) *Nature 344*, 565–567.
Robertson, D. L., & Joyce, G. F. (1990) *Nature 344*, 467–468.
Ruffner, D. E., Dahm, S. C., & Uhlenbeck, O. C. (1989) *Gene 82*, 31–41.
Sampson, J. R., Sullivan, F. X., Behlen, L. S., Direnzo, A. B., & Uhlenbeck, O. C. (1987) *Cold Spring Harbor Symp. Quant. Biol. 52*, 267–275.
Sheldon, C. C., & Symons, R. H. (1989a) *Nucleic Acids Res. 17*, 5679–5685.
Sheldon, C. C., & Symons, R. H. (1989b) *Nucleic Acids Res. 17*, 5665–5677.
Talbot, V., & Bruening, G. (1988) *Nature 334*, 196–197.
Uhlenbeck, O. C. (1987) *Nature 328*, 596–600.
Walker, G. T. (1988) *Nucleic Acids Res. 16*, 3091–3099.