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Factors Altering Ribozyme-Mediated Cleavage of Tumor Necrosis Factor-α mRNA *in Vitro*

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Hammerhead ribozymes are capable of cleaving RNA in a sequence specific manner *in vitro*. However, the complex environment of the cell differs dramatically from the conditions *in vitro*. Therefore, we explored cleavage of full-length target RNA with two ribozymes targeted against the murine tumor necrosis factor-alpha (TNF-α) mRNA. These ribozymes cleaved TNF-α mRNA within a pool of total cellular RNA *in vitro,* but less efficiently than previously reported for similar ribozymes. Although there may be several factors that could affect ribozyme activity, two of these factors were tested. The first factor was whether non-target polynucleotides inhibited ribozyme-mediated cleavage. Total cellular RNA and to a lesser degree DNA inhibited ribozyme activity. This inhibition was a combination of competitive and non-competitive inhibition. Non-target RNA with minimal complementarity to the ribozyme or target showed no effect on cleavage rates. The second factor was whether denaturing conditions improved ribozyme cleavage efficiency. Hammerhead ribozymes with 24 complementary bases had increased cleavage efficiency in formamide. Thus, the ribozymes may have had too long of an antisense flanking sequence which hybridized with the target RNA and resulted in a high melting temperature. These studies demonstrate that ribozyme cleavage was influenced by the amount of non-target polynucleotide and the strength of the ribozyme-substrate interaction.

One option for the specific inhibition of cytokine expression is ribozyme-mediated cleavage of target mRNA. Hammerhead-type ribozymes are low molecular weight RNA molecules of 30-100 bases which catalytically cleave target RNA at specific sites (1, 2). This cleavage can result in inactivation of the target mRNA (3, 4). For optimal target cleavage, ribozymes must be able to identify, hybridize, catalytically cleave, and dissociate from the target RNA. If the hybridization between ribozyme and target was too strong, the ribozyme would be unable to dissociate, thus decreasing ribozyme activity (5). Before such a tool can be effectively used, the factors influencing ribozyme activity must be delineated. For example, temperature, length of antisense flanking domain, magnesium concentration, neomycin, and tetracycline have been shown to alter ribozyme activity (6-8). To elucidate additional variables that may alter ribozyme cleavage, we have chosen to concentrate on other inhibitors and the effect of denaturing conditions.

Tumor necrosis factor-α is a multifunctional cytokine produced by macrophages and other cells during inflammatory responses (9). Although this cytokine has been shown to be cytotoxic or cytostatic to some solid tumors *in vivo* and several transformed cell lines *in vitro,* the functional role of this cytokine during the initiation and maintenance of immune responses has been difficult to study (10). *In vitro* actions of TNF-α include cytolysis or cytostasis of tumor cell lines, inhibition of lipoprotein lipase in adipocytes, induction of acute phase proteins by hepatocytes, and induction of cytokine production by T-cells (11). TNF-α has been implicated in several clinical syndromes, most notably septic shock (12). That response can be duplicated in mice by the administration of TNF-α or IL-1β protein (11). Moreover, administration of antibodies against those cytokines has been shown to protect animals against lipopolysaccharide (LPS) induced septic shock when given prior to LPS treatment (13). Because of these observations, there has been great interest in the past few years in blocking TNF-α activity in order to treat and prevent septic shock.

The purpose of this study was to use hammerhead

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Abbreviations: IL-1α, interleukin-1α; LPS, lipopolysaccharide; SIV, simian immunodeficiency virus; Tm, melting temperature; TNF-α, murine tumor necrosis factor-alpha.

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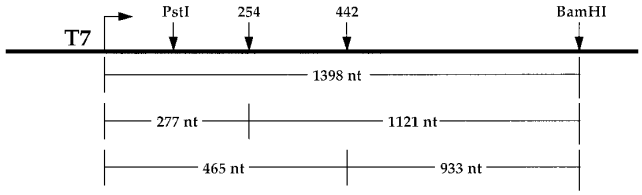

FIG. 1. Schematic of target RNA and sites of cleavage products by rz254 and rz442.

ribozymes to specifically cleave TNF-α mRNA. For that, we assessed the potential of select non-target polynucleotides to inhibit ribozyme activity.

MATERIALS AND METHODS

Ribozyme design and synthesis. Two ribozymes were designed targeted to two different sites of the TNF-α mRNA (Fig. 1). Ribozyme rz254 (5′GGG CGA AUU CGA GCU CGG UAC CCG GGG AUC CCG AAG UUC AGU ACU GAU GAG AGU CCG UGA GGA CGA AAC AGA AGA GCG UCU GCA GGC AUG CA) and rz442 (5′GGG CGA AUU CGA GCU CGG UAC CCG GGG AUC CAA CCU GGG AGU UAC UGA UGA GAG UCC GUG AGG ACG AAA CAA GGU ACU UCC UGC AGG CAU GCA) were 92 and 93 bases respectively. Rz254 had a GC content of 58% and was targeted to cleave at position 254 of the TNF-α cDNA. Rz442 had a GC content of 57% and was targeted to cleave at position 442 of the TNF-α cDNA (Fig. 1). The base positions in figure 1 reflect the 23 bases of the plasmid polylinker that were transcribed along with the full-length mTNF-α mRNA. Site 254 was chosen to cleave the 5′ untranslated region from the majority of the TNF-α coding sequence, and site 442 was selected to create fragments more equal in length for ease of kinetic analysis.

Appropriate oligonucleotides encoding the ribozyme RNA were subcloned into the bacterial expression vector pGEM-3z (Promega, La Jolla, CA) between the *Bam*HI and *Hind* III restriction sites. Plasmids were linearized with *Hind* III and transcribed *in vitro* with T7 RNA polymerase (Promega) as per the manufacturer's instructions. Unincorporated nucleotides were removed by chromatography on G-25 spin columns (Boehringer-Mannheim, Indianapolis, IN). RNA was diluted in 50 mM Tris, 1 mM EDTA and stored at −70°C. RNA was quantitated as described below.

Substrate synthesis. cDNA encoding the TNF-α was generously provided by Dr. Anthony Cerami (14). The cDNA was 1375 bases and contained a *Pst* I restriction site at the 5′ end and a *Bam*HI restriction site at the 3′ end. This cDNA was subcloned into the plasmid pGEM-4z (Promega). The plasmid was linearized with *Hind* III and transcribed *in vitro* with T7 RNA polymerase (Promega) as per the manufacturer's instructions in order to produce TNF-α substrate RNA. For kinetic determinations, TNF-α substrate RNA was radiolabelled internally by the addition of γ-^{32}P-CTP during transcription. mRNA was separated by electrophoresis in a 1% low melting temperature agarose gel containing 0.7% formaldehyde. The band corresponding to the full length transcript was isolated from the gel. The RNA was purified by chromatography with the RNAaid Kit (Bio 101, La Jolla, CA) as per the manufacturer.

Total cellular RNA from the murine macrophage cell lines RAW 264.7 and J774A.1 (American Type Culture Collection, Rockville, MD.) was isolated by a single extraction with an acid guanidine thiocyanate-phenol-chloroform mixture (15). To enhance expression of TNF-α mRNA, confluent macrophage cultures were stimulated with 100 ng/ml phenol extracted lipopolysaccharide (LPS) derived from, *Escherichia coli* serotype 0111:B4 (Sigma, St. Louis, MO) in medium. That concentration was found to be optimal for induction of TNF-α mRNA and protein expression (data not presented). Cellular RNA was precipitated in 70% ethanol and 100 mM sodium acetate and stored at −70°C.

Cleavage reactions. Ribozymes and substrates were prepared separately as described above. Cellular RNA was dried with vacuum and resuspended in 50 mM Tris, 1 mM EDTA. Ribozymes and substrates were heated separately for 1 minute at 95°C to disrupt aggregates and cooled to room temperature prior to coincubation (13). Following this, ribozymes and substrates were heated again separately to the reaction temperature (either 40°C or 50°C) in the presence of 10 mM $MgCl_2$. All reactions were performed in the absence of 10% formamide, except as noted. Ribozymes were then added to the substrates and the reactions allowed to proceed for various lengths of time. Reactions were stopped by the addition of an equal volume of gel loading buffer which consisted of 97% deionized formamide, 5 mM EDTA, 0.01% bromphenol blue, and 0.01% xylene cyanol and placed on ice. Reaction products were separated on a 5% polyacrylamide gel containing 1× TBE and 7 M urea and transferred to Nytran. Northern blot analysis was carried out using an internally ^{32}P-labeled TNF-α cDNA as a probe. β-actin and murine IL-1α probes were used as controls for ribozyme specificity as previously described (16).

Quantization of ribozymes, substrates, and cleavage products. To determine polynucleotide concentration, RNA was separated via polyacrylamide gels electrophoresis. The gel was then soaked in distilled water to remove urea and stained with EZ Blue (Boehringer Mannheim). The gel was scanned by two dimension densitometry at 585 nm, which was the absorbance maximum of the EZ Blue stain (unpublished observation) with a CS-9000 dual wavelength flying spot scanner (Shimadzu, Concord, CA). Densitometry values for the RNA were compared to standards in each gel to determine the exact amount of RNA present. Values reported here represent the mean of at least three lanes.

Cleavage inhibition reactions. For these experiments, three different non-target polynucleotide inhibitors were used, each in two different concentrations. The inhibitors used were total cellular RNA from the murine macrophage cell line RAW 264.7, salmon sperm DNA (Sigma), or an *in vitro* transcribed 422 base RNA from the simian immunodeficiency virus (SIV) GAG gene, clone 1α11 (17). The salmon sperm DNA had been sheared by passage through a 27 gauge needle, heated to 95°C, and then snap cooled. The various inhibitors were added to the ribozyme:substrate mixture at either 0.1 mg/ml or 0.2 mg/ml at the zero time point and allowed to incubate for varying lengths of time. Reactions were stopped as described above.

Determination of kinetic parameters. Km and Vmax values were determined from initial cleavage rates of *in vitro* transcribed TNF-α target RNA by rz254. Ribozyme cleavage was assayed at six different substrate concentrations (20, 50, 100, 200, 500, and 1000 nm). All experiments were repeated three times and results are reported as the mean of the three samples. Initial cleavage rates varied up to 50% at 40°C, but less than 10% at 50°C (data not shown). Therefore, only the results from the cleavage assays performed at 50°C are shown.

Estimation of duplex melting temperatures, activation energy, and dissociation constants. The binding of ribozyme to substrate forms an RNA duplex. The activation energy (Ea) for RNA duplexes with 6 to 12 paired bases can be calculated from the number of hydrogen bonds in the duplex by the following equation (18):

$$Eq = 152.99 \times \log(\#\text{ of hydrogen bonds}) - 141.87 \qquad [1]$$

Activation energy can then be used to estimate the dissociation constants (Kd) for the RNA duplexes according to the equation:

TABLE 1

Estimates of Dissociation Constants, Melting Temperatures, and Activation Enthalpies for Several Duplexes Which Could Be between rz254 and Target RNA

	Hybridizing sequence	
	UCUGCGAGAAGACA	AUGACUUGAACG
ΔH (kcal/mol)	−136	−102.5
ΔS_{nn} (eu)	−364.9	−280.7
Tm (°C)	79.2	66.5
Kd		
40°C	4.68	7.09×10^{-1}
50°C	5.13×10^{2}	4.17×10^{1}

$$Kd = Ae^{-Ea/RT} \quad [2]$$

where R is the gas constant (1.987 cal/(mol × °K)) and T is the temperature in degrees Kelvin. A is a constant that can be calculated using the following equation (18).

$$Ln\ A = 10.989 \times (\#\ of\ base\ pairs) - 0.586 \quad [3]$$

The theoretical melting temperature (Tm) of potential duplex regions between the ribozymes and substrate RNA were calculated according to the equation (19):

$$Tm = \Delta H/(A + \Delta S_{nn} + R\ ln(Ct)) - 273.15 \quad [4]$$

where A is a constant equal to −2.8 eu, which is a correction for symmetry. R is the gas constant. ΔH and ΔS_{nn} are the change in enthalpy and entropy, respectively, and were calculated according to previously reported values for duplex propagation (19). Ct is the total concentration of single strands which was taken to be 0.1 mM as used previously (19). The calculated values for the 12 base pair duplexes formed between rz254 and TNF-α mRNA are shown in Table 1.

RESULTS

Cleavage of TNF-α mRNA in a Pool of Total Cellular RNA

Since our ultimate goal was to utilize ribozymes to alter *in vivo* cytokine production, we wanted to determine whether the ribozymes rz254 and rz442 would cleave TNF-α mRNA *in vitro* in the presence of total cellular RNA (Fig. 2). Cellular RNA was isolated from the murine macrophage cell lines RAW 264.7 or J774A.1. Two different macrophage cell lines were used to exclude the possibility that the results were unique to one particular cell line. The cells had previously been stimulated with 100 ng/ml LPS in order to induce expression of TNF-α mRNA. Cellular RNA from RAW 264.7 cells was incubated with or without addition of rz254 at 40°C for 1 hr (Fig 2A). Cellular RNA from J774A.1 was also incubated with or without the addition of rz442 at 40°C for 1 hr (Fig 2B). In the absence of ribozyme, no cleavage was detected (Fig. 2A, lane 2; Fig. 2B, lane 2). However, in the presence of ribozyme a small amount of cleavage product was detected (Fig. 2A, lane 1; Fig. 2B, lane 1). To determine whether other abundant RNA in LPS stimulated macrophages, such as β-actin and IL-1α, were cleaved by these ribozymes, the Northern blots were reprobed with for β-actin and murine IL-1α. No cleavage products were detected (data not shown).

Effect of Non-target Polynucleotide on Ribozyme-Mediated Cleavage

The efficiency of rz254 and rz442-mediated cleavage within a pool of total cellular RNA differed markedly from the efficiency previously reported for other similar ribozymes (20). Our ribozymes were assayed for cleavage of full-length target RNA within a pool of total cellular RNA rather than cleavage of a purified 15-mer target RNA sequence as used by other investigators (20). Therefore, it was possible that non-target cellular RNA inhibited ribozyme-mediated cleavage. To test that possibility, we examined the kinetic parameters of ribozyme-mediated cleavage when varying amounts of non-target polynucleotide were present in the reaction. Rz254 was assayed for cleavage of *in vitro* transcribed TNF-α RNA in the presence or absence of (A) total cellular RNA; (B) salmon sperm DNA; or (C) *in vitro* transcribed RNA from the SIV GAG gene (Fig. 3). The addition of cellular RNA from unstimulated RAW 264.7 cells increased Km and decreased Vmax (Fig. 3A). This outcome was not consistent with only competitive or noncompetitive inhibition, but rather a mixture of both.

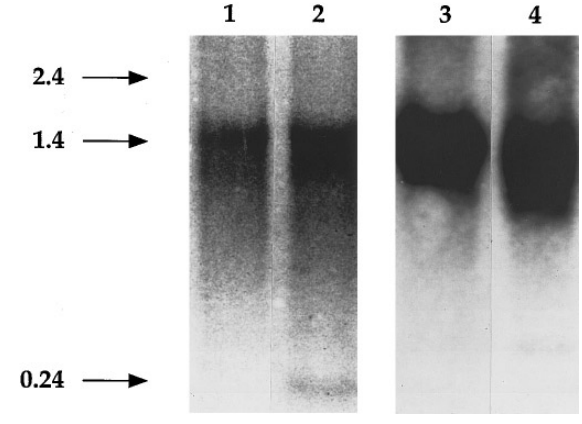

FIG. 2. Cleavage of TNF-α mRNA by ribozyme RNA in a pool of total cellular RNA. Ribozyme RNA (160 ng) was incubated at 40°C for 1 hr with total cellular RNA (20 μg) from murine macrophage cells treated with 100 ng/ml LPS. Northern blot analysis was performed to detect cleavage of TNF-α mRNA. RNA from stimulated RAW 264.7 cells was co-incubated without (Lane 1) or with rz254 (Lane 2). RNA from stimulated J774A.1 cells was co-incubated without (Lane 3) or with rz442 (Lane 4).

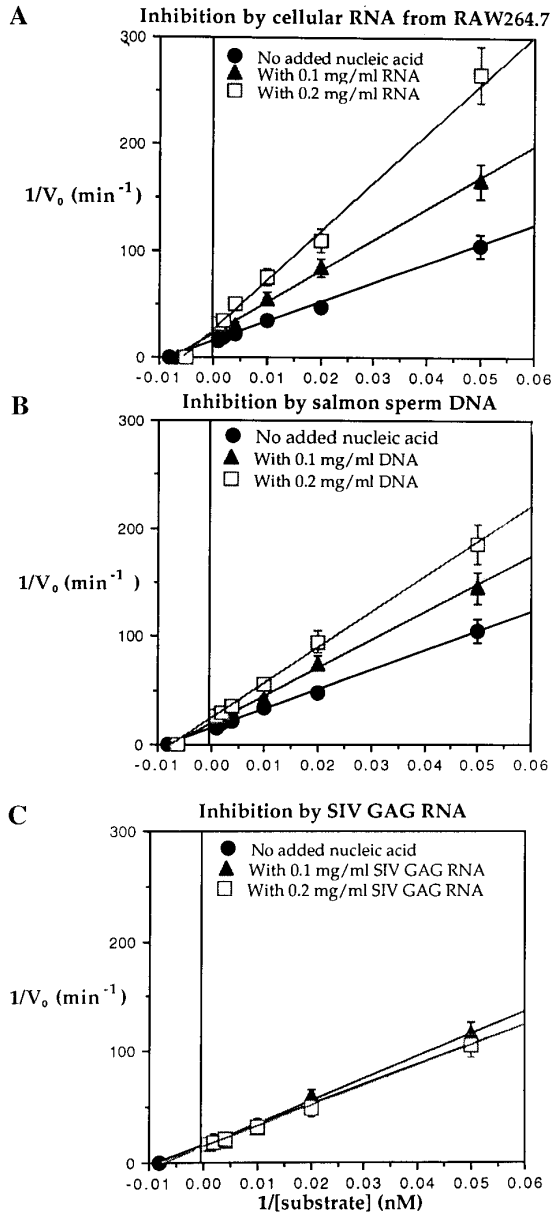

FIG. 3. Lineweaver-Burk plots of ribozyme cleavage reactions in the presence and absence of various polynucleotide inhibitors. Rz254 was incubated with increasing amounts of *in vitro* transcribed TNF-α RNA. Various polynucleotide inhibitors were added to the cleavage reactions at either 0.1 or 0.2 mg/ml. The different polynucleotide inhibitors were: (A) total cellular RNA from unstimulated RAW 264.7 cells; (B) salmon sperm DNA; or (C) *in vitro* transcribed RNA from the SIV GAG gene.

To test the effect of non-target DNA on ribozyme-mediated cleavage, rz254 was assayed for cleavage of TNF-α mRNA in the presence of Salmon sperm DNA. The addition of the Salmon sperm DNA resulted in 60% less inhibition as compared to when total cellular RNA was added (Fig. 3B). Again the inhibition was not consistent with only competitive or noncompetitive inhibition, but rather a mixed inhibition.

Next, we wanted to determine whether the inhibition previously observed was due to either sequence similarity between the ribozyme and the non-target polynucleotides or macromolecular crowding caused by excess non-target polynucleotides. Therefore, rz254 was assayed for cleavage of the TNF-α mRNA in the presence of *in vitro* transcribed RNA from the SIV GAG gene (Fig. 3C). This RNA is a 422 base length RNA that has a maximum complementary length of 4 bases with rz254. The addition of the SIV GAG gene RNA resulted in no evidence of ribozyme inhibition at the concentrations tested (Fig. 3C) The kinetic parameters, of Km, Kcat, and Kcat/Km are summarized in Table 2.

Effect of Formamide on Ribozyme Activity

Another possible reason as to why the ribozymes rz254 and rz442 showed suboptimal cleavage, is the length of the complimentary binding arms. We hypothesize that the antisense flanking sequences on our ribozymes were too long which resulted in an elevated Tm of the ribozyme-substrate complex and thus a slow resultant ribozyme turnover rate. Since ribozymes require substrate turnover for efficient cleavage, decreased turnover would result in suboptimal ribozyme activity. To test this hypothesis, we assayed for the ability of rz442 to cleave *in vitro* transcribed substrate RNA in the absence or presence of formamide (Fig. 4). Formamide increases the stringency required for RNA-RNA interactions. Therefore, formamide would help to facilitate ribozyme-target RNA dissociation following cleavage. This in turn would allow the ribozyme to cleave multiple target RNA and increase the efficiency

TABLE 2

Kinetic Parameters for rz254 Cleavage of *in Vitro* Transcribed TNF-α mRNA in the Presence of Non-Target Nucleic Acids

Source and amount of nucleic acids added	Kinetic parameter		
	K_{cat}	K_m (*nM*)	K_{cat}/K_m
Cellular RNA			
0 mg/ml	2.7	124	0.0218
0.1 mg/ml	1.9	140	0.0136
0.2 mg/ml	1.5	187	0.0080
Salmon sperm DNA			
0 mg/ml	2.7	124	0.0218
0.1 mg/ml	2.1	139	0.0151
0.2 mg/ml	1.7	144	0.0118
SIV GAG RNA			
0 mg/ml	2.7	124	0.0218
0.1 mg/ml	2.8	144	0.0194
0.2 mg/ml	2.8	129	0.0217

Note. The correlation coefficient for the regression lines from which the data was calculated were all greater than 0.90.

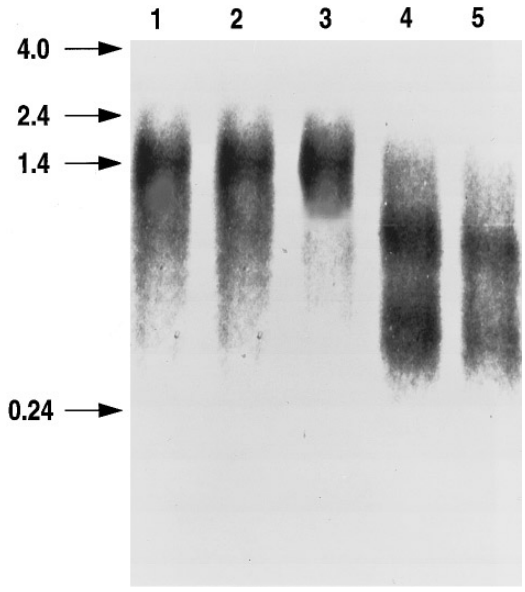

FIG. 4. Cleavage of TNF-α mRNA in the presence of formamide. Rz442 (5 nM) was incubated with *in vitro* transcribed TNF-α RNA (500 nM) at 40°C in the absence of formamide for 30 (Lane 1) or 60 (Lane 2) minutes. TNF-α mRNA incubated with 10% formamide, no ribozyme for 60 minutes (Lane 3). Incubation of TNF-α mRNA and rz442 in the presence of 10% formamide for 30 (Lane 4) or 60 (Lane 5) minutes.

of ribozyme-mediated cleavage. Rz442 was incubated with *in vitro* transcribed TNF-a RNA in the absence of formamide at 40°C for 30 or 60 min and no cleavage was detected (Fig. 4, lanes 1 and 2). However, when 10% formamide was added to the reaction mixture a high level of cleavage was demonstrated (Fig. 4, lanes 3 and 4). To exclude the possibility that the formamide itself resulted in the cleavage, target RNA was incubated with formamide in the absence of ribozyme (Fig. 4, lane 3). No cleavage was evident.

DISCUSSION

In this study, we have designed and synthesized two hammerhead ribozymes targeted against two different sites in the TNF-α mRNA. Both rz254 and rz442 catalytically cleaved target TNF-α mRNA within a pool of cellular RNA *in vitro.* However, cleavage of the target RNA was less than previously reported for other similar hammerhead ribozymes (20, 21). Two main differences existed between our ribozymes and the assay systems used and those previously reported (20, 21). The first major difference was that our ribozymes were assayed for cleavage of full length mRNA within a pool of cellular RNA rather than assayed for cleavage of an isolated, purified, small target RNA. Thus, we tested whether non-target polynucleotides could inhibit ribozyme-mediated cleavage. Non-target RNA or DNA inhibited ribozyme catalysis in a manner consistent with both competitive and non-competitive inhibition. The second major difference was the length of the antisense flanking sequence; ours were longer than previously used by other investigators. To evaluate this, we assayed our ribozymes with denaturing conditions and showed increased cleavage. This suggests that the length of the complementary binding domains were too long, resulting in a strong interaction between ribozyme and target RNA, with decreased ribozyme turnover (5, 7, 22).

It has previously been shown that cleavage of full-length target RNA was 1,000 fold less efficient than cleavage of small (<20 nt) target RNA (23). Moreover, the addition of total poly(A) RNA isolated from a leaf resulted in 71% inhibition of ribozyme cleavage (24). To further characterize the inhibition of ribozyme activity by unrelated RNA, we assayed ribozyme mediated cleavage of full length *in vitro* transcribed TNF-α mRNA in the presence and absence of several non-target polynucleotides. The addition of total cellular RNA from unstimulated RAW 264.7 cells to mixtures of rz254 and *in vitro* transcribed target RNA resulted in decreased Vmax and increased Km. The addition of salmon sperm DNA to the reaction mixture resulted in 60% less inhibition than total cellular RNA, but with similar alterations of Vmax and Km. The inhibition was neither completely competitive nor noncompetitive, but rather a mixture of the two. This study, however, cannot determine whether the inhibition resulted from interaction between the ribozyme or the substrate with the exogenous polynucleotides.

One possible explanation for the inhibition resulting from the addition of exogenous polynucleotides was that a bulk phenomena, such as macromolecular crowding. This would result in decreased interaction between the ribozyme and substrate. That possibility was examined by the addition of various amounts of RNA of known size and sequence to a mixture of ribozyme and *in vitro* transcribed TNF-α mRNA. The RNA used was a 422 base portion of the GAG gene of SIV (17). The longest complementarity of this sequence with either ribozyme or substrate was 4 bases. This RNA was used to eliminate any possible base pairing between either the ribozyme or substrate and the exogenous RNA. Therefore, any inhibition would be solely due to macromolecular crowding. After the addition of the 422 base RNA molecule, Km and Vmax values changed only slightly and remained within experimental error. These results rule out non-sequence specific effects at the concentrations tested.

The decreased Vmax and increased Km observed is consistent with a combination of competitive and non-competitive inhibition. A competitive inhibitor may bind to the active site of the enzyme and form an inactive complex. Since the active site of a ribozyme is de-

fined as the part of the ribozyme that interacts with the substrate, all sequences with sufficient complementarity to form duplexes with this part of the ribozyme are potential inhibitors. We propose that there is sufficient complexity of sequence present in total cellular RNA and salmon sperm DNA that could interact with the ribozyme antisense flanking domain to act as a competitive inhibitor.

Non-competitive inhibition of ribozyme activity would result from interaction between the ribozyme-substrate complex and the inhibitor. This could occur if the polynucleotides were able to interact with the ribozyme-substrate complex and help stabilize the complex. This stabilization would decrease the dissociation of ribozyme and substrate and therefore decrease ribozyme turnover. Ribozyme turnover is vital for successful ribozyme-mediated cleavage. Thus, a decrease in ribozyme turnover would decrease ribozyme activity.

The second possible explanation for the low level of ribozyme-mediated cleavage observed was that our ribozymes had too long of an antisense flanking sequence and thus bound target RNA too tightly. The optimal length of the ribozyme complementary domain for *in vitro* activity has been reported to be 12-16 nts (7, 23) and for *in vivo* cleavage more than 33 nts (25). Other investigators have used complementary domains in the range of 18-25 nts and shown high levels of *in vitro* cleavage (4, 26, 27). However, in some of those previous studies, the ribozymes were assayed at 50°C or 60°C, rather than the closer to physiological temperature of 40°C used in this current study (4, 27). Increased temperature would facilitate ribozyme-substrate dissociation and thus raise Kd. An increase in temperature from 40°C to 50°C would increase Kd greater than 50 fold. Therefore, ribozyme-substrate interactions that were too strong due to an extended antisense flanking sequence could be altered by an increase in temperature. Increased rates of ribozyme cleavage have been observed when assays were conducted at higher temperatures (8, 24, 28). Similar results could be obtained by the addition of urea or formamide.

The melting temperature (Tm) of duplex RNA can be estimated by calculation of ΔH and ΔS (19). Such calculations for rz254 show that the predicted Tm of each 13 base antisense flanking sequence is in the range 65-80°C. However, the accuracy of using these calculations to predict the Tm of longer RNA duplexes (>9 bases) has not been demonstrated. Thus, if the length of the antisense flanking sequences were reduced, the Tm of the ribozyme-substrate interaction would also be reduced and cleavage efficacy could be increased. This principle can be demonstrated experimentally by performing the ribozyme cleavage assay in the presence of a denaturing agent that would lower effective Tm. The addition of 10% formamide to the ribozyme reaction resulted in dramatically increased cleavage rates. These results support the findings of others, that formamide can markedly increase ribozyme cleavage activity (25, 28, 29). Other investigators, however, have failed to show a benefit to the addition of formamide (6). Thus, length of complementation must be taken into consideration when designing ribozymes for *in vitro* or *in vivo* utilization.

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