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# Trivalent Lanthanide Ions Do Not Cleave RNA in DNA-RNA Hybrids

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Compounds that promote RNA transesterification are of interest because they may be more selective than are reagents that promote oxidative cleavage of RNA.[1-11] Metal complexes are among the most efficient compounds that promote RNA transesterification and there are now several examples of metal complexes that promote RNA cleavage at 37 °C and neutral pH.[10,11] These metal complexes act on single-stranded RNA as a substrate, and there is little information on how RNA structure will affect cleavage rates. This is surprising considering the uses that have been proposed for RNA transesterification catalysts. For example, a major impetus to the design of RNA cleavage catalysts lies in the search for improved antisense oligonucleotides. An antisense oligonucleotide with an attached transesterification catalyst might be effective in translation arrest if, following formation of the DNA-RNA hybrid between antisense oligonucleotide and target m-RNA, the m-RNA were to be rapidly cleaved.[12] Thus, it would be of interest to determine whether metal ions cleave RNA in a DNA-RNA hybrid. Here we demonstrate that RNA structure has a dramatic effect on RNA cleavage by metal complexes. The Eu(III) hexadentate Schiff-base macrocycle ($Eu(L^1)^{3+}$) is an efficient RNA transesterification catalyst for single-stranded RNA.[11] However, $Eu(L^1)^{3+}$ or $Eu(CH_3CO_2)_3$ are unable to cleave RNA in a DNA-RNA hybrid. When a short DNA oligonucleotide is annealed to a longer fragment of RNA, the complementary RNA sequence is protected from cleavage and a footprint of the DNA-RNA hybrid is produced. Because DNA-RNA hybrids are important structures in antisense oligonucleotide strategies, our results may have a significant effect on the design of oligonucleotide-artificial ribonuclease conjugates.

Cleavage of $t\text{-RNA}^{phe}$ by $Eu(L^1)^{3+}$ produces fragments that comigrate with fragments produced by RNase $T_1$, $Zn(NO_3)_2$, or $Eu(CH_3CO_2)_3$ (Figure 1).[13,14] It has been shown previously that RNA cleavage reactions with $Ln(L^1)^{3+}$ (Ln = La, Eu, Tb, Gd) produce fragments containing a cyclic 2′,3′-phosphate monoester and a 5′-hydroxyl terminus.[11] Cleavage sites are assigned by comparison to an RNase $T_1$ digestion and the RNA ladder produced by $Eu(L^1)^{3+}$, which is similar to that produced by alkaline hydrolysis of RNA. An identical pattern is observed with $La(L^1)^{3+}$ (data not shown). A 20-base oligodeoxynucleotide was annealed to $t\text{-RNA}^{phe}$ by heating to 65 °C followed by cooling to 0 °C. The solution of RNA with the oligodeoxynucleotide was incubated with $Eu(L^1)^{3+}$ for 3 h at 37 °C (Figure 2). The DNA-RNA hybrid is shown schematically[15] (structure I). Sites in the RNA sequence complementary to the DNA strand ($A_{38}$–$G_{57}$) were protected from cleavage by $Eu(L^1)^{3+}$ with the exception of sites at the ends of the hybrid where fraying of the ends of the hybrid probably occurs ($\Psi_{55}$, $C_{56}$, $G_{57}$). (Longer incubation times led to some cleavage at $A_{38}$ as well). Cleavage at *every* nucleotide of RNA was eventually observed with the exception of those sites protected by the oligodeoxynucleotide. Similar results are obtained with $Eu(CH_3CO_2)_3$ as a transesterification catalyst; no cleavage of the DNA-RNA hybrid is observed after several hours although all other sites on the RNA are cleaved (data not shown). Preliminary studies indicate that the DNA-RNA hybrid is inert to cleavage by other metal ions including $Cd^{2+}$ and $Zn^{2+}$.

RNA transesterification proceeds by attack of the 2′-hydroxyl at the phosphate diester; hence, reactivity will be modulated by

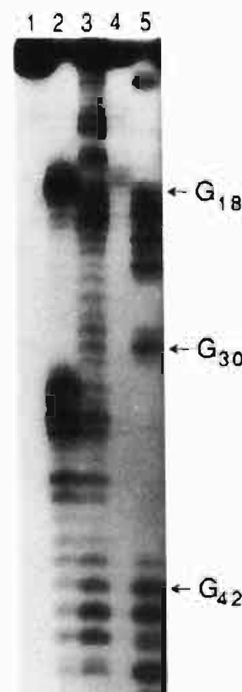


**Figure 1.** Cleavage of $t\text{-RNA}^{phe}$ by $Eu(CH_3CO_2)_3$, $Zn(NO_3)_2$, and $Eu(L^1)^{3+}$ ($L^1$ is 2,7,13,18-tetramethyl-3,6,14,17,23,24-hexaazatricyclo-[17.3.1.1]tetracosa-1(23),2,6,8,10,12(24),13,17,19,21-decane).[29] Autoradiograms are of 8 M urea denaturing polyacrylamide sequencing gels of $t\text{-RNA}^{phe}$ labeled with $^{32}P$ at the 3′-end.[30] Approximately $1 \times 10^5$ cpm of labeled $t\text{-RNA}^{phe}$ was loaded on the gel for each sample. Cold $t\text{-RNA}^{phe}$ was added to give a total concentration of t-RNA of 20 μM (1.3 mM nucleotide). Metal complex concentrations were 1.0 mM and hepes buffer was 0.4 M. Reactions with metal ions were run at pH 7.86, 37 °C for 5 h. Nucleotides are counted from the 5′-end, and labels denote the nucleotide which was cleaved (i.e., "$G_{18}$" signifies that the phosphate ester 3′ to $G_{18}$ has been cleaved). Key lane 1, control; lane 2, $Eu(CH_3CO_2)_3$; lane 3, $Eu(L^1)^{3+}$; lane 4, $Zn(NO_3)_2$; lane 5, RNase $T_1$ (G specific, weak cleavage at sites other than G is sometimes observed).

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(13) Reaction conditions are given in Figures 1 and 2.
(14) All standard precautions were taken to avoid ribonuclease contamination. Solutions were made fresh and were autoclaved and gloves were worn in all stages of experiments. In addition, the following experiments were carried out. Different samples of $Eu(L^1)^{3+}$ were recrystallized from chloroform and found to give identical cleavage results. A concentrated stock solution of $Eu(L^1)^{3+}$ was extensively dialyzed against water, and the resulting solution was incubated with $t\text{-RNA}^{phe}$ for 5 h at 37 °C under the conditions given in Figure 1. Cleavage experiments with this solution gave autoradiograms identical to the control.
(15) Cloverleaf sequence of $t\text{-RNA}^{phe}$; Y is a modified purine nucleotide: Kim, S. H.; Sussman, J. L.; Suddath, F. L.; Quigley, G. J.; McPherson, A.; Wang, A. H. J.; Seeman, N. C.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4970–4974.

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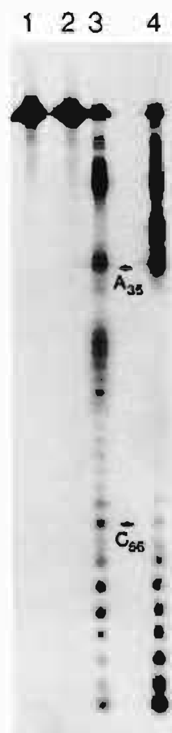
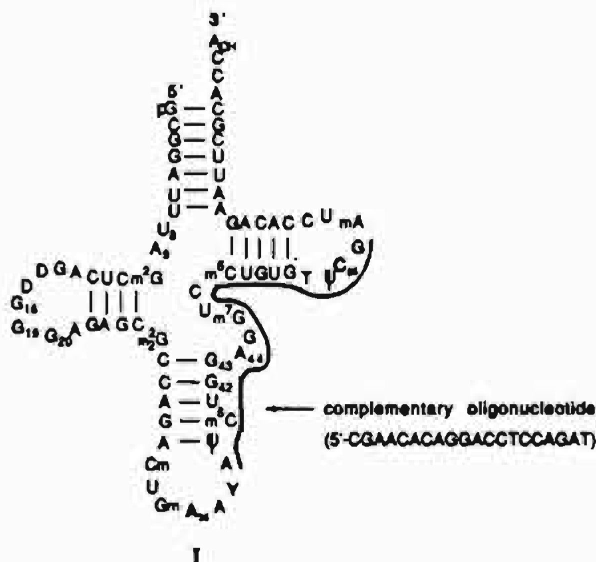



**Figure 2.** Cleavage of t-RNA$^{phe}$. Concentrations of metal complex and RNA are described in Figure 1. Incubation times were for 3 h. Key: lane 1, control; lane 2, control with oligonucleotide (20 $\mu$M); lane 3, Eu(L$^1$)$^{3+}$; lane 4, 20 $\mu$M oligonucleotide annealed to t-RNA then incubated with Eu(L$^1$)$^{3+}$. Cleavage sites were assigned from comparison to RNase $T_1$ digests and an RNA ladder as in Figure 1.




proximity and relative orientation of the 2'-hydroxyl and phosphate diester in the RNA backbone. In general, double-stranded RNA has much less conformational flexibility[16] than does single-stranded RNA. In the double-stranded A-form of RNA, the conformation of the sugar–phosphate backbone may not be favorable for transesterification. It has been noted that the ribose and phosphate are oriented such that nucleophilic attack of the 2'-hydroxyl and displacement of the 5'-hydroxyl cannot occur by an in-line displacement mechanism.[17] DNA–RNA double helices are structurally similar to RNA–RNA double helices;[16] thus, geometric constraints for phosphate ester transesterification may also be similar. Our results concur with studies which have demonstrated the inert character of RNA–RNA helices toward cleavage by organic compounds[18] and by studies on the structure dependent cleavage of t-RNA by metal ions.[19–23] Reactivity differences may also result from differences in lanthanide ion coordination to single- and double-stranded RNA. Lanthanide ions are known, however, to bind strongly to both single- and double-stranded nucleic acids.[24–26]

There are many synthetic options for attaching groups such as metal complexes to oligodeoxynucleotides.[27] Positioning the transesterification catalyst to interact with a phosphate ester may be important in the design of compounds for sequence-specific cleavage. The length and conformation of the tether may be important variables.[28–30] The resistance of RNA to cleavage by metal complexes when the RNA is in a DNA–RNA hybrid suggests that it may be advantageous in the construction of antisense oligonucleotides to place the RNA transesterification catalyst on the end of the oligonucleotide proximate to a flexible section of RNA rather than in the center of the oligonucleotide sequence. RNA reactivity differences arising from RNA structure should be considered in the design of antisense oligonucleotides that incorporate metal ion transesterification catalysts.

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