Site-Specific Hydrolysis of RNA by Europium(III) Texaphyrin Conjugated to a Synthetic Oligodeoxyribonucleotide

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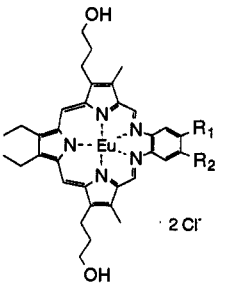
There is considerable interest in the development of antisense agents that effect the hydrolysis of their RNA complement without the participation of endogenous nucleases.[1] One of the more attractive ways of achieving this goal involves the use of metal complexes. For some time it has been known that certain lanthanide(III) metal cations and complexes catalyze the hydrolysis of RNA.[2-4] Recently, we found this is also true for europium(III) chelated by the monoanionic, pentadentate texaphyrin ligand (EuTx, **1**). In this communication we describe a DNA–EuTx conjugate based on the functionalized texaphyrin **2**. To the best of our knowledge, this "ribozyme analogue" (cf., **3**) provides the first example of an oligodeoxynucleotide-directed hydrolysis of a complementary RNA oligomer by a covalently attached metal complex. This communication thus links reports of metal cation-catalyzed hydrolysis of RNA[5,6] to those of oligodeoxynucleotide-directed cleavage of DNA using oxidative[7] and alkylation-based[8] chemistry.



1 $R_1 = R_2 = OCH_2CH_2CH_2OH$
2 $R_1 = OCH_2CO_2H$, $R_2 = H$
3 $R_1 = OCH_2CO\text{-DNA}$, $R_2 = H$

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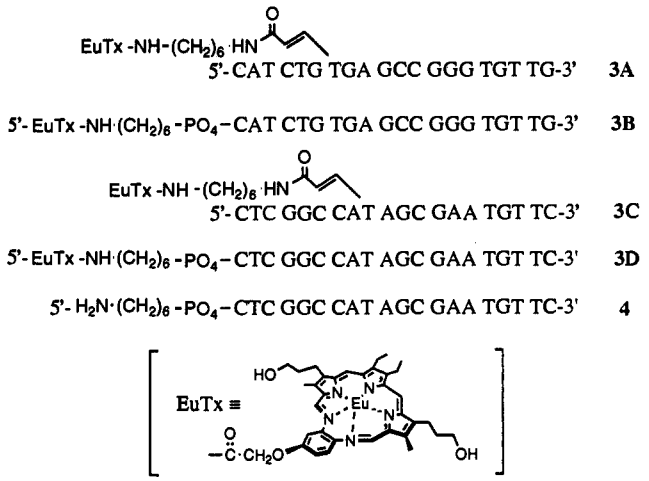

Figure 1. The four europium(III) texaphyrin (EuTx)–DNA conjugates used in this study.

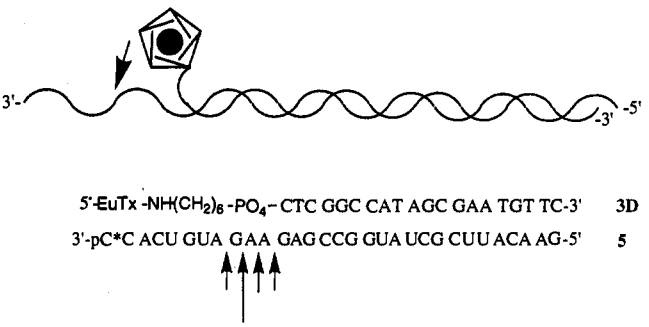

Figure 2. Schematic representation of cleavage of RNA 30-mer by EuTx–DNA conjugate; the arrows show sites of metal-catalyzed hydrolysis.

To prepare the texaphyrin-based ribozyme analogues, two 20-mer sequences were machine-synthesized to contain alkylamine groups at either the 5-position of an internal thymine residue or the 5′-end terminal phosphate.[9] Reaction of the carboxylic acid functionalized europium(III) texaphyrin complex **2** with carbodiimide and *N*-hydroxysuccinimide produced the corresponding activated ester, which was added directly to a solution of the chosen oligodeoxynucleotide amine. The resulting DNA–EuTx conjugates (Figure 1) were purified by electrophoresis.

A synthetic RNA 30-mer (**5**, Figure 2) was prepared as substrate, with a sequence selected from a unique site within the gene transcript for multiple drug resistance.[10] The 3′-^{32}P-labeled substrate was incubated with an excess of oligodeoxynucleotide conjugate at 37 °C for 18–24 h in a buffered salt solution, ethanol precipitated, and assayed on a 20% denaturing polyacrylamide

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(9) Oligodeoxynucleotide amines modified on the 5-position of thymine were purchased from Oligo's Etc.; oligodeoxynucleotide amines modified on the 5′-end were purchased from Keystone Laboratories, Inc. Oligonucleotides were HPLC purified and precipitated using LiCl prior to use.

(10) RNA 30-mer was purchased from Keystone Labs, Inc. Sequence is complementary at 1562 bases posttranscriptional start site in mouse multidrug resistance protein mRNA.

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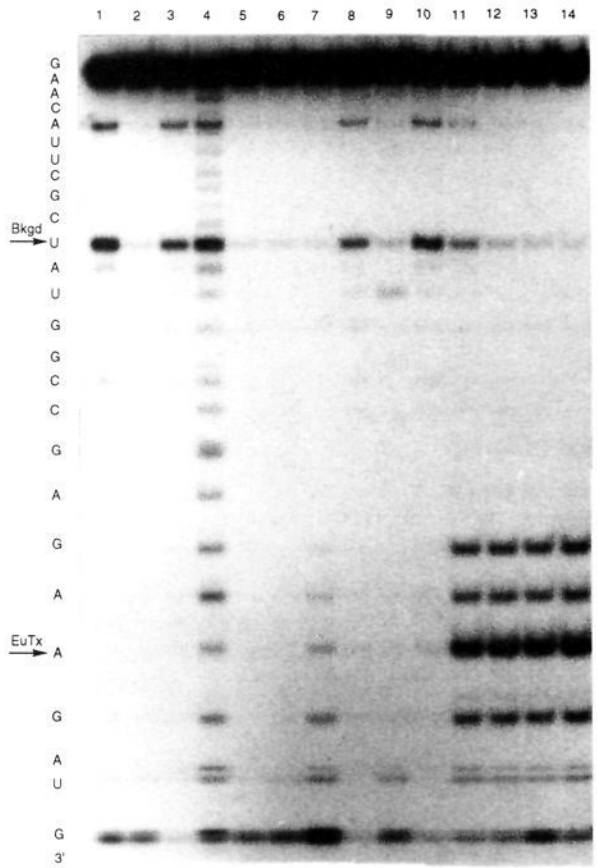

Figure 3. Cleavage of synthetic RNA 30-mer **5** by EuTx–DNA conjugates or free europium complex **2**. Autoradiograph of a 20% denaturing polyacrylamide gel of the oligoribonucleotide labeled with ^{32}P at the 3′-end.[13] Approximately 1×10^5 cpm of substrate was incubated for ca. 24 h at 37 °C in a total volume of 20 μL of buffer containing 50 mM HEPES, pH 7.5, 100 mM NaCl, 25 μM EDTA, and 5 μg/mL calf thymus DNA. Key: lane 1, oligodeoxyribonucleotide-free control; lane 2, control with unmodified oligodeoxynucleotide **4**; lane 3, 2.5 μM **2**; lane 4, 25 μM **2**; lanes 5–7, **4** and 250 nM, 2.5 μM, and 25 μM **2**, respectively; lane 8, **3A**; lane 9, **3C**; lane 10, **3B**; lanes 11–14, **3D** at 2.5 nM, 25 nM, 250 nM, and 2.5 μM, respectively. All other oligodeoxynucleotides were at 2.5 μM final concentration. Nucleotide sequence analysis was determined by partial digestion with base-specific ribonucleases,[14] cf. the supplementary material. Bkgd, background autocleavage; EuTx, europium(III) texaphyrin–DNA conjugate-mediated cleavage.

gel. As illustrated schematically in Figure 2 and shown explicitly in Figure 3, ca. 30% cleavage[11] occurred near the expected location of the europium(III) texaphyrin complex upon hybridization with conjugate **3D**. The corresponding cleavage bands were not observed when this same substrate was incubated with oligonucleotides that were noncomplementary in sequence, unmodified, or modified internally with the complex (Figure 3, lanes 8–10). Control reactions (cf. supplementary material) indicate that ambient light, calf thymus DNA, type of buffer (Tris acetate or HEPES, EDTA,[12] pH 6.0–8.0), and presence or absence of oxygen had no apparent effect on cleavage efficiency.

The cleavage fragments comigrate with bands in sequencing lanes produced by incubation of substrate under alkaline conditions or subjected to partial digestion with a series of base-specific ribonucleases.[15] This observation is consistent with a hydrolytic mechanism, presumably involving the EuTx acting as a Lewis acid that facilitates an intramolecular attack of the 2′-hydroxyl group to effect cleavage.[2,3] Interestingly, there are bands indicating site-specific cleavage of the ribonucleotide target sequence in the absence of any added cleavage reagents (lane 1, Figure 3). Although the source of this background cleavage is unknown, we believe it is the direct result of a higher order structure (i.e., a hairpin) of the oligoribonucleotide, since hybridization with any complementary oligonucleotide dramatically inhibits the cleavage (e.g., lanes 2, 5–7, 9, Figure 3). This type of structure-dependent cleavage behavior has been seen previously with oligoribonucleotides.[5,6]

Maximal cleavage activity of the DNA–EuTx was observed down to 25 nM conjugate. We assume the decreased cleavage below this level was due to a decrease in hybridized material (as judged by increased background cleavage of the target RNA).[16] By means of comparison, the free EuTx complex nonspecifically hydrolyzed the RNA substrate at 25 μM (lane 4, Figure 3). In the control reaction containing both free EuTx complex and the nonderivatized complementary DNA oligomer (lane 7, Figure 3), cleavage occurred predominantly in the single-stranded region, although still at lower efficiency than the EuTx–DNA conjugate at 2.5 nM. Thus, attachment of the EuTx to the DNA probe increases its effective concentration ca. 10 000-fold.[17] This augurs well for the use of such conjugates in antisense applications. Efforts directed toward improving the cleavage efficiency and catalytic turnover of this system are underway.

Acknowledgment. J.L.S. thanks the NIH (Grant No. AI 28845) for partial support of this work. **Note Added in Proof:** The dysprosium(III) analogue of **3D** has been found to effect ca. 2-fold greater cleavage of the RNA under conditions identical to those reported herein (i.e., concentration, pH, temperature, etc.).

Supplementary Material Available: Characterization data for **2**, polyacrylamide gel autoradiographs (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(11) Cleavage yield was measured by densitometry and calculated as the ratio of cleavage band to intact material.

(12) EDTA inhibits cleavage by free lanthanide(III) cations as observed in ref 3a.

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(15) Cleavage of 5′-labeled RNA **5** produced fragments of appropriate length and phosphate termini. For an example of a similar end-product analysis, see ref 5.

(16) RNA concentration in cleavage reactions was ca. 1 nM.

(17) A target RNA without the secondary structure observed here would likely allow for cleavage at lower DNA–EuTx concentrations.