Sequence-Specific Photocleavage of DNA by an Expanded Porphyrin with Irradiation above 700 nm

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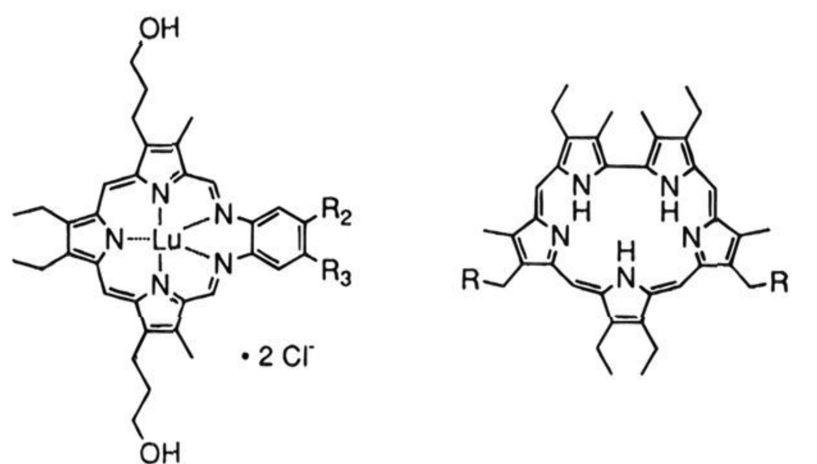
The development of chemically modified oligonucleotides as a means of regulating gene expression is a topic of current widespread interest.[1] One approach being pursued involves using antisense agents containing photoactivatable groups.[2–6] Such a strategem offers the advantage of providing sequence-specific reagents that are inert in the dark, yet subject to being "switched on" *via* irradiation at a particular wavelength. Currently, oligonucleotide conjugates containing azido derivatives,[2] ellipticines,[3] psoralens,[4] proflavins,[5] and porphyrin moieties[6] are known. All of these derivatives modify DNA when irradiated at wavelengths below 700 nm. For use *in vivo*, however, photoactivation in the 700–900 nm region would be preferable as it is here that bodily tissues are most transparent.[7] In this communication, we describe the photocleavage capabilities of two expanded porphyrins, the lutetium(III) texaphyrin complex (LuTx) **1** and the nonmetalated sapphyrin **4**, both of which cleave DNA upon irradiation at wavelengths above 700 nm. The more efficacious of these two chromophores, LuTx **1**, has also been incorporated into oligonucleotide conjugates (e.g., **3**). The latter provide the first example of oligonucleotide-directed photocleavage of DNA with irradiation above 700 nm.



1 $R_2 = R_3 = OCH_2CH_2CH_2OH$
2 $R_2 = OCH_2CO_2H$, $R_3 = H$
3 $R_2 = OCH_2CO$-RNA (2'-OMe), $R_3 = H$
4 $R = CH_2CON(CH_2CH_2OH)_2$

LuTx **1** and sapphyrin **4** were screened for their photocleavage capabilities against the known photocleaving agent

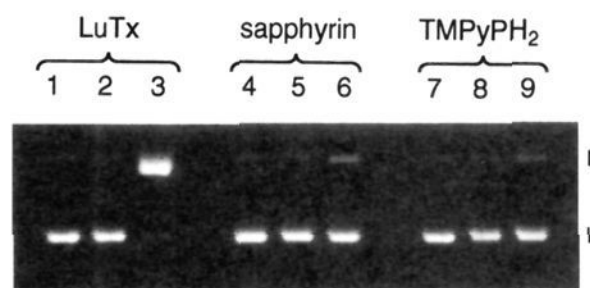


Figure 1. Photograph of a 0.8% agarose gel containing ethidium bromide showing the results of a pBR 322 plasmid DNA cleavage study. In accord with the general procedure of refs 8 and 10, the DNA was irradiated in quartz cuvettes in the presence of either LuTx **1**, sapphyrin **4**, or $TMPyPH_2$. Irradiation was effected at room temperature using light from a high-pressure xenon lamp (Oriel) passed through a 700 nm filter (CVI Laser). The sample received approximately 280 mW/cm^2. The following conditions pertained. DNA: 20.4 ng/μL (all cases). Lanes 1, 4, 7: 0 μM chromophore, $t = 60$ min. Lanes 2, 5, 8: 4 μM chromophore, $t = 0$ min. Lanes 3, 6, 9: 4 μM chromophore, $t = 60$ min.

meso-tetrakis(4-*N*-methylpyridyl)porphine ($TMPyPH_2$),[8,9] using a general pBR 322 plasmid DNA assay.[8,10] DNA cleavage was followed by monitoring of the conversion of supercoiled (form I) plasmid DNA to the nicked circular (form II) DNA. At wavelengths above 300 nm, all three compounds showed efficient photocleavage (cf. supplementary material). When, however, the shorter wavelengths were blocked out with a 700 nm filter, cleavage efficiencies of 8%, 17%, and 93% were recorded for the porphyrin control, sapphyrin **4**, and LuTx **1**, respectively (Figure 1).[11,12]

In order to test the ability of LuTx to cleave DNA within a double-stranded helix, the LuTx complex **2** was conjugated with two 2'-*O*-methyl RNA 15-mers (Figure 2, **3A** and **3B**).[13–15] Synthetic DNA 36-mers **5** and **6** were selected as substrates,[14] each complementary in sequence to only one of the LuTx

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(11) The factors influencing the relative photocleavage efficiencies of the different chromophores are complex and could reflect such effects as differences in absorption maxima, singlet oxygen quantum yields, binding affinities, etc.

(12) Cleavage yields were measured by densitometry and calculated as in ref 8.

(13) 2'-OMe derivatives were chosen because they were thought to be more useful for *in vivo* work. Unmodified DNA conjugates were also made. However, the conjugates prepared using 2'-*O*-methyl RNA proved more effective. Cf. footnote 22.

(14) 2'-OMe derivatized oligoribonucleotide amines modified on the 5' end were purchased from Keystone Laboratories, Inc., as were the DNA 36-mer substrates. All conjugates and substrates were PAGE purified and ethanol precipitated prior to use.

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(16) Photochemical reactions were carried out by treating 20 μL samples through the side wall of a 1.6 mL centrifuge tube with a dye laser (Coherent) using a power density of 150 mW/cm^2.

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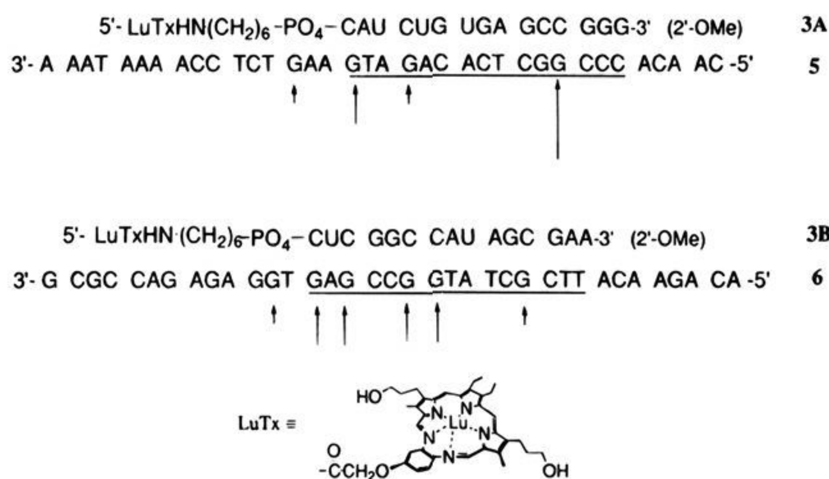

Figure 2. Synthetic DNA 36-mers **5** and **6** used as complementary targets for the LuTx-2'-*O*-methyl RNA conjugates **3A** and **3B**. Arrows indicate positions of strong (40%), intermediate (15–25%), or weak (≤10%) DNA photomodification as exposed by treatment with piperidine.[24]

conjugates. The 5'-^{32}P-labeled 36-mers were hybridized briefly with an excess (50 nM) of conjugate, whereupon samples were irradiated for 15 min using a dye laser tuned to 732 nm.[16] No cleavage was detected in the absence of light (Figure 3, lanes 1, 2, 7, and 8). The presence of the complementary LuTx conjugate, but not the control noncomplementary conjugate, led to photodegradation of each of the DNA targets (Figure 3, lanes 3 and 10). Cleavage products were found to comigrate exclusively with bands generated by the Maxam–Gilbert sequencing (G) reaction,[17] consistent with a mechanism involving singlet oxygen generation.[18] pBR 322 cleavage studies in the presence of sodium azide, a known singlet oxygen quencher,[19] and under nitrogen and oxygen atmospheres also support this mechanism.[20,21] Interestingly, the sites of reaction primarily lie across the minor groove from the position of LuTx attachment within the duplex, although some cleavage is found also to occur across the adjacent major groove.[22] The cleavage of DNA at these sites totals 70–80% on each substrate.[23]

The efficacy of **3A** and **3B** in effecting photocleavage in primarily double stranded regions leads us to suggest that LuTx-derived photoactivatable conjugates could be prepared to target duplex DNA within a triple-helical motif using irradiation above 700 nm.[3,25]

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(20) Irradiation at ≥700 nm for 30 min. Cleavage: 63% and 3% in the presence of 150 mM NaCl and NaN_3, respectively; 68%, 83%, and 14% under air, oxygen, and nitrogen, respectively.

(21) LuTx is known to be an efficient singlet oxygen generator and is being studied as a potential photosensitizer for use in photodynamic therapy. See: Sessler, J. L.; Hemmi, G.; Mody, T. D.; Murai, T.; Burrell, A.; Young, S. W. *Acc. Chem. Res.* **1994**, *27*, 43–50.

(22) The relative amounts of cleavage appear to depend on factors affecting duplex conformation such as sequence and the type of backbone of the conjugate. The nature of these effects is currently being studied.

(23) Samples were irradiated for 15 min to emphasize cleavage yield. Under these conditions, multiple-hit kinetics may be operative. Indeed, preliminary time course experiments, which compared 5, 10, 30, and 60 min, showed a shift in the distribution of cleavages to shorter products which appeared to be complete within 30 min.

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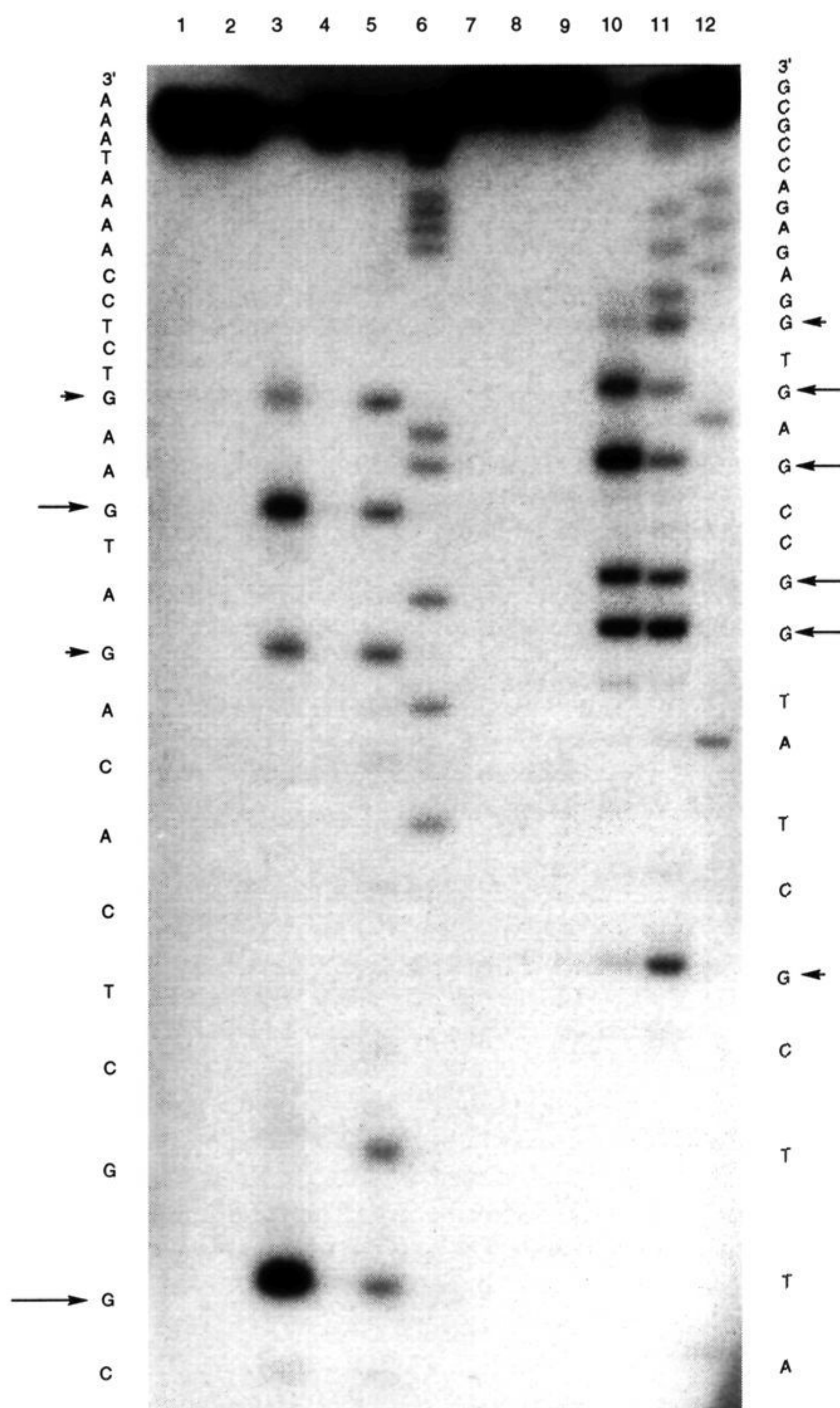

Figure 3. Cleavage of synthetic DNA 36-mers **5** and **6** by LuTx-2'-*O*-methyl RNA conjugates **3A** and **3B**. Autoradiograph of a 20% denaturing polyacrylamide gel of the oligodeoxyribonucleotides labeled with ^{32}P at the 5'-end. Approximately 1 × 10^5 cpm of substrate was heated briefly at 60 °C and allowed to cool to ambient temperature 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. Samples were irradiated for 15 min at 732 nm.[16] Key: lanes 1–6, substrate **5**; lanes 7–12, substrate **6**; lanes 1, 3, 7, and 9, 50 nM **3A**; lanes 2, 4, 8, and 10, 50 nM **3B**; lanes 1, 2, 7, and 8, dark controls; lanes 5 and 11, Maxam–Gilbert (G) sequencing reaction;[17] lanes 6 and 12, Iverson–Dervan (A) sequencing reaction.[26] Final substrate DNA concentration: ca. 1 nM. Arrows indicate sites of LuTx-induced photocleavage as exposed by treatment with piperidine.[24]

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Supplementary Material Available: pBR 322 gels (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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