N-Methylmesoporphyrin IX Exhibits G-Quadruplex-Specific Photocleavage Activity


N-Methylmesoporphyrin IX (NMM) has long been known as a G-quadruplex DNA (G4) ligand. However, there has been little investigation into its G-quadruplex photocleavage activity. Herein, we demonstrate that NMM is a highly selective photocleavage agent for G4 structures but not duplex DNA. Analysis of the cleavage products by PAGE demonstrates that G4 photocleavage by NMM occurs at sites similar to those cleaved by TMPyP4, a nonselective DNA photocleavage agent. Although NMM is shown here to generate singlet oxygen in the presence of both duplex and G4, the lack of increased photocleavage in D2O indicated that singlet oxygen is not involved in the photocleavage of G4 by NMM.

The ability of G-rich DNA sequences to adopt non-B-form, G-quadruplex (G4) structures is well-established; these G4 structures are highly polymorphic, forming different topologies based on both DNA sequence and environmental factors.[1] The growing interest in understanding the dynamic nature and potential biological roles of G4 structures in vivo has led to the search for molecular probes capable of highly selective photocleavage of these structures.[2] The porphyrin TMPyP4 (Scheme 1) has been shown to photocleave G4, but lacks selectivity, binding to and effecting photocleavage of duplex DNA with similar efficiency.[3] N-Methylmesoporphyrin IX (NMM; Scheme 1) has been shown to bind selectively to G4 structures,[4] and inhibit the unwinding of G4 by Bloom’s syndrome-associated G4 helicase BLM.[5] More recently, the ability of NMM to induce topological changes in G4 structures and the integral role of the nonplanar, N-methylated porphyrin ring of NMM in this selectivity has been demonstrated.[6]

Unlike TMPyP4 and related planar porphyrins, which have demonstrated DNA photocleavage activity, no studies investigating the DNA-photocleavage activity of NMM have been reported. One published abstract has interestingly reported that NMM photosensitized the generation of singlet oxygen in the presence of G4 but not duplex DNA.[7] We have therefore investigated the G4 photocleavage potential of NMM, and report here that this nonplanar porphyrin is an extremely selective photocleavage agent for G4 versus duplex DNA.

We have previously described a fluorescence-based assay for G4 cleavage agents that uses dual-labeled G4-forming oligonucleotides (Figure 1A).[2b] We employed this assay by using two dual-labeled G4-forming sequences: a 22-mer derived from the cMYC promoter region (F-cMyc-T) and a 21-mer derived from the human telomeric sequence (F21T). Irradiation with 420 nm lamps of solutions of these two G4 structures (100 nM) in the presence of NMM (1 μM) in either Na+ or K+–containing buffers demonstrated a significant extent of G4 photocleavage, particularly for the cMyc sequence in the presence of K+ (Figure 1B, C). NMM photocleavage of this sequence was also observed with visible-light irradiation (fluorescence bulbs), albeit at about 20% the efficiency (see the Supporting Information). In contrast, neither G4 sequence afforded significant photocleavage in the presence of Na+. NMM is known to preferentially interact with parallel-stranded G4 structures formed in K+–containing buffers, and can also induce the formation of parallel-stranded topologies when co-annealed with G4-forming sequences.[8] We thus explored the effect of annealing these G4-forming sequences in the presence or absence of NMM on the photocleavage. Surprisingly, the topological changes induced upon annealing these G4-forming sequences in the presence of NMM result in complexes that are less efficiently photocleaved (see the Supporting Information).

In order to determine the selectivity of NMM photocleavage, a mixed-sequence dual-labeled hairpin duplex oligonucleotide (F-mixed-T) was employed. As shown in Figure 2A, no significant cleavage of this duplex was observed over a range of NMM concentrations, in contrast to the F-cMyc-T G4 sequence.
We also examined the ability of NMM to photocleave F-cMyc-T in the presence of other DNA structures. Photocleavage was carried out in the presence of a tenfold excess (relative to F-cMyc-T concentration) of unlabeled single-stranded, duplex, triplex, and i-motif DNA as well as duplex and triplex RNA and unlabeled cMyc (Figure 2B). There was no significant inhibition of the photocleavage of the labeled F-cMyc-T in the presence of competitor unlabeled cMyc. Among the non-G4-forming sequences, the highest photocleavage inhibition was 20% in the presence of excess triplex DNA, and even in the presence of 100-fold excess duplex DNA there was only a 20% inhibition of F-cMyc-T photocleavage. Finally, we compared the ability of NMM and TMPyP4 to effect DNA nicking by using a supercoiled plasmid assay (Figure 2C). Irradiation of supercoiled plasmid in the presence of NMM produced insignificant photocleavage, even at 50 μM concentration. In contrast, TMPyP4 at 10 μM effects nearly complete photocleavage of supercoiled DNA to relaxed and duplex linear products. The selectivity of NMM photocleavage of G4 DNA versus duplex DNA is reflected in the binding selectivity of NMM. UV/Vis titrations show that NMM binds more strongly to the K+ stabilized cMyc G4 ($K_d = 0.6$ μM) than to duplex DNA ($K_d = 24$ μM; see the Supporting Information). However, the photocleavage selectivity of NMM exceeds this binding selectivity, thus indicating that the NMM–duplex DNA complex, which likely involves external association, is not competent for photocleavage.

In order to gain more insight into the photocleavage of cMyc G4 by NMM, the DNA photoproducts were analyzed by PAGE and compared to those produced by TMPyP4 (Figure 3). The overall pattern of photocleavage by these two agents is remarkably similar, despite their widely different selectivity. Both NMM and TMPyP4 produce small quantities of frank DNA...
breakage products that are observed immediately after irradiation; however, the majority of photocleavage products are revealed only after piperidine/heat treatment. In both cases, the major sites of cleavage occur at G residues on either 5’- or 3’-end of runs of G’s. More detailed analysis of the sequence selectivity of NMM photocleavage was obtained by quantifying the bands corresponding to cleavage products and mapping the cleavage sites onto the NMR-derived structure of this G4 (Figure 4).[36]

The cleavage pattern shown in Figure 4 has elements that are similar to both those reported for singlet oxygen (¹O₂)-based cleavage (equal amounts of cleavage of terminal G-tetrads) and single electron oxidation (preferential cleavage of S’ G’s in G tracks, e.g., G17) of G4.[37] Thus, we carried out additional experiments to determine the role of singlet oxygen in photochemical cleavage by NMM. Photochemical cleavage reactions carried out in D₂O afforded similar levels of cleavage to those carried out in H₂O (Figure 5A). As the lifetime of ¹O₂ is longer in D₂O than H₂O, this result indicates that photochemical cleavage by NMM is not due to ¹O₂ generation. However, we also examined the ability of NMM to photogenerate ¹O₂ in the presence of G4 and duplex DNA by using a highly sensitive fluorescence sensor for ¹O₂ generation (Figure 5B). Surprisingly, more ¹O₂ was detected in samples containing NMM and duplex or G4 DNA compared to NMM alone (Figure 5B, gray bars). The increased production of ¹O₂ by NMM in the presence

![Figure 3](image-url)  
**Figure 3.** PAGE analysis of the cMyc photocleavage products formed by NMM (1 µM) and TMPyP4 (0.25 µM). The photocleavage products were either immediately loaded on a 20% polyacrylamide gel or first treated with 10% piperidine at 90°C prior to loading on the gel. The gel was directly visualized by using 6-carboxyfluorescein (FAM) fluorescence (λex = 488 nm, λem = 530 nm).

![Figure 4](image-url)  
**Figure 4.** Analysis of the DNA photocleavage products of cMyc mapped onto the NMR structure of the cMyc G4. A) The band intensities of the gel shown in Figure 3 for the lane corresponding to cleavage in the presence of NMM following piperidine/heat treatment after subtraction of the bands in the ligand-free irradiated sample. B) Representation of the topology of the cMyc sequence G4 with arrows signifying the sites of photocleavage by NMM. The length of the arrows is proportional to the intensity of the corresponding band shown in (A).

![Figure 5](image-url)  
**Figure 5.** Singlet oxygen is not involved in the NMM-mediated photocleavage of G4 DNA. A) Photocleavage of F-cMyc-T (250 nm) with NMM (1 µM) was carried out in H₂O or D₂O, and the products were analyzed by PAGE both before and after piperidine/heat treatment. No significant difference in the amount of DNA cleavage products was observed in the D₂O versus H₂O reactions (e.g., boxed bands). B) The production of ¹O₂ in photochemical reactions of NMM (1 µM) alone or in the presence of cMyc G4 DNA (10 µM) or calf thymus duplex DNA (10 µM base-pair) in K⁺-containing buffer as measured by the increase in fluorescence due to Singlet Oxygen Sensor Green (SOSG, 1 µM). Samples were subjected to irradiation (420 nm lamps) for 15 min, and the fluorescence intensity of the SOSG ¹O₂ adduct (λex = 504 nm, λem = 525 nm) was measured and normalized to that of a sample containing the singlet oxygen sensitizer erythrosine B (1 µM). Gray bars are from reactions where NMM was added from a 155 µM stock in water; red bars are from reactions in which NMM was added from a stock in DMSO, with a final DMSO concentration in all reactions of 1% v/v.
of DNA appears to be at least partially due to disaggregation of the ligand; when NMM is added from a stock solution in DMSO (final DMSO concentration = 1%), there is significantly more $^{18}O_2$ produced by NMM alone, and the amounts of $^{18}O_2$ produced in the presence of duplex and G4 DNA are similar (Figure S5, red bars). However, as NMM does not cause extensive photocleavage of duplex DNA, the levels of $^{18}O_2$ detected do not appear to be relevant to the DNA cleavage observed.

In conclusion, NMM is a highly selective photocleavage agent for specific G4 DNA structures, with negligible duplex photocleavage activity. NMM photocleavage appears to favor parallel-stranded G4 topologies, and the slight topological changes induced upon co-annealing G4 with NMM afford complexes with diminished photocleavage propensity. Although NMM can afford $^{18}O_2$ upon irradiation in the presence of both duplex and G4 DNA, this does not correlate with the photochemical cleavage, which is highly selective for G4 and unaffected by carrying out photocleavage in D$_2$O. Further studies of the mechanism of the selective G4 photocleavage by NMM and its application to the study of G4 formation and dynamics in biological systems are on-going.

**Experimental Section**

**General:** HPLC-purified F21T (5'-FAM-dGGGTTTGGGTTGGTTGGTAG-TAM-3'), F-chMy-cT (5'-FAM-dTGGAGTTGGATGGTTGGTAG-TAM-3'), and F-mixed-cT (5'-FAM-GCATGCTTTTTGATGCTG-TAM-3') and the unlabeled oligonucleotide F-cMy (5'-GAGGTTGGATGGTTGGTAG) were purchased from Integrated DNA Technologies (IDT, Coralville, Iowa, USA) and used without further purification. The plasmid pX174 RF-1 was purchased from Fisher and used without further purification. Singlet Oxygen Sensor Green (SOSG) was purchased from Fisher. NMM was purchased from Frontier Scientific (Logan, Utah, USA). Calf thymus DNA was purchased from Sigma–Aldrich and used without further purification. Buffers consisted of Na$^+$ or K$^+$ cacodylate (50 mM) prepared from cacodylic acid and NaOH or KOH, used to adjust the pH to 7.4.

**Photocleavage reactions:** Photocleavage reactions were carried out in a Luzchem photoreactor with 420 nm lamps, as previously described.$^{[38]}$ Briefly, solutions of dual-labeled DNA (100 nm) in buffer with or without added NMM (1 µM) in 96-well plates were irradiated and treated with a dissociation buffer (5 mM K$^+$-cacodylate, pH 7.4, 10 µg/mL $^1$-calf thymus DNA), and the plates were sealed, heated to 85 °C for 30 min, and subjected to centrifugation (2250g, 10 min). The plates were analyzed for FAM fluorescence on a Cary Eclipse spectrophuorimeter or a PerkinElmer Victor 3 V plate reader. The percent cleavage was calculated as previously described,$^{[38]}$ and the values reported are averages of three separate reactions $\pm$ standard deviation. Alternatively, the photocleavage reactions were analyzed by PAGE on 20% polyacrylamide gels either immediately after irradiation or following treatment with 10% pyridine at 90 °C for 30 min. After electrophoresis, the gels were imaged on a BioRad Pharos imager ($\lambda_{em}$ = 485 nm, $\lambda_{ex}$ = 535 nm). Band intensities were quantified by exporting the raw image file and analyzed by ImageJ.

**Singlet oxygen determination:** Singlet oxygen was detected by co-incubating reaction mixtures with SOSG (1 µM) in quartz fluorescence cells followed by irradiation in the photochemical reactor. The resulting fluorescence of the singlet oxygen adduct of SOSG was directly measured on a Cary Eclipse spectrophuorimeter ($\lambda_{em}$ = 504 nm/$\lambda_{ex}$ = 525 nm) and expressed as the normalized fluorescence by taking the ratio of the observed fluorescence to the fluorescence of an irradiated solution containing 1 µM erythrosine B and SOSG.

**Acknowledgements**

We would like to acknowledge the Cancer Prevention and Research Institute of Texas (grant RP160852) and Texas State University for support of this work.

**Conflict of Interest**

The authors declare no conflict of interest.

**Keywords:** G-quadruplexes • photocleavage • porphyrins • singlet oxygen

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Manuscript received: January 1, 2019
Revised manuscript received: February 28, 2019
Accepted manuscript online: March 8, 2019
Version of record online: May 27, 2019