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DNA-Programmed Control of Photosensitized Singlet Oxygen Production

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Singlet molecular oxygen, $O_2(a^1\Delta_g)$, plays a major role in the degradation of materials and in photoinduced cell death.[1] Although the direct transition from oxygen's triplet ground state, $O_2(X^3\Sigma_g^-)$, to $O_2(a^1\Delta_g)$ is forbidden, the process can be mediated by energy transfer to $O_2(X^3\Sigma_g^-)$ from the triplet state of a photosensitizer.[2] This is applied in photodynamic therapy, a medical treatment wherein $^1O_2$ thus produced is used to kill cells and destroy undesired tissue (e.g., cancerous tumors).[3]

In biological systems, the distance over which $^1O_2$ will diffuse in its lifetime is much smaller than the diameter of a cell.[4] Therefore, it is desirable to design sensitizers that not only selectively target diseased cells but also target pertinent subcellular organelles.[5] It is also desirable to develop methods by which the efficiency of $^1O_2$ production can be selectively controlled. For example, Chen et al. recently developed a system in which a photosensitizer was tethered to a $^1O_2$ quencher by a short peptide sequence.[6] In the presence of a specific protease, the peptide chain was cleaved, which, in turn, led to an increase in the amount of $^1O_2$ that could be optically detected. In the same vein, McDonnell et al.[7] have developed a sensitizer system that responds to the pH of the medium.

In this report, we demonstrate the phenomenon of DNA sequence-controlled on-and-off switching of a $^1O_2$ sensitizer. In our approach, the photosensitizer pyropheophorbide-*a* (**P**) is attached to a short 15-mer nucleotide sequence. Similarly, the so-called "black hole quencher 3" (**Q**) is attached to a 21-mer oligonucleotide which complements the **P**−DNA strand (Figure 1).

By DNA-programmed assembly, the two **P** and **Q** conjugates are brought into close proximity.[8] In analogy with work on DNA-based molecular beacons,[9] the singlet state of **P** is then quenched via Förster resonance energy transfer (FRET) or by contact-mediated electron exchange. This quenching leads to smaller yields of both fluorescence and triplet state production in **P**. In turn, and most importantly, the latter influences the $^1O_2$ yield.

We demonstrate that, for the **P**−DNA system, both the fluorescence of **P** and $^1O_2$ production are almost completely shut down in the presence of the complementary **Q**−DNA conjugate. Furthermore, we show that the addition of a third DNA sequence can displace and release the **P**−DNA conjugate from the **P**−**Q** pair and turn the production of $^1O_2$ back on (Figure 1). In this process, we thus model a benign drug (the **P**−**Q** pair) that becomes active only in the presence of a specific targeted nucleotide sequence.

The sensitizer **P** (Figure 2A) has an absorption maximum at 415 nm and a fluorescence maximum at ∼670 nm. However, S→T intersystem crossing also occurs to produce a triplet state from which energy transfer to oxygen can occur. We have measured a $^1O_2$ quantum yield for **P** in toluene of 0.53 ± 0.04, which is in good agreement with reported values of ∼0.45 for derivatives of **P** in benzene.[10]

For the formation of the **P**−DNA conjugate, commercially available **P**-COOH was converted into the NHS ester[11] and acylated with 3-aminopropanol. The latter was converted into the phosphoramidite (Figure 2B) and incorporated at the 5′-terminus of a 15-mer nucleotide sequence by automated DNA synthesis (**P**−**a**, Figure 2C).

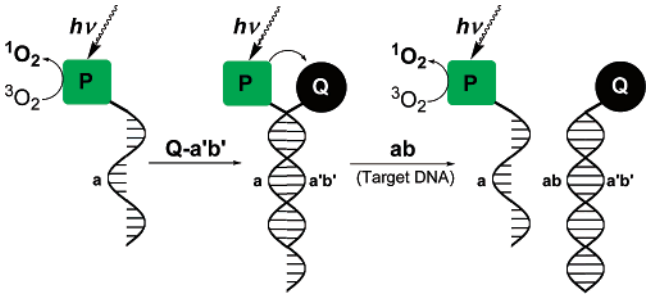

Figure 1. DNA sequence-specific control of $^1O_2$ generation.

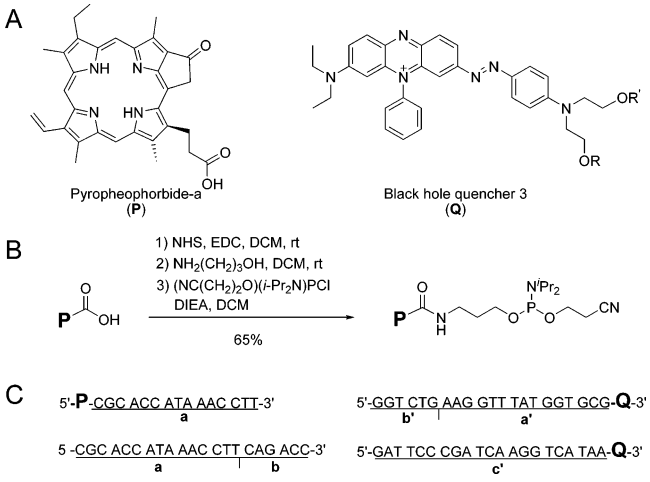

Figure 2. (A) Structures of **P** and **Q**, (B) conversion of **P** to a phosphoramidite for incorporation with DNA, and (C) nucleotide sequences in the DNA strands used.

Q is commercially available as a phosphoramidite which can be incorporated at the 3′-end of a DNA strand, and the conjugate **Q**−**a′b′** was obtained by standard procedures (Figure 2C). The conjugates were purified or analyzed by HPLC and characterized by mass spectrometry and UV absorption.

Events depicted in Figure 1 were monitored using an emission microscope that has been described.[4b,c] Through the use of specific band-pass filters and a detector sensitive to a broad range of wavelengths, this system is suited to the detection of both $^1O_2$ phosphorescence and **P** fluorescence. All experiments were performed in a deuterated aqueous buffer, since $^1O_2$ has a larger quantum efficiency of phosphorescence in D_2O compared to H_2O.[1,2]

In initial experiments, $^1O_2$ production from 5 μM **P**−**a** conjugate was monitored. The background signal from the buffer solution was subtracted, and the data were normalized to yield a reference standard (Figures 3 and 4, column 1). To study the efficiency of the quenching and the required stoichiometry, **P**−**a** was titrated

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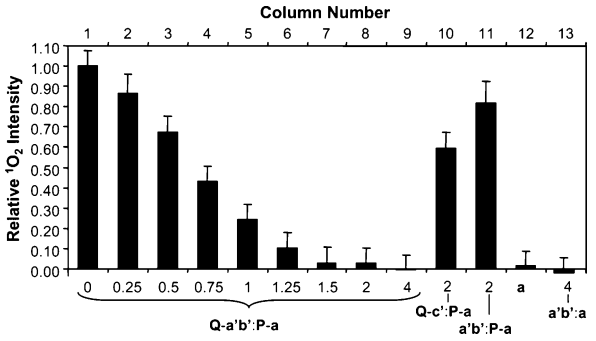

Figure 3. Quenching of $^1O_2$ production from **P**−**a**, monitored using $^1O_2$ phosphorescence at 1270 nm: column 1, **P**−**a** reference; columns 2−9, titration with 0.25−4 equiv of **Q**−**a′b′**; column 10, noncomplementary **Q**−**c′**; column 11, **P**−**a**, **a′b′**; column 12, **a**; column 13, **a**, **a′b′**.

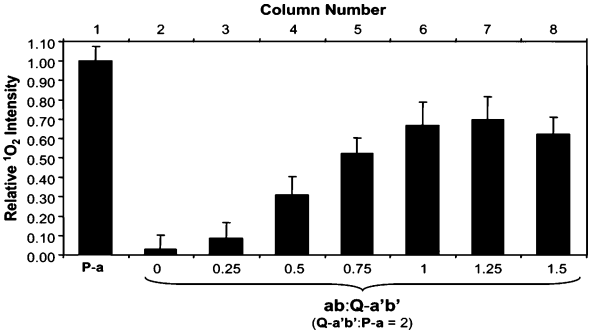

Figure 4. Efficiency of $^1O_2$ generation upon competitive release of **P**−**a** by addition of a target DNA sequence: column 1, **P**−**a**; column 2, **Q**−**a′b′**, **P**−**a** (2:1); columns 3−8, titration of **Q**−**a′b′**, **P**−**a** with up to 1.5 equiv of **ab** (relative to **Q**−**a′b′**).

with up to 4 equiv of **Q**−**a′b′** (Figure 3, columns 2−9). In the presence of 1 equiv of **Q**−**a′b′**, the $^1O_2$ production from **P**−**a** was reduced to 25% of the initial value. With **Q**−**a′b′** equivalents greater than 1, efficient quenching of $^1O_2$ production was obtained. A similar trend was observed for the quenching of **P**−**a** fluorescence (see Supporting Information).

If **Q** is linked to a nucleotide sequence that does not complement that in **P**−**a**, such as **Q**−**c′** (Figure 2C), the addition of 2 equiv of **Q**−DNA leads to only moderate quenching of **P**−**a**; $^1O_2$ production is reduced to 60% of the original value (Figure 3, column 10). These data likely reflect quenching from free **Q**−**c′**. In control experiments, it was demonstrated that hybridization of **P**−**a** with an **a′b′** sequence lacking **Q** did not decrease the $^1O_2$ production significantly (Figure 3, column 11). Moreover, in the absence of **P**, the sequences **a** or a mixture of **a** and **a′b′** gave rise to a signal that was almost identical to that from the buffer alone (Figure 3, columns 12 and 13).

We have shown that $^1O_2$ production from **P**−**a** is efficiently quenched in the presence of 2 equiv of **Q**−**a′b′**. We next investigated the ability of a third "target" DNA sequence, **ab** (Figure 2C), to disrupt the 15-mer duplex between **P**−**a** and **Q**−**a′b′** by formation of a stronger 21-mer duplex. This process would release **P** from its proximal position to **Q** and be manifested in an increased production of $^1O_2$. As shown in Figure 4, the mixture of **P**−**a** and **Q**−**a′b′** (1:2) was subjected to titration with various amounts of the competing sequence **ab**. As expected, only limited recovery of $^1O_2$ production is observed in the presence of small amounts of **ab**, since this initial addition primarily hybridizes with the excess **Q**−**a′b′** in solution. However, with larger amounts of added **ab**, we have been able to record up to 85% recovery of $^1O_2$ production.

We believe these latter data indeed reflect the phenomenon of competing hybridization as illustrated in Figure 1. This was supported by polyacrylamide gel electrophoresis, demonstrating the hybridization of the couples **P**−**a**, **Q**−**a′b′**, and **Q**−**a′b′**,**ab** (see Supporting Information).

Fluorescent probes are widely used as molecular beacons for the detection of specific DNA sequences and for monitoring the expression of m-RNA in cells. We have shown here that similar hybridization events can be used for the controlled production of a reactive species, singlet oxygen. Because the latter plays a critical role in mechanisms of photoinduced cell death, the results presented here could potentially influence the development of new methodologies used in photodynamic therapy.

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Supporting Information Available: Experimental procedures; HPLC chromatograms; UV, mass, and NMR spectra; PAGE gels; fluorescence studies of **P**−**a** quenching and release. This material is available free of charge via the Internet at http://pubs.acs.org.

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