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Singlet Oxygen

Reversible pH-Regulated Control of Photosensitized Singlet Oxygen Production Using a DNA i-Motif**

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Singlet molecular oxygen ($^1O_2$) is a reactive intermediate that is important in fields that range from materials science to medicine.[1] Singlet oxygen has a characteristic chemistry in which molecules are oxygenated that sets it apart from the ground triplet state of oxygen ($^3O_2$). These oxygenation reactions can be important in processes that include polymer degradation and cell death.

Singlet oxygen is commonly and conveniently produced by photosensitization.[2] In this process, light is used to create an excited state of a given molecule, the photosensitizer, which in turn transfers its energy of excitation to $^3O_2$ to generate $^1O_2$. The judicious use of light and sensitizers facilitates a great deal of control in the production of $^1O_2$,[3] which can then be applied, for example, in the selective killing of undesired cells (e.g., the medical procedure of photodynamic therapy, PDT, whereby cancerous tissue can be removed[4]).

Even though significant progress has been made in the development and use of biologically relevant $^1O_2$ sensitizers, the available systems are still often indiscriminate, which in turn limits their usefulness. Thus, the development of sensitizer systems that provide more control and selectivity over the photosensitized production of $^1O_2$ has received increasing attention.[1,3,5] In particular, systems that allow reversible switching of the photosensitizer between "on" and "off" configurations have gained interest.

A convenient method to control the ability of a photosensitizer to produce $^1O_2$ is to alter the efficiency with which energy can be transferred from the excited state of the sensitizer to $^3O_2$. This principle has been demonstrated by selectively placing the $^1O_2$ sensitizer close to a molecule that can quench the excited state of the sensitizer by using a positioning system that can then be manipulated to change the distance between the sensitizer and the quencher. Use of positioning systems based on cleavable peptides[6,7] or dynamic DNA structures[8,9] has resulted in appreciable control in the production of $^1O_2$. Moreover, this approach has been successfully applied in vivo.[9]

In 2005, O'Shea and co-workers reported a related approach by which a change in pH could be used to control photosensitized $^1O_2$ production with a sensitizer closely linked to a pH-responsive amine. [10] At high pH values, the excited state of the sensitizer was quenched by electron transfer from the amine, whereas at low pH values, protonation of the lone pair of electrons on the amine precluded quenching by electron-transfer, thereby allowing the photosensitized production of $^1O_2$. The amount of $^1O_2$ produced varied by a factor of 10.6 upon changing from high to low pH values.[10]

We report herein a new approach to reversibly control photosensitized $^1O_2$ production by using a DNA i-motif as a pH-sensitive regulator. The i-motif is a four-stranded DNA structure formed from sequences that contain stretches of cytosine (C) residues.[11] The structure is stable at slightly acidic pH values, where the Cs are partially protonated and form a quadruplex structure of interdigitated C–CH(+) base pairs. Under alkaline conditions, however, the Cs are fully deprotonated and the i-motif is no longer stable, thus leading to denaturation and stretching of the DNA sequence. The i-motifs have been explored intensively as nanoscale switching devices.[12]

In our current design, a $^1O_2$ sensitizer and a quencher of the sensitizer are covalently linked to each end of a DNA sequence that contains an i-motif. The principle of the pH-regulated $^1O_2$ sensitizer/quencher/i-motif (SQI) is illustrated in Figure 1a. Under acidic conditions ($pH < 5$) the i-motif quadruplex is stable and holds the sensitizer and the quencher in close proximity. Upon irradiation, the fluorescent state of the sensitizer is efficiently deactivated by the adjacent quencher, thereby precluding the ultimate formation of the triplet state of the sensitizer which is the immediate precursor to $^1O_2$. As the pH is raised above 5, the C residues are deprotonated and the i-motif is no longer stable. In turn, the increased distance between the sensitizer and the quencher has the consequence that the sensitizer is no longer immediately deactivated and $^1O_2$ production can take place. To more efficiently separate the sensitizer and the quencher in the open state, a complementary oligonucleotide strand can be added to form a rigid DNA helix between the two moieties (Figure 1a). At low pH values, the formation of the i-motif will efficiently compete with the formation of the DNA hybrid, thus providing the basis for an effective pH-responsive switch.

The SQI conjugate was generated by synthesizing a phosphoramidite analogue of the $^1O_2$ sensitizer pyropheo-

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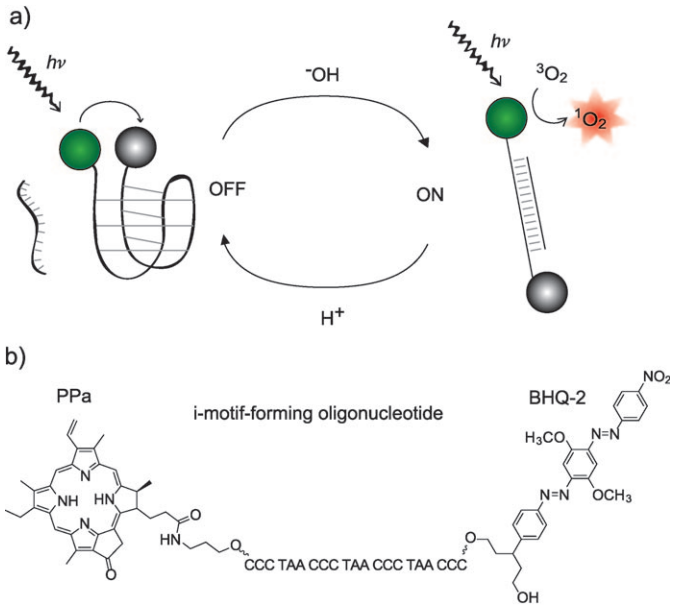

Figure 1. a) The photosensitized production of $^{1}O_2$ can be switched from an "OFF" state to an "ON" state by a pH-dependent change in the distance between the sensitizer (green) and the quencher (gray). The complementary DNA strand that binds in the "ON" state is also shown. b) Structure of the SQI. Both the photosensitizer (PPa) and the quencher (BHQ-2) are introduced during standard automated DNA synthesis.

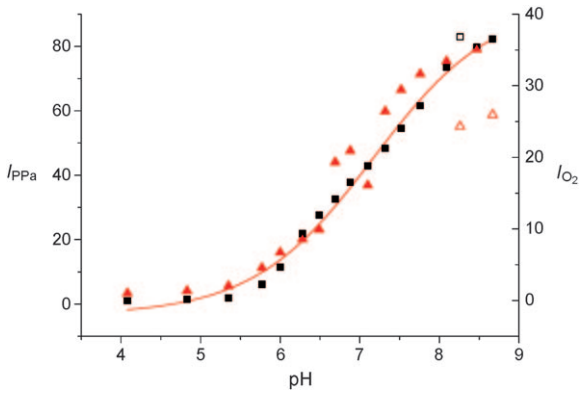

Figure 2. $^{1}O_2$ phosphorescence (I_{O_2}) and integrated PPa fluorescence (I_{PPa}) signals from the SQI in air-saturated solutions at various pH values after irradiation at 400 nm. Red triangles: $^{1}O_2$ phosphorescence intensity, black squares: PPa fluorescence intensity. The open triangles and the open square at high pH values presumably reflect aberrant data. All optical signals were corrected for changes in the sensitizer absorbance caused by dilution of the system.

phorbide a (PPa), which was introduced into the SQI together with an efficient excited state quencher molecule, the so-called "black hole quencher 2" (BHQ-2, see the Supporting Information) by using standard automated DNA synthesis (Figure 1 b).

The spectroscopic properties of SQI were examined as a function of pH. The fluorescence spectrum recorded at pH 8.67, where the i-motif is denatured and the SQI is in the "on" state, showed a maximum emission at 680 nm, which is consistent with that expected for PPa. Fluorescence spectra recorded at 18 different pH values over the range 4.1–8.7 revealed no change in the wavelength-dependent profile; only the emission intensity changed. At pH values below 5.5, low emission intensities were observed which is in agreement with the expectation that the excited state PPa is quenched in the i-motif configuration. Raising the pH value above 5.5 leads to a significant increase in the PPa fluorescence intensity (Figure 2); the intensity observed at pH 8.7 was 94 times higher than the intensity observed at pH 4.1. To the best of our knowledge, this is the best on/off ratio observed for i-motif beacons to date. This improvement in the on/off ratio may, among other factors, reflect our choice of fluorophore and quencher. These pH-dependent changes in PPa fluorescence intensity were fully reversible over the pH range used. However, at pH values above 10 we observed irreversible changes, presumably as a consequence of OH^{-} induced structural changes in PPa.

SQI-sensitized $^{1}O_2$ production was detected directly by the phosphorescence of $^{1}O_2$ at 1275 nm in time-resolved experiments. As in the PPa fluorescence experiments, the photosensitizer system was excited at 400 nm, and experiments were performed in buffered D_2O, which was used because $^{1}O_2$ has a larger phosphorescence quantum efficiency in D_2O than in H_2O.[13] The $^{1}O_2$ detection system is described elsewhere.[14] The overall signal intensity, which is directly proportional to the amount of singlet oxygen produced, was obtained by integration of the time-resolved signal at 1275 nm (see the Supporting Information).

The SQI-sensitized production of $^{1}O_2$ was monitored at various pH values in the range 4.1–8.7 (Figure 2). At pH values lower than 5.5, very low singlet oxygen production was observed, which is consistent with the fact that at these pH values the excited state of PPa is efficiently quenched by BHQ-2 (see above). Moreover, the pH dependence of the $^{1}O_2$ production effectively mirrored the pH dependence of the PPa fluorescence intensity (Figure 2), again as expected given the model shown in Figure 1. The amount of $^{1}O_2$ produced at pH 8.7 was 35 times larger than that produced at pH 4.1; thus the on/off ratio of the pH-dependent change in $^{1}O_2$ yield is smaller than the observed on/off ratio for the corresponding PPa fluorescence intensity. Among other factors, this difference partially reflects the fact that not all the excited states of PPa are quenched by $^{3}O_2$ in water, where the oxygen concentration is low[15] (see the Supporting Information).

For reference, both the fluorescence and the $^{1}O_2$ production experiments were performed by using free PPa at different pH values. When changing from acidic to alkaline media, both the PPa fluorescence and the $^{1}O_2$ phosphorescence intensities increased by a factor less than 3, that is, there is only a small inherent pH-dependent change in the fluorescence and in the $^{1}O_2$ yields of the PPa chromophore in comparison to the magnitude of the effect observed in the SQI construct (see the Supporting Information). It is known that pH changes can inherently affect fluorescence and photosensitized $^{1}O_2$ yields.[16,17]

We did not observe any pH dependence of the $^{1}O_2$ lifetime, and consistently recorded values of 67 μs, which are in agreement with the expected value in D_2O.[13] This behavior

is consistent with the model in which BHQ-2 will deactivate only the excited state of PPa, not $^1O_2$.

Reversible switching of the SQI motif between the "on" and "off" configurations was achieved by sequentially altering the pH between 8.7 and 4.3, as demonstrated by the intensity of the $^1O_2$ phosphorescence signal recorded after each switch (Figure 3). The first six "on/off" experiments of the SQI motif

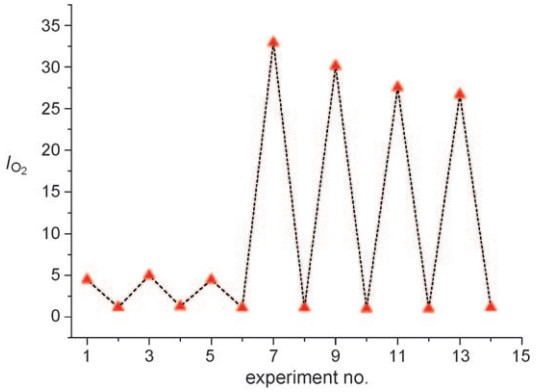

Figure 3. Intensity of the $^1O_2$ phosphorescence signal from the SQI motif when the pH is alternated between high (ca. 8.7, odd-numbered experiments) and low (ca. 4.3, even-numbered experiments) values. Points 1–6 and 7–14 show data recorded in the absence and presence of a complementary DNA strand, respectively. The $^1O_2$ phosphorescence signals were corrected for changes in the sensitizer absorbance caused by dilution of the system.

were performed in the absence of a complementary DNA strand and, as expected, only a moderate modulation of the $^1O_2$ signal was observed (a factor of 5 difference between high and low pH values). After these oscillations, the short 17-mer complementary DNA strand was added to the solution, facilitating a more efficient separation of the PPa and BHQ-2 moieties in the open configuration (Figure 1). As expected, the pH-dependent differences in the $^1O_2$ signal intensity increased dramatically. Thereafter, the slight decrease in $^1O_2$ signal intensity with an increase in the number of oscillation is ascribed to SQI photobleaching, which thereby reduces the effective number of sensitizers in the system (Figure 3, also see the Supporting Information).

In conclusion, we have developed a new pH-dependent approach to exert control over the photosensitized formation of singlet oxygen, which is a cytotoxic species. To the best of our knowledge, the DNA i-motif method leads to the largest reversible environment-dependent change in $^1O_2$ yield that has been reported to date. This result provides a promising outlook for the development of a plethora of molecule-based $^1O_2$-releasing systems.

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