JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
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Photocontrolled Binding and Binding-Controlled Photochromism within Anthracene-Modified DNA

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Supporting Information

ABSTRACT: Modified DNA strands undergo a reversible light-induced reaction involving the intramolecular photodimerization of two appended anthracene tags. The photodimers exhibit markedly different binding behavior toward a complementary strand that depends on the number of bases between the modified positions. By preforming the duplex, photochromism can be suppressed, illustrating dual-mode gated behavior.

Within the field of light-activated molecular and supramolecular systems, the photocontrol of biomolecular binding processes in chemical or biological environments is particularly appealing, due to the prospect of light-triggered uptake or release of agents for various therapeutic and nanodevice applications.[1] An effective way of achieving reversible control over such events is to attach photochromic groups to biomolecular components in a way that allows a structural change at the photochrome to impart a change in binding affinity. As far as nucleic acids are concerned, *trans*−*cis* isomerism (in particular that within azobenzene) has been utilized extensively, allowing photocontrol over factors such as secondary structure, binding, and catalysis.[2,3] Until now, anthracene photochromism has been used considerably less in this capacity,[4] but it is quite unusual among photochromic systems in that it involves a photodimerization reaction, which can lead to a profound change in structure when two anthracenes are appended to one binding motif. We have used this approach effectively in the past to demonstrate the photoswitched binding of cations[5a] and also neutral molecules[5b,c] using supramolecular receptor systems. Here we show how the incorporation of two anthracene groups into one oligonucleotide strand[6] leads to an unusual example of a system in which DNA duplex formation can either control, or alternatively be controlled by, a photochromic process (Scheme1).

The sequences of the DNA strands made for this study are presented in Table 1, and the structures of the anucleosidic groups **X** and **Y** incorporated into the modified strands are depicted in Figure 1. Sequences **S1-A**, **S2-A**, and **S3-A** were designed to monitor the effect of increasing the number of bases between the two anthracene tags **X**. In addition, various control strands containing the propyl linker **Y** were synthesized, with letter codes **B**, **C**, and **D** identifying the particular combination of **X** and **Y** used. The anucleosidic threoninol unit in **X** (in this study used in its D-configuration) has previously been shown to be readily incorporated into oligonucleotides via

Scheme 1. The S3-A↔S3-AP Photochromic System, in Which the Anthracene Photodimer Consists of the Head-to-Tail Isomer

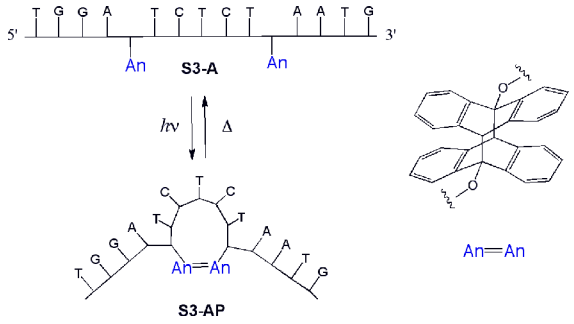


Table 1. Sequences of the 14 DNA Strands Synthesized[a]

S1-A	5′-TGGACT**X**T**X**TCAATG-3′
S2-A	5′-TGGAC**X**CTC**X**CAATG-3′
S3-A	5′-TGGA**X**TCTCT**X**AATG-3′
S1-B	5′-TGGACT**X**T**Y**TCAATG-3′
S2-B	5′-TGGAC**X**CTC**Y**CAATG-3′
S3-B	5′-TGGA**X**TCTCT**Y**AATG-3′
S1-C	5′-TGGACT**Y**T**X**TCAATG-3′
S2-C	5′-TGGAC**Y**CTC**X**CAATG-3′
S3-C	5′-TGGA**Y**TCTCT**X**AATG-3′
S1-D	5′-TGGACT**Y**T**Y**TCAATG-3′
S2-D	5′-TGGAC**Y**CTC**Y**CAATG-3′
S3-D	5′-TGGA**Y**TCTCT**Y**AATG-3′
T0	3′-ACCTGAGAGAGTTAC-5′
S0	5′-TGGACTCTCTCAATG-3′

[a]Each strand composition is identified by the letter codes in Figure 1, so that, for example, **S2-B** is the strand where anthracene tag **X** and propyl linker **Y** are separated by three bases.

Received: May 4, 2012
Published: June 13, 2012

   dx.doi.org/10.1021/ja304205m | J. Am. Chem. Soc. 2012, 134, 10791−10794

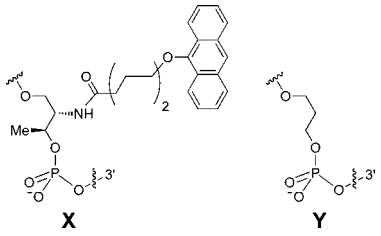


Figure 1. Structures of the anucleosidic groups **X** and **Y** when incorporated into DNA (phosphodiester groups toward 3′ end).

the corresponding phosphoramidite monomer.[2a,b,7] The anthracene tag was connected to the threoninol unit according to a procedure described previously for related systems,[7] prior to DNA incorporation via standard automated synthesis. All the strands, including the target strand **T0** and its unmodified complementary **S0**, were purified by reversed-phase HPLC and characterized by ESI mass spectrometry (see the Supporting Information (SI)).

Upon photoirradiation with filtered light from a high-pressure Hg-Xe lamp (365 ± 5 nm) of Ar-degassed solutions of each of the doubly tagged strands **S1-A**, **S2-A**, and **S3-A** (ca. 20 μM, 10 mM phosphate buffer, pH 7.0, 100 mM NaCl), the characteristic anthracene band centered at ca. 360 nm was observed to decrease significantly over a period of 40 min. HPLC runs of these irradiated solutions indicated a clean photoreaction with generally high conversion (see the SI), with the appearance in each case of one new major peak and one new minor peak in addition to the residual starting material. Mass spectrometry analysis of the isolated major photoproduct from each reaction (designated **S1-AP**, **S2-AP**, and **S3-AP**, respectively) revealed a mass identical to that of the corresponding starting material in each case, in agreement with the formation of an intramolecular photodimer. The absence of any photoreactivity in the singly tagged **B** and **C** control strands for **S1**, **S2**, and **S3** excludes the occurrence of other significant intermolecular (e.g., between anthracenes on separate strands) or intramolecular (e.g., with DNA bases) photoinduced processes. At room temperature, each major photoproduct was found to be quite stable, whereas the minor product readily converted back to the starting material. In line with previous work on related anthracene systems,[5b,c,8] this trend indicates a head-to-tail (see Scheme 1) and a head-to-head orientation for the major and minor photoadducts, respectively.

A series of thermal reversion studies were then undertaken on buffered solutions of the three major photoproducts (ca. 5 μM). In each case, no significant changes were noted below 55 °C, but upon continued heating at 80 °C for 16−20 h, each compound reverted back cleanly to its respective starting material, as indicated by HPLC. The opening rate constants at 80 °C were determined to be 2×10^{-3}, 1.9×10^{-3}, and 2.6×10^{-3} s^{-1}, respectively for **S1-AP**, **S2-AP**, and **S3-AP**. The data indicate that the base separation between the photoligated units influences the reversion kinetics to some extent, with the five-base separation giving the fastest rate, presumably due to greater destabilization of the cyclodimer by the longer oligonucleotide spacer.

The extent to which DNA duplex formation could affect, or be affected by, anthracene photochromism was then investigated. Each of the doubly tagged **A** strands was found to form a stable duplex at room temperature with the complementary target strand **T0**, as evidenced by melting temperatures from variable-temperature UV/vis spectroscopy (phosphate buffer, 250 mM NaCl, strand concentration = 5 μM). The T_m values are presented in Table 2. The duplex between strands **S2-A** and **T0** is the most stable, which is consistent with it containing two more GC base pairs. Significantly, the values for the **A** duplexes are all higher than those for most of the control duplexes involving strands **B**, **C**, and **D** that contain the propyl linker **Y** instead of the anthracene tag **X** at one or both positions.[9] This indicates that the anthracene groups stabilize their respective duplexes through an intercalative interaction with the base-pair stack, in agreement with our previous findings[7b] on the same 15-mer sequence. A striking trend is apparent when comparing the T_m data for the three photoproducts with those for the corresponding starting materials. For the **S1-A** system, there is essentially no change in duplex stability upon photocyclization. However, the ΔT_m value is 20 °C for the **S2-A** system, and for the five-base-separated system, no inflection was observed at all (Figure 2), indicating no duplex formation whatsoever between **S3-AP** and **T0** under the conditions used. These differences in duplex stability are comparable with the best results obtained in other photoswitchable systems[2b,3b] where normally more than one photochromic unit is required to generate large ΔT_m values. These studies indicate that the greater the base separation between the reacting anthracene units, the greater the structural change upon photodimerization, which then hinders or even prevents duplex formation with the complementary strand.

Table 2. T_m Data (°C) for Duplexes Formed by Various Strands with the Complementary Target Strand T0[a]

modification	S1	S2	S3
A	37.5	45	35
AP	37	25	<5[b]
B	38	40	28.5
C	37.5	40	27
D	30	31.5	19.5

[a]Conditions: 5 μM, pH 7.0, 10 mM sodium phosphate buffer, 250 mM NaCl. [b]No duplex formation observed down to 5 °C, the lowest temperature the conditions allowed for in water.

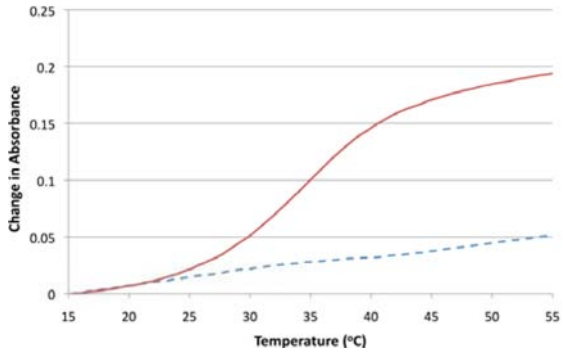


Figure 2. Normalized graphs showing the change in absorbance as a function of temperature for **T0** in the presence of **S3-A** (red) and **S3-AP** (dashed blue).

To further probe these dramatic differences in duplex stability, two other independent sets of experiments were undertaken. First, CD spectroscopy was performed at 20 °C under the same conditions as the melting curves. For both **S1-**

AP and **S2-AP** in the presence of **T0**, the characteristic negative and positive bands associated with duplex B-DNA were observed. However, in the corresponding scan for **S3-AP**, the negative band at ca. 245 nm correlating to duplex helicity[10] was absent, with the observed spectrum essentially the same as that for the two strands measured independently and then mathematically added together (Figure 3).

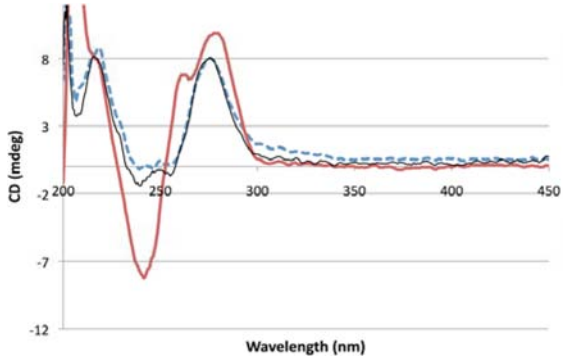


Figure 3. CD spectra of **T0** with 1 molar equivalent of **S3-A** (red), with 1 molar equivalent of **S3-AP** (dashed blue), and measured alone and then mathematically added to an independent spectrum of **S3-AP** (black). Conditions: 5 μM, pH 7.0, 10 mM sodium phosphate buffer, 250 mM NaCl, 293 K.

Second, a series of native gel electrophoresis experiments was undertaken. Under the conditions used, which required a lower NaCl concentration of 25 mM, neither **S2-AP** nor **S3-AP** could form a stable duplex with **T0** at 20 °C, as illustrated for the three-base-separated system in Figure 4. Ethidium bromide

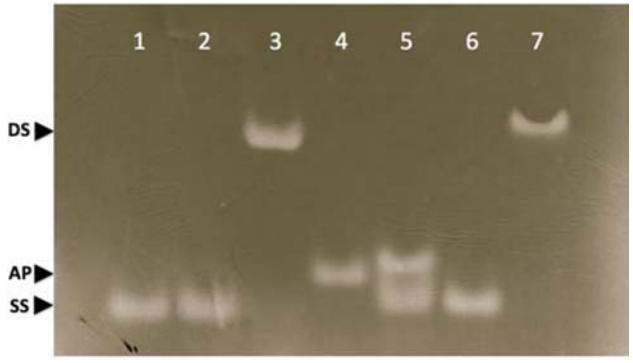


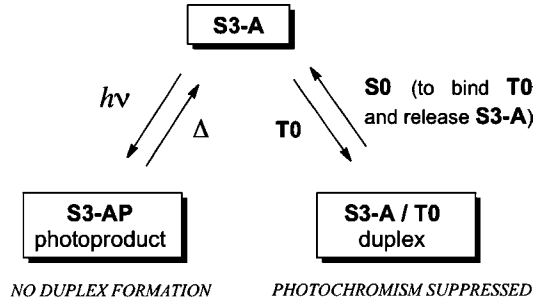
Figure 4. 20% Native-PAGE experiment illustrating differing duplex-forming abilities with **T0** between strands **S0**, **S2-A**, and **S2-AP**: lane 1, **S0**; lane 2, **T0**; lane 3, **S0** + **T0**; lane 4, **S2-AP**; lane 5, **S2-AP** + **T0**; lane 6, **S2-A**; lane 7, **S2-A** + **T0**. Conditions: 25 mM NaCl, 1× TB, 24 h at 100 V, 293 K.

staining experiments (ethidium binds preferentially to duplex DNA, see the SI) confirmed unambiguously that only the undimerized strands **S2-A** and **S3-A** formed a duplex under these conditions.

Finally, whereas the photoreaction of the single strands **S1-A**, **S2-A**, and **S3-A** was straightforward, little photoreactivity was observed upon photoirradiation of their corresponding duplexes (again formed with the complementary strand **T0**) under the same conditions (see SI). This supports the notion of the anthracene units interacting with the base-pair stack (*vide supra*), which precludes their availability for photodimerization.

This system therefore represents an example of *gated photochromism*,[11] where a separate external input (in this case, a binding process via the addition of DNA strand) can control a photochromic process by switching it ON or OFF. The addition of **T0** restricts photochromism, but by then adding the competing strand **S0**, the stronger duplex **S0**/**T0** is formed, thereby unlocking the system and allowing photochromism to recommence (Scheme 2). Therefore, in this particular system, it is possible to demonstrate both photocontrolled duplex formation and binding-controlled photochromism.

Scheme 2. Representation of Photocontrolled Binding of T0 and Binding-Controlled Photoreactivity within the S3-A↔ S3-AP Photochromic System



To conclude, these studies further demonstrate the scope and potential of photochromism in the design of functional and controllable nanodevices comprising biomolecular components. Further studies are now underway to explore and exploit these findings further in related nucleic acid and peptide systems.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures for new compounds, oligomer characterization (HPLC, MS), selected UV/vis and fluorescence spectra, CD spectra, electrophoresis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was funded by an EPSRC Leadership Fellowship to J.H.R.T. (EP/G007578/1). Advantage West Midlands and staff within the School of Chemistry Analytical Facility are gratefully acknowledged. J.H.R.T. thanks Prof. Alison Rodger (Warwick) for helpful suggestions in the preparation of this manuscript.

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