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Manchester, J., Bassani, D. M., Duprey, J-L. H. A., Giordano, L., Vyle, J. S., Zhao, Z., & Tucker, J. H. R. (2012). Photocontrolled Binding and Binding-Controlled Photochromism within Anthracene-Modified DNA. Journal of the American Chemical Society, 134(26), 10791-10794. DOI: 10.1021/ja304205m

Published in:
Journal of the American Chemical Society

Document Version:
Publisher's PDF, also known as Version of record

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Photocontrolled Binding and Binding-Controlled Photochromism within Anthracene-Modified DNA

Jack Manchester,* Dario M. Bassani,† Jean-Louis H. A. Duprey,* Luciana Giordano,* Joseph S. Vyle,‡ Zheng-yun Zhao,§ and James H. R. Tucker*§

§School of Chemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.
†Université de Bordeaux, CNRS, ISM UMR 5255 351, Cours de la Libération, 33400 Talence, France
‡School of Chemistry and Chemical Engineering, David Keir Building, Queen’s University Belfast, Stranmillis Road, Belfast BT9 5AG, U.K.

Supporting Information

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†

| Strands   | Sequences                                |
|-----------|------------------------------------------|
| S1-A      | 5′-TGACTTXTCAATG-3′                      |
| S2-A      | 5′-TGACTXXCTCAAATG-3′                    |
| S3-A      | 5′-TGACTXCTCAAATG-3′                     |
| S1-B      | 5′-TGACTXXCTCAAATG-3′                    |
| S2-B      | 5′-TGACTXTYCTCAAATG-3′                   |
| S3-B      | 5′-TGACTXTYCTCAAATG-3′                   |
| S1-C      | 5′-TGACTXTCATCAATG-3′                    |
| S2-C      | 5′-TGACTXTCATCAATG-3′                    |
| S3-C      | 5′-TGACTYCTCAAATG-3′                     |
| S1-D      | 5′-TGACTXTCAATG-3′                       |
| S2-D      | 5′-TGACTXCTCAATG-3′                      |
| S3-D      | 5′-TGACTYCTCAAATG-3′                     |
| T0        | 3′-ACCTGAGAGATTCAC-5′                    |
| S0        | 5′-TGACTCTCTCAATG-3′                     |

†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.

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...
the corresponding phosphoramidite monomer. The anthracene tag was connected to the threoninol unit according to a procedure described previously for related systems, 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, this trend indicates a head-to-tail 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 Tm 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. 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 on the same 15-mer sequence. A striking trend is apparent when comparing the Tm 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 ΔTm 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 where normally more than one photochromic unit is required to generate large ΔTm 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.

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 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 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.

AUTHOR INFORMATION

Corresponding Author

j.tucker@bham.ac.uk

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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