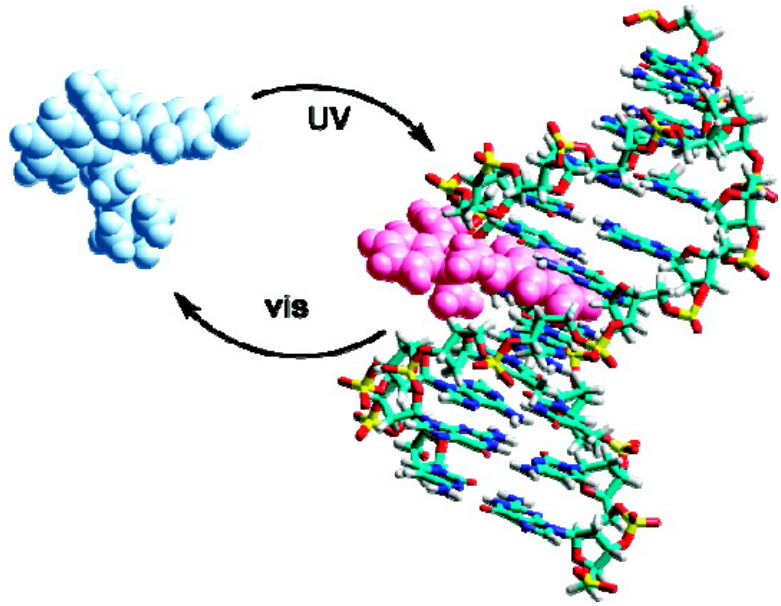
Communication

Photoswitched DNA-Binding of a Photochromic Spiropyran

Johanna Andersson, Shiming Li, Per Lincoln, and Joakim Andre#asson

J. Am. Chem. Soc., **2008**, 130 (36), 11836-11837 • DOI: 10.1021/ja801968f • Publication Date (Web): 13 August 2008



J|A|C|S
COMMUNICATIONS

Published on Web 08/13/2008

Photoswitched DNA-Binding of a Photochromic Spiropyran

Johanna Andersson, Shiming Li, Per Lincoln, and Joakim Andréasson*

Department of Chemical and Biological Engineering, Physical Chemistry, Chalmers University of Technology, SE-412 96 Göteborg, Sweden

Received March 17, 2008; E-mail: a-son@chalmers.se

One of the many challenges of chemical biology is the design and the understanding of the mechanisms of small DNA-binding molecules.[1,2] Since most of the DNA-associated processes occurring in the cell could be inhibited by a molecule with a high DNA-binding affinity, controlling the action using external stimuli would be of tremendous utility for, e.g., chemotherapeutics.[3,4] Ultimately, a molecule for this purpose should show no or very small affinity for the DNA double helix in the "passive" form. Upon activation the molecule should interconvert to a form that binds strongly to DNA to inhibit the targeted process. Here, we report a photochromic molecule from the spiropyran[5] (SP) family that can be activated using UV light from a form that shows no signs of interaction with DNA to a form that binds to DNA by intercalation. The corresponding deactivation can be achieved by visible light; i.e., the DNA-binding process is fully reversible using light as the only stimulus. The idea of switching the state of photochromes to control various DNA-associated processes has been demonstrated before.[6−13] However, in the vast majority of these pioneering studies, the approach has been to covalently link photochromic units to short single-stranded oligonucleotides,[6−12] while other authors have been targeting mismatch-containing 11-mers.[13] Our approach surmounts the need of covalently modifying the targeted DNA molecules and the principle is applicable on naturally occurring DNA, making it much more suitable for any practical biological application.

Chart 1 shows the structures and the isomerization scheme of photochromic spiropyran **1** used in this work. The thermally stable spiro form **1c** is isomerized to the ring-opened merocyanine form **1o** by exposure to UV light. Shown in Chart 1 is the *trans*−*trans*−*cis* form of **1o**, which has been suggested to be the thermodynamically stable **1o**-isomer.[14−16] The photostationary distribution after exposure to 254 nm light is ca. 54/46 **1c/1o** as judged by NMR measurements whereas visible light ($600 > \lambda > 475$ nm, ∼10 mW/cm^2) converts the sample to 100% **1c** with a time constant of ∼1 min. By contrast, the time constant for establishment of thermal equilibrium at 23 °C is 124 min. The equilibrium composition is ca. 67/33 **1c/1o**.

As shown in Figure 1, **1c** does not absorb light significantly in the visible region; its longest-wavelength absorption band is at 348 nm. **1o** displays absorption in the visible region with the strongest absorption band centered at 512 nm.

The absorption spectra of **1c** with and without addition of calf-thymus (CT) DNA are virtually superimposable as illustrated in Figure 1 (top panel, **1c**), indicating no or very weak interactions between **1c** and DNA. The corresponding spectra of **1o** are shown in the lower panel. Here, the spectral changes upon addition of DNA are dramatic as seen by comparing the spectrum of **1o** without DNA (blue line) with that in the presence of DNA (red line). In fact, the spectral perturbation is even stronger since some unbound **1o** remains in solution. A spectrum representing 100% DNA-bound **1o** (green line) shows that the longest-wavelength band is shifted from 512 to 420 nm; i.e., there is a 92 nm shift toward shorter wavelengths upon DNA addition. Furthermore, the band has experienced a ca. 45% hypochromic shift, i.e., a decrease in the absorbance (see Supporting Information (SI) for details on the spectral deconvolutions). These observations clearly show that **1o** interacts strongly with DNA.

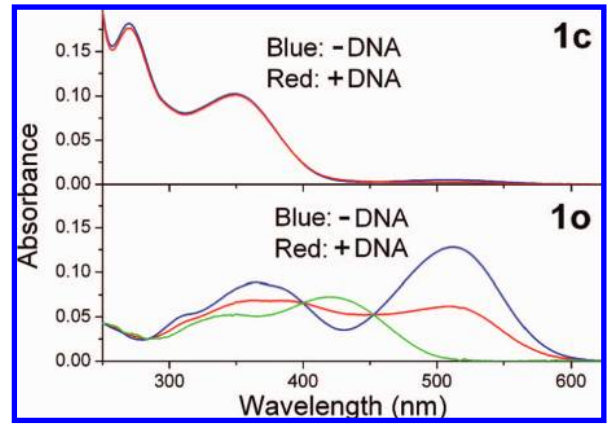


Figure 1. Absorption spectra of **1c** (top panel) and **1o** (lower panel) in the absence (blue lines) and presence (red lines) of calf-thymus DNA. The contribution from DNA to the overall absorption has been subtracted for ease of comparison. Likewise, the contribution from **1c** has been subtracted from the spectra of **1o** shown in the lower panel. The green line corresponds to a sample of 100% **1o** bound to DNA as the contribution from unbound **1o** has been corrected for (see Supporting Information for details). The total concentration of **1** was $\sim 1.5 \times 10^{-5}$ M. The concentration of DNA was 11.6×10^{-5} M, and the NaCl concentration of the solution was 8.6×10^{-3} M.

Chart 1. Structures of Photochromic Spiropyran **1**

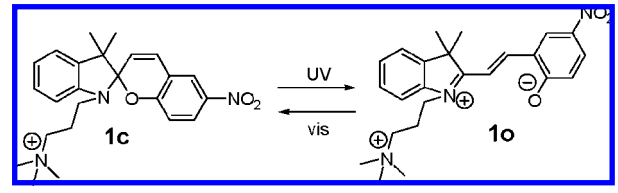


Figure 2 shows the results from flow-oriented linear dichroism (LD) measurements[17] where **1** was exposed to alternating UV and visible light irradiation in the presence of calf-thymus DNA. The first spectrum was recorded on a sample containing **1c** and DNA and shows a weak negative LD signal in the visible, originating from a small fraction of thermally formed **1o**. UV irradiation of the sample for 5 min converted a substantial fraction to the open form **1o**, and the isomerization process is paralleled by an amplification of the negative LD signal by more than a factor 3. Subsequent exposure to visible light for 5 min decreased the LD response to almost the initial value, signaling that the DNA-binding process can be light-switched both ways. Indeed, upon exposure to a second 5 min portion of UV light the previous high-amplitude

 10.1021/ja801968f CCC: $40.75

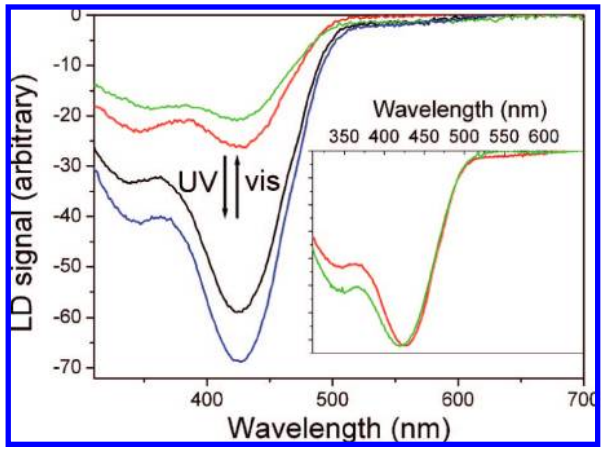


Figure 2. Flow-oriented LD spectra on a sample of **1** and calf-thymus DNA after the following irradiation sequence: no light exposure (green line), 5 min UV irradiation (blue line), 5 min visible light irradiation (red line), and 5 min more UV irradiation (black line). The inset shows the LD signal observed for **1o** + DNA (red line) and the absorption spectrum of DNA-bound **1o** (green line). The negative of the absorption spectrum is shown for ease of comparison. The total concentration of **1** was $\sim 9 \times 10^{-5}$ M. The concentration of DNA was 13.3×10^{-5} M, and the NaCl concentration of the solution was 8.6×10^{-3} M.

value of the LD signal was nearly recovered, proving that the observed changes in the LD signal are due to a fully reversible, light-controlled DNA-binding process rather than photodecomposition. As seen in the inset, the bands of the LD spectra virtually coincide with the absorption bands of DNA-bound **1o**.

The reduced LD (LD/Abs) may be analyzed in terms of the angle between the transition moment of the DNA-binding molecule and the DNA helix axis to provide information about binding geometries.[18] Here it was found that the angle between the transition of **1o** centered at 420 nm and the helix axis is 80 ± 5°, which is a strong indication for intercalation (see SI for details).

Upon intercalation of a reagent between the DNA bases, the absorption of the reagent typically experiences a hypochromic shift. In Figure 1 it is seen that the absorption of **1o** is indeed decreased by 45% upon binding to DNA. Furthermore, the spectrum is shifted 92 nm toward shorter wavelengths. This observation could be explained by inspecting the two different resonance structures of **1o**. The zwitterionic structure shown in Chart 1 is accompanied by a resonance structure where the nitrogen in the heterocycle and the phenolic oxygen both are formally charge neutral. This form should be favored in a hydrophobic environment, e.g., between the DNA bases, whereas the zwitterionic form should be more likely to be found in a highly polar medium such as water. This notion is supported by the fact that in chloroform, where the formally charge neutral structure is more likely, the longest-wavelength absorption band of **1o** is centered at 438 nm (not shown). The corresponding value in water is 512 nm; i.e., there is a 74 nm shift toward shorter wavelengths in going from a highly polar water environment to less polar chloroform.[19] The pronounced solvatochromism for **1o** makes it an excellent candidate for a nucleic acid probe, as the color changes drastically upon changes in the polarity of the environment.

The absorption changes observed upon addition of DNA shown in Figure 1 can also be used to roughly estimate the binding constant for **1o** (see SI). The resulting number of ca. 2×10^4 M^{-1} is not very high but would be more than sufficient for applications where the difference in binding affinity between **1c** and **1o** is the key parameter. A comparison between the absorption spectra of **1o** recorded in the presence of AT-DNA ($[poly(dA\text{-}dT)]_2$) and GC-DNA ($[poly(dG\text{-}dC)]_2$) shows that the binding affinity of **1o** increases in the following order: GC-DNA < CT-DNA < AT-DNA (see SI for details).

In conclusion, we have demonstrated the dramatically different DNA-binding properties of the two isomeric forms of a photochromic spiropyran. The closed, nonbinding form is activated by UV light to the open form that intercalates the DNA bases. The open form in turn can be deactivated and dissociated by visible light, making the DNA-binding equilibrium completely tunable by light.

Acknowledgment. This work was supported by the Swedish Research Council (VR).

Supporting Information Available: Details on the synthesis, the experimental procedures, the deconvolution of the absorption spectra, and the DNA-binding studies. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2215–2235.
(2) Nelson, S. M.; Ferguson, L. R.; Denny, W. A. *Mutat. Res.* **2007**, *623*, 24–40.
(3) Willner, I. *Acc. Chem. Res.* **1997**, *30*, 347–356.
(4) Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4900–4921.
(5) Bertelson, R. C. In *Organic Photochromic and Thermochromic Compounds*; Crano, J. C., Guglielmetti, R. J., Eds.; Plenum Press: New York, 1998; Vol. 1, pp 11−83..
(6) Asanuma, H.; Ito, T.; Yoshida, T.; Liang, X.; Komiyama, M. *Angew. Chem., Int. Ed.* **1999**, *38*, 2393–2395.
(7) Nishioka, H.; Liang, X.; Kashida, H.; Asanuma, H. *Chem. Commun.* **2007**, *n/a*, 4354–4356.
(8) Asanuma, H.; Takarada, T.; Yoshida, T.; Tamaru, D.; Liang, X.; Komiyama, M. *Angew. Chem., Int. Ed.* **2001**, *40*, 2671–2673.
(9) Liu, M.; Asanuma, H.; Komiyama, M. *J. Am. Chem. Soc.* **2006**, *128*, 1009–1015.
(10) Yamazawa, A.; Liang, X.; Asanuma, H.; Komiyama, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 2356–2357.
(11) Liang, X.; Asanuma, H.; Komiyama, M. *J. Am. Chem. Soc.* **2002**, *124*, 1877–1883.
(12) Matsunaga, D.; Asanuma, H.; Komiyama, M. *J. Am. Chem. Soc.* **2004**, *126*, 11452–11453.
(13) Dohno, C.; Uno, S.; Nakatani, K. *J. Am. Chem. Soc.* **2007**, *129*, 11898–11899.
(14) Aubard, J. In *Organic Photochromic and Thermochromic Compounds*; Crano, J. C., Guglielmetti, R. J., Eds.; Kluwer Academic Publishers: Hingham, MA, 1999; Vol. 2, pp 369−370..
(15) Sheng, Y.; Leszcynski, J.; Garcia, A. G.; Rosario, R.; Gust, D.; Springer, J. *J. Phys. Chem. B* **2004**, *108*, 16233–16243.
(16) Wohl, C. J.; Kuciauskas, D. *J. Phys. Chem. B* **2005**, *109*, 22186–22191.
(17) Nordén, B.; Tjerneld, F. *Biophys. Chem.* **1976**, *4*, 191–198.
(18) Nordén, B.; Kubista, M.; Kurucsev, T. *Q. Rev. Biophys.* **1992**, *25*, 51–170.
(19) As the wavelength shift upon intercalation is larger than the corresponding shift in going from water to chloroform, it is likely that the intercalation process induces a conformational change to a more nonplanar structure of **1o** compared to solution.

JA801968F