Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters 16 (2006) 3184–3187
Bioorganic & Medicinal Chemistry Letters

8-(Pyren-1-yl)-2′-deoxyguanosine as an optical probe for DNA hybridization and for charge transfer with small peptides

Linda Valis, Elke Mayer-Enthart and Hans-Achim Wagenknecht*

University of Regensburg, Institute for Organic Chemistry, D-93040 Regensburg, Germany

Received 13 February 2006; revised 14 March 2006; accepted 16 March 2006
Available online 18 April 2006

Abstract—8-(Pyren-1-yl)-2′-deoxyguanosine (Py-G) was incorporated synthetically as an optical probe into oligonucleotides. The Py-G group in DNA does not discriminate between any of the four natural nucleosides as a counterbase and exhibits altered optical properties in single strands versus double strands. Thus, the duplex hybridization of Py-G-modified DNA can be observed by both fluorescence *and* absorption spectroscopy. Moreover, Py-G in DNA can be applied as photoinducable donor for charge transfer processes with small peptides.

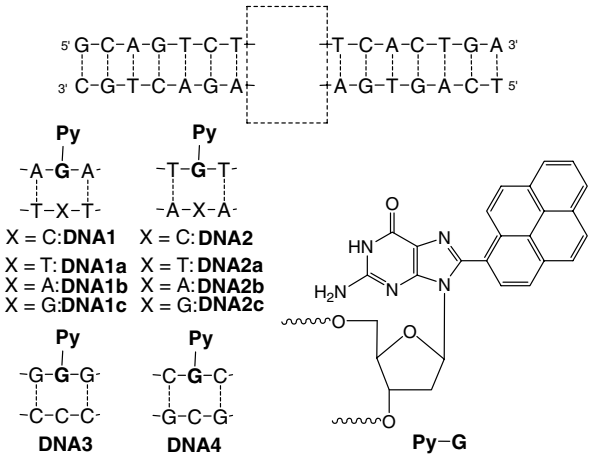
Important analytical problems in biotechnology as well as for molecular diagnostics in biomedicine require powerful DNA labels for optical spectroscopy. Various organic dyes have been applied as covalently attached probes for DNA analytics.[1–6] Especially the detection of single nucleotide polymorphisms by fluorescent DNA base analogs or surrogates has been successfully achieved using ethidium,[7] thiazole orange derivatives[8] or pyrene.[9] Additionally, there is an increasing demand for optical DNA labels that change not only their emission but also their absorption properties as a result of the duplex formation.[10] One important way to create the desired optical properties is to attach suitable organic chromophores covalently to DNA bases. Recently, we applied this modification strategy for the investigation of DNA-mediated electron transfer processes.[11,12] Moreover, we introduced similarly the 1-ethynylpyrene moiety as a versatile and tunable fluorescent probe for DNA.[13,14] Herein, we want to describe our recent studies of 8-(pyren-1-yl)-2′-deoxyguanosine (Py-G) as a duplex-sensitive optical probe for DNA and as a fluorescent donor for the investigation of charge transfer processes between DNA and peptides.

We chose this fluorescent probe since we expected based on literature[15] and our own experience[16,17] that it exhibits an exciplex-type fluorescence with a strong solvent dependence that we could apply as a DNA duplex-sensitive emission. The modified nucleoside Py-G was prepared via a Suzuki-Miyaura-type cross-coupling strategy from 8-bromo-2′-deoxyguanosine and 1-pyrenyl boronic acid as the starting materials.[17] It is important to point out that according to NMR measurements (NOESY) the attachment of the pyrene moiety forces the Py-G nucleoside into the *syn*-conformation.[17] Hence, it is expected that the local DNA conformation is perturbed in such a way that the pyrene moiety is partially intercalated. Using the phosphoramidite DNA building block strategy we prepared a range of four Py-G-modified duplexes DNA1–DNA4 (Scheme 1). The sequences of the four DNA duplexes are identical except for the base pairs that are placed adjacent to the Py-G modification site.

First, we measured the UV/Vis absorption of the hybridized Py-G-modified DNA1–DNA4 at eight different temperatures between 20 and 90 °C (Fig. 1, top). At low temperatures the maximum of the pyrene absorption range (320–430 nm) can be found at ca. 370 nm. This indicates a strong stacking interaction of the pyrene chromophore with the adjacent base pairs inside the DNA duplex since at high temperatures the absorption maximum is shifted by approximately 20 nm to ca. 350 nm. The latter absorption of the DNA samples is similar to that of the corresponding single-stranded Py-G-modified oligonucleotide at rt. Remarkably, the pyrene absorption of all four duplexes DNA1–DNA4 is very similar with a maximum at 370 nm that does

Keywords: Charge transfer; DNA; Fluorescence; Oligonucleotide; Peptide; Pyrene.
* Corresponding author. Tel.: +49 941 943 4802; fax: +49 941 943 4617; e-mail: achim.wagenknecht@chemie.uni-regensburg.de

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.03.063



Scheme 1. Sequences of the Py-G-modified DNA1a–DNA1c, DNA2–DNA2c, DNA3, and DNA4.

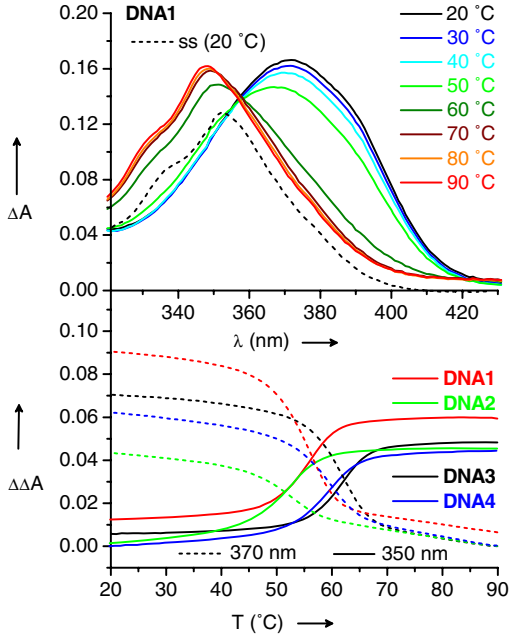


Figure 1. Temperature-dependent UV/Vis spectra of the single-stranded (ss) and duplex DNA1, and melting curves of DNA1–DNA4 (5 μM DNA in buffer: 5 mM Na_2HPO_4, 5 mM NaH_2PO_4, and 250 mM NaCl, pH 7.0).

not depend significantly on the different neighboring base pairs.

The isosbestic point at ca. 360 nm indicates a single transition between the pyrene in duplex DNA and the dehybridized chromophore in the single strand. According to the absorption spectra, this thermal dehybridization of the DNA duplexes occurs between 50 and 60 °C, and seems to be correlated to the DNA melting temperature (T_m). Thus, we measured the absorption changes at two characteristic wavelengths (350 and 370 nm, Fig. 1, bottom) in a temperature-dependent way, and compared the results to the DNA melting behavior at 260 nm (Table 1). Interestingly, the dehybridization temperatures observed at 350 and 370 nm track very well with

Table 1. Melting curves and temperatures (T_m) of DNA1–DNA4 (buffer: 5 mM Na_2HPO_4, 5 mM NaH_2PO_4, and 250 mM NaCl, pH 7.0) at three different wavelengths

Duplex	T_m (°C)
DNA1	58.1 (260 nm)
	57.0 (350 nm)
	56.5 (370 nm)
DNA1a	57.0 (260 nm)
DNA1b	56.1 (260 nm)
DNA1c	59.5 (260 nm)
DNA3	63.1 (260 nm)
	65.5 (350 nm)
	63.5 (370 nm)
DNA2	52.2 (260 nm)
	53.5 (350 nm)
	54.0 (370 nm)
DNA2a	53.1 (260 nm)
DNA2b	52.5 (260 nm)
DNA2c	55.5 (260 nm)
DNA4	59.2 (260 nm)
	60.0 (350 nm)
	60.5 (370 nm)

the DNA denaturation profiles and are nearly identical with the T_m values at 260 nm. As mentioned previously, the *syn*-conformation of Py-G intercalates the pyrene at least partially and forces the guanine part of Py-G and/or the counterbase C into a perturbed position outside the helix. The Py-G modification decreases the thermal stability of the DNA duplex significantly.[18] In fact, this structural scenario can explain the significant absorption differences of the pyrene which are observed in comparison between the full duplex (at 20 °C), the dehybridized DNA (at 90 °C), and the single-stranded oligonucleotide (at rt).

We measured the steady-state fluorescence of all four duplexes at excitation wavelengths between 350 and 410 nm. As expected, all fluorescence spectra of the Py-G-modified duplexes DNA1–DNA4 display an exciplex-type emission profile. The different fluorescence intensities of the duplexes DNA1–DNA4 reflect the different exciplex stabilization and the charge transfer influence by the adjacent base pairs. Remarkably, at all excitation wavelengths the emission quantity of the duplexes is significantly higher than the emission of the corresponding single-stranded oligonucleotide. In order to elucidate the fluorescence abilities of the Py-G label more detailed, we measured the relative fluorescence intensity as a ratio between the integrated emission of the double-stranded DNA and the integrated emission of the single-stranded oligonucleotides (Fig. 2). The recorded ratio is highest (up to 25) in the excitation range between 390 and 410 nm where also the absorption differences between duplex DNA and single-stranded oligonucleotide are highest. It must be pointed out that the ratio of the integrated emission intensities behaves very similar as the ratio of the emission maxima. Quantum yield (Φ) measurements support this result. Representatively, at an excitation wavelength of 360 nm $\Phi_{ds\text{-}DNA}$:$\Phi_{ss\text{-}DNA}$ is 0.34:0.11 for DNA2 and 0.25:0.08 for DNA4. Hence in both cases the ratio is 3.1 that is nearly identical to the corresponding ratio of emission intensities.

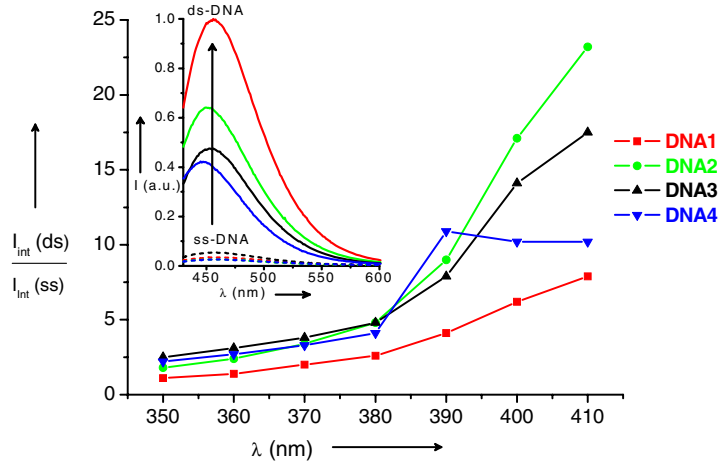


Figure 2. Relative fluorescence intensity (duplex vs single-stranded) of DNA1–DNA4 (5 μM DNA in buffer: 5 mM Na_2HPO_4, 5 mM NaH_2PO_4, and 250 mM NaCl, pH 7.0) at different excitation wavelengths.

With respect to the potential applicability of Py-G as both a base analog and an optical label for DNA hybridization, the influence of the counterbase to the Py-G modification site represents an important parameter. Hence, we varied representatively in DNA1 and DNA2 the base that is opposite to Py-G. In both duplex series the relative emission intensity of the 'matched' duplexes (DNA1 or DNA2) is similar compared to that of the 'mismatched' duplexes (DNA1a–DNA1c or DNA2a–DNA2c, Supporting Information). From these experiments it becomes clear that the counterbase seems not to have any significant influence on the fluorescence of Py-G indicating that there is no significant interaction via hydrogen bonding to the counterbase. This interpretation is supported by the T_m values of the 'mismatched' duplexes which are in the same range as the 'matched' ones (Supporting Information).

In recent investigations, we applied pyrene-modified nucleosides as models for electron transfer in DNA due to their charge-separated character in the excited state[19–21] and we studied charge transfer processes between DNA and small interacting peptides.[22] Similar fluorescence titration experiments with the Py-G-modified DNA1 are shown in Figure 3. The photoexcitation of Py-G initiates the ultrafast formation of the charge-separated state $Py^{\cdot-}$ $G^{\cdot+}$.[19] A crude estimation of the driving force for this process (using E_{00} = 3.25 eV for Py^* and E_{red} = −2.1 V (vs NHE) for Py-G/$Py^{\cdot-}$-G (measured by cyclic voltammetry) and E_{ox} = +1.3 V for $dG^{\cdot+}$/dG)[23] reveals a ΔG value of −0.15 eV. The 4-nitrobenzoyl (Nb) moiety represents a suitable electron trap due to its potential of E_{red} = −0.46 V for Nb/$Nb^{\cdot-}$[24] and due to the synthetically simple attachment to the N-terminus of peptides. The smallest possible DNA-binding peptides represent tripeptides, for example, Lys-Trp-Lys,[25] binding primarily through the electrostatic interactions of the positively charged amino groups of the lysine side chains with the negatively charged phosphodiester backbone. The binding constants can be found in the range of $2–6 \times 10^{-4}$ M. Both Nb-modified peptides Nb-Lys-Lys-Lys and Nb-Lys-Trp-Lys quench the fluorescence significantly. In analogy to our previous results this quenching can be interpreted as an electron transfer from the pyrene moiety of the DNA to the Nb group in the peptides. An additional amount of quenching is observed with Nb-Lys-Trp-Lys that can be attributed to a transfer of

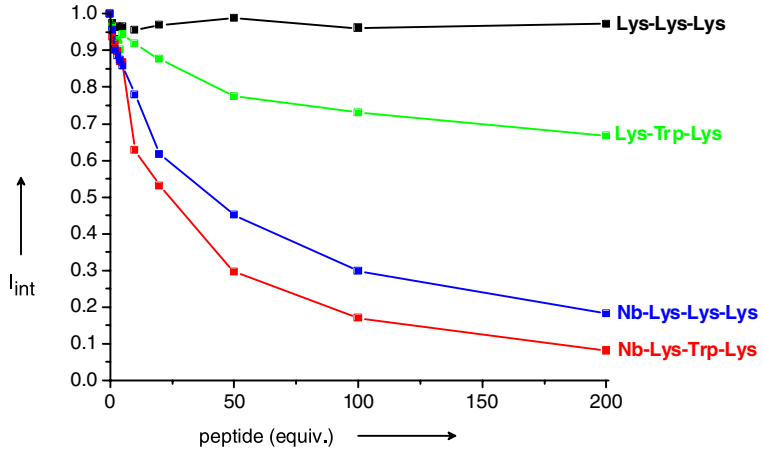


Figure 3. Fluorescence quenching titration experiments of the Py-G-modified DNA1 in the presence of Lys-Lys-Lys, Lys-Trp-Lys, Nb-Lys-Lys-Lys, and Nb-Lys-Trp-Lys (5.0 μM DNA in buffer: 5 mM Na_2HPO_4, 5 mM NaH_2PO_4, and 250 mM NaCl, pH 7.0).

the positive charge from $dG^{\cdot+}$ to the more easily oxidizable indole side chain of Trp. Interestingly, this oxidative-type charge transfer is also observed in the presence of the Nb-unmodified peptide Lys-Trp-Lys. Consequently, Nb-Lys-Trp-Lys exhibits the strongest fluorescence quenching and thus is able to trap both the negative and positive charges from the $Py^{\cdot-}G^{\cdot+}$ moiety in the DNA.

In conclusion, 8-(pyren-1-yl)-2′-deoxyguanosine modification represents an interesting and promising optical label for DNA analytical and electron transfer studies. Remarkably, the DNA duplex hybridization can be observed by both fluorescence and absorption spectroscopy since the Py-G group exhibits altered properties of single strands versus double strands for both types of spectroscopy methods. Hence, it can be envisioned that the Py-G group represents an important label for time-resolved studies of DNA dynamics and stacking interactions,[26] and could be applied especially for assays in which conformational changes or base-flipping processes are crucial to be observed, such as the investigation of DNA–protein complexes with DNA repair proteins.

Acknowledgments

This work was supported by the Volkswagen-Stiftung (Az I/78 101), the Deutsche Forschungsgemeinschaft (Wa 1386/9), the Fonds der Chemischen Industrie, and the University of Regensburg.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2006.03.063.

References and notes

1. Wojczewski, C.; Stolze, K.; Engels, J. W. *Synlett* **1999**, 1667.
2. Johansson, M. K.; Cook, R. M. *Chem. Eur. J.* **2003**, *9*, 3466.
3. Wan, C.-W.; Burghart, A.; Chen, J.; Bergström, F.; Johansson, L. B.-A.; Wolford, M. F.; Kim, T. G.; Topp, M. R.; Hochstrasser, R. M.; Burgess, K. *Chem. Eur. J.* **2003**, *9*, 4430.
4. Tan, W.; Wang, K.; Drake, T. J. *Curr. Opin. Chem. Biol.* **2004**, *8*, 547.
5. Tyagi, S.; Marras, S. A. E.; Kramer, F. R. *Nat. Biotechnol.* **2000**, *18*, 1191.
6. Ranasinghe, R. T.; Brown, T. *Chem. Commun.* **2005**, 5487.
7. Valis, L.; Amann, N.; Wagenknecht, H.-A. *Org. Biomol. Chem.* **2005**, *3*, 36.
8. Köhler, O.; Jarikote, D. V.; Seitz, O. *Chem. Bio. Chem.* **2005**, *6*, 69.
9. Okamoto, A.; Kanatani, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 4820.
10. Asanuma, H.; Kashida, H.; Liang, X.; Komiyama, M. *Chem. Commun.* **2003**, 1536.
11. Kaden, P.; Mayer-Enthart, E.; Trifonov, A.; Fiebig, T.; Wagenknecht, H.-A. *Angew. Chem. Int. Ed.* **2005**, *44*, 1637.
12. Wagner, C.; Wagenknecht, H.-A. *Chem. Eur. J.* **2005**, *11*, 1871.
13. Mayer, E.; Valis, L.; Wagner, C.; Rist, M.; Amann, N.; Wagenknecht, H. A. *Chem. Bio. Chem.* **2004**, *5*, 865.
14. Wagner, C.; Rist, M.; Mayer-Enthart, E.; Wagenknecht, H.-A. *Org. Biomol. Chem.* **2005**, *3*, 2062.
15. Grabowski, Z. R.; Rotkiewicz, K.; Rettig, W. *Chem. Rev.* **2003**, *103*, 3899.
16. Trifonov, A.; Buchvarov, I.; Wagenknecht, H.-A.; Fiebig, T. *Chem. Phys. Lett.* **2005**, *409*, 277.
17. Mayer, E.; Valis, L.; Huber, R.; Amann, N.; Wagenknecht, H.-A. *Synthesis* **2003**, 2335.
18. The T_m value at 260 nm decreases by ca. 10 °C in case of the following representative DNA sequences: 3′-AGT-CAC-TAX-GTC-TAC-G-5′ from X = G (T_m = 65.0 °C) to X = Py-G (T_m = 55 °C).
19. Huber, R.; Fiebig, T.; Wagenknecht, H.-A. *Chem. Commun.* **2003**, 1878.
20. Amann, N.; Pandurski, E.; Fiebig, T.; Wagenknecht, H.-A. *Angew. Chem. Int. Ed.* **2002**, *41*, 2978.
21. Raytchev, M.; Mayer, E.; Amann, N.; Wagenknecht, H.-A.; Fiebig, T. *Chem. Phys. Chem.* **2004**, *5*, 706.
22. Mayer-Enthart, E.; Kaden, P.; Wagenknecht, H.-A. *Biochemistry* **2005**, *44*, 11749.
23. Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617.
24. Harriman, A.; Millward, G. R.; Neta, P.; Richoux, M. C.; Thomas, J. M. *J. Phys. Chem.* **1988**, *92*, 1286.
25. Hélene, C.; Maurizot, J. C. *CRC Crit. Rev. Biochem.* **1981**, *10*, 213.
26. Trifonov, A.; Raytchev, M.; Buchvarov, I.; Rist, M.; Barbaric, J.; Wagenknecht, H.-A.; Fiebig, T. *J. Phys. Chem. B* **2005**, *109*, 19490.