Fluorescent DNA

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Perylene Bisimide Dimers as Fluorescent "Glue" for DNA and for Base-Mismatch Detection**

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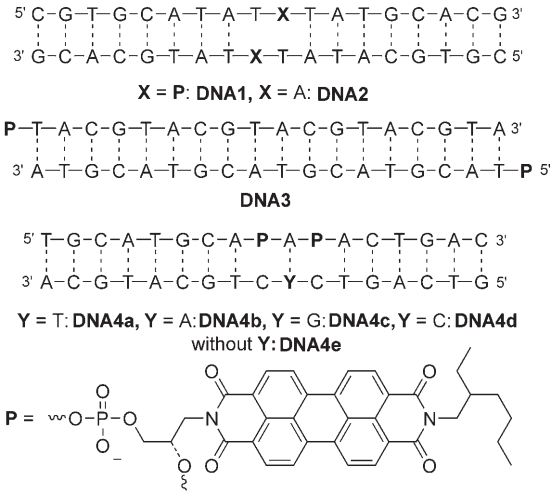
Perylene-3,4:9,10-tetracarboxylic acid bisimide ("PB") and its derivatives are applied as fluorescent dyes in organic materials owing to their excellent photochemical stability as well as the high fluorescence quantum yields.[1,2] The strong hydrophobic stacking interactions between the PB chromophores make this dye an important building block for functional supramolecular architectures.[1,3] Based on these properties, PB in the dimeric form should be also of potential interest as a probe for fluorescent DNA/RNA analytics as well as for functionalized DNA-based architectures. Its noncovalent DNA-binding interactions have been studied with PB derivatives that had been modified with spermine[4] or other amines.[5] Moreover, an increasing number of publications about covalent modifications of oligonucleotides with PB have appeared over the last few years.[6–12] Recently, we presented a facile route for the synthetic incorporation of PB as an artificial DNA base in order to study the stacking interactions of this dye at specific sites in duplex DNA.[13] Herein, we present the evaluation of fluorescent PB dimers for the optical functionalization of DNA using three representative duplexes (**DNA1**, **DNA3**, and **DNA4a**). For the synthetic modification of the corresponding oligonucleotides with the PB chromophore (Scheme 1), the 2-deoxyribofuranoside moiety was replaced by an acyclic linker system which is tethered to one of the imide nitrogens of the PB dye.[13] This linker allows the chromophore to intercalate in the base stack while providing high chemical stability during the automated phosphoramidite chemistry.[13,14]

DNA1 bears one interstrand PB dimer inside the duplex, whereas **DNA3** contains a PB monomer outside the duplex at each 5′ end. Both duplexes contain palindromic sequences. When **DNA1** and **DNA3** are excited at 505 nm, the fluorescence spectra of both duplexes (Figure 1) are dominated by a broad band at ≈660 nm without fine structure. This band corresponds to the excimer-type emission of the PB dimer that has been observed in nanoaggregates of perylene bisimides.[3,8,15] The UV/Vis spectra of **DNA1** and **DNA3** (Figure 2) at low temperatures show two major bands that are hyposochromically (506 nm) or bathochromically (545 nm) shifted in comparison to the 0→1 and 0→0 vibronic transitions of the PB monomer.[16] This result shows the strong π–π excitonic interactions of the two PB chromophores inside (**DNA1**) and outside (**DNA3**) of the duplex.[15]



Scheme 1. PB-modified duplexes **DNA1**–**DNA3** and **DNA4a**–**DNA4e**.

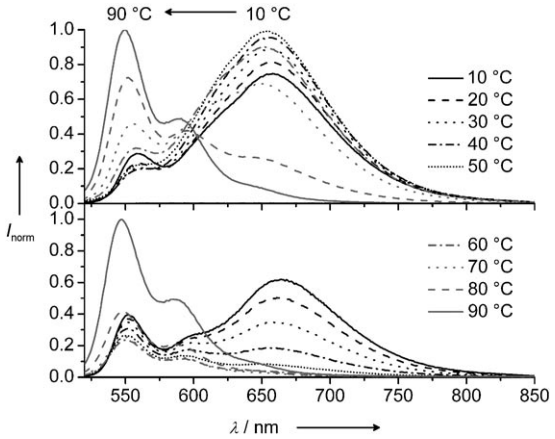


Figure 1. Temperature-dependent fluorescence spectra of **DNA1** (top) and **DNA3** (bottom), 1.25 μM duplex, 10 mM Na-P_i buffer, 250 mM NaCl, pH 7, excitation at 505 nm.

Both the excimer-type fluorescence band and the shifted absorption bands of **DNA1** vanish at higher temperatures. It is remarkable that this occurs cooperatively at a temperature (75.9 °C) that corresponds to the cooperative thermal dehybridization of the whole DNA duplex, which is typically measured at 260 nm (T_m = 78.6 °C). Apparently the intact helical duplex is required as a framework for the PB dimer formation. In comparison to the unmodified **DNA2** (T_m = 76.2 °C) the duplex **DNA1** is stabilized by 2.4 °C through the

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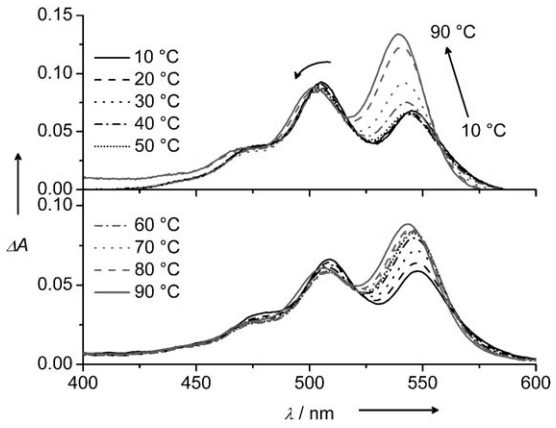

Figure 2. Temperature-dependent UV/Vis absorption spectra of **DNA1** (top) and **DNA3** (bottom), 1.25 μM duplex, 10 mM Na-P_i buffer, 250 mM NaCl, pH 7.

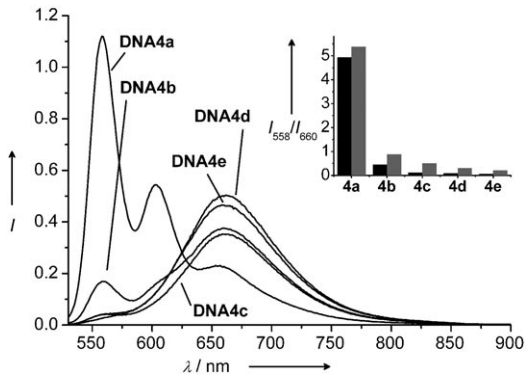

Figure 3. Fluorescence spectra of **DNA4a**–**DNA4e**, 2.5 μM duplex in 10 mM Na-P_i buffer, 250 mM NaCl, pH 7, 0 °C, excitation at 505 nm. Inset: Ratio of fluorescence intensities at 558 nm and 660 nm with 2.5 μM DNA duplex (black) and 250 nM DNA duplex (gray).

interstrand hydrophobic interactions between the two PB chromophores. This is a remarkable result since the glycol linker as a substitute for the the 2-deoxyribofuranoside in a single modification typically destabilizes the duplex stability significantly.[14,17,18] Hence, the PB dimer in DNA could be regarded as a hydrophobically and diagonally interacting base pair similar to the biphenyl and phenanthrenyl base pairs recently published by Leumann et al.[20] and Häner et al.[19] In contrast to these published examples, the PB base pair exhibits a fluorescence and absorption output signal as a result of the base interaction.

In the case of **DNA3**, the excimer-type fluorescence can be explained only by duplex aggregation through the hydrophobic interactions of the 5′-terminal PB caps, which function as fluorescent glue between the duplexes. In contrast to **DNA1**, the excimer band of **DNA3** vanishes gradually and not cooperatively with increasing temperature. This indicates that the thermally induced interruption of the PB interactions appears as a separate process and is not coupled to the thermal dehybridization of the DNA duplex architecture that occurs at $T_m = 50.7\,^{\circ}C$.

The duplex set **DNA4a**–**DNA4e** (Scheme 1) was designed to evaluate the potential of the PB excimer fluorescence for the detection of single base mismatches and single base deletions. The duplexes contain an intrastrand PB dimer that is separated by one intervening base pair. It is very astonishing that only in the presence of the correct counterbase (T in **DNA4a**) are the two chromophores well-separated and not able to induce the excimer-type fluorescence at 660 nm (Figure 3). In this case, only the PB monomer fluorescence at 558 nm is observed. In the presence of the oligonucleotides with the wrong counterbases (A, G, C) the excimer fluorescence persists and no (**DNA4c**, **DNA4d**) or only very little (**DNA4b**) PB monomer fluorescence is obtained. The latter result is also observed with duplex **DNA4e**. The complementary strand of this duplex contains one base less, representing the corresponding single-base-deletion mutant. Using the ratio of the fluorescence intensity at 558 nm vs. that at 660 nm, the detection of the right counterstrand is clearly possible at concentrations below the μM range. Thus, the sensitivity is comparable to that of other fluorescence methods.[21] In contrast to the previously published experiments of Asanuma et al.,[22] we apply the PB chromophore as the smallest representative of the very photostable rylene dyes.[23] The strong interaction of the two PB dyes as a clamp around a questionable base pair makes it possible not only to distinguish clearly between the correct counterstrand and the wrong ones but also to quantify how much of the correct counterstrand is present in a mixture (Figure 4). The PB monomer (558/603 nm) and the PB

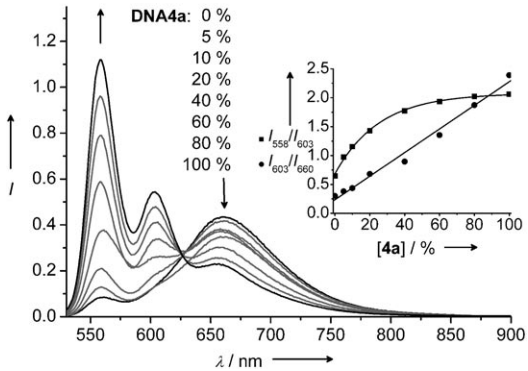

Figure 4. Fluorescence spectra of single-stranded **DNA4** hybridized with an increasing amount (0→100%) of matched counterstrand and an decreasing amount (100→0%) of an equimolar mixture of wrong counterstrands in 10 mM Na-P_i buffer, 250 mM NaCl, pH 7, 0 °C, excitation at 505 nm. Inset: Ratio of fluorescence intensities at 558 nm and 603 nm (squares) and at 603 nm and 660 nm (circles).

excimer-type fluorescence (660 nm) are well-separated by 100 nm. The ratio between the fluorescence intensities at 558 nm and 603 nm and between 603 nm and 660 nm serve as references in the determination of the amount of matched counterstrand in a mixture of counterstrands that differ only in a single base (Figure 4, inset).

In conclusion, it is evident that PB dimers as artificial DNA base substitutions yield a strong and characteristic excimer-type fluorescence inside and outside of the DNA duplex. The interstrand PB dimer can be regarded as a

hydrophobically and diagonally interacting base pair with a fluorescence readout signal for the pairing interaction. The intrastrand PB dimer as a clamp around a questionable site of base mismatches or base deletions allows the detection and quantification of the amount of matched counterstrand. The excitation wavelength of the PB dimer (505 nm) is in the range of typical bioanalytical fluorescence readers. Moreover, the high photostability of PB as the smallest representative of the rylene dyes[22] makes PB dimer-based DNA assays superior to the application of other organic chromophores including pyrene, perylene, and cyanine derivatives.

Experimental Section

The PB-modified oligonucleotides were prepared and purified according to a published procedure.[12] The only difference was the purification of the PB–DNA building block by flash chromatography (SiO_2, CH_2Cl_2/acetone 10:1) prior to the oligonucleotide synthesis. The synthetic PB–DNA building block coupled as efficiently as the commercially available phosphoramidites for the natural nucleosides A, C, G, and T. MS (ESI): single-stranded(ss)-**DNA1**: calcd. 5821,5, found $m/z = 1468$ [M^{4-}], 1945 [M^{3-}]. ss-**DNA2**: calcd. 5496.0, found $m/z = 1376$ [M^{4-}], 1834 [M^{3-}]. ss-**DNA3**: calcd. 6134.6, found $m/z = 1543$ [M^{4-}], 2047 [M^{3-}]. ss-**DNA4**: calcd. 5836,1, found $m/z = 1461$ [M^{4-}], 1947 [M^{3-}]. The PB-modified oligonucleotides were quantified by their absorbance in DMSO on a Varian Cary 100 spectrometer using $\varepsilon_{528} = 62\,500\ L\,mol^{-1}\,cm^{-1}$ for ss-**DNA1** and ss-**DNA3**, and using $\varepsilon_{500} = 79\,600\ L\,mol^{-1}\,cm^{-1}$ for ss-**DNA4**. Duplexes were formed by heating to 90 °C (10 min) followed by slow cooling. Spectroscopic measurements were recorded using quartz glass cuvettes (10 mm). The UV/Vis absorption spectra and the melting temperatures (2.5 μM DNA, 10–90 °C, 0.7 °C min^{-}, step width 0.5 °C) were recorded on a Varian Cary 100 spectrometer equipped with a 6 × 6 cell changer unit. **DNA1**: $T_m = 78.6$ °C; **DNA2**: $T_m = 76.2$ °C; **DNA3**: $T_m = 50.7$ °C; **DNA4a**: $T_m = 56.5$ °C; **DNA4b**: $T_m = 54.8$ °C; **DNA4c**: $T_m = 54.1$ °C; **DNA4d**: $T_m = 55.8$ °C; **DNA4e**: $T_m = 57.9$ °C. The fluorescence spectra were measured on a Jobin-Yvon Fluoromax 3 fluorimeter with a step width of 1 nm and an integration time of 0.2 s. All spectra were recorded with an excitation and emission bandpass of 2 nm and are corrected for Raman emission from the buffer solution.

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