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Excimer formation by interstrand stacked pyrenes†

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Received (in Cambridge, UK) 19th August 2004, Accepted 8th September 2004
First published as an Advance Article on the web 9th November 2004

Non-nucleosidic, pyrene-derived base surrogates form excimers *via* interstrand stacking in duplex DNA.

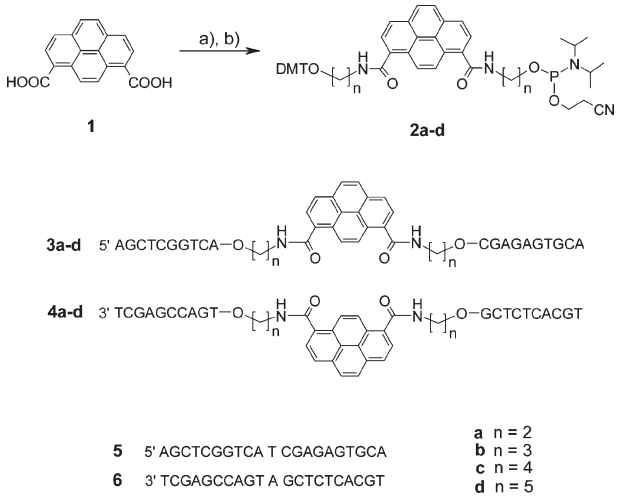
Besides their importance as genetic material, nucleic acids are increasingly gaining interest as nanometer-sized, functional matter.[1–4] Due to the repetitive, well-defined arrangement of their building blocks, nucleic acids and related types of oligomers[5–7] are ideal objects for the designed construction of larger assemblies and architectures.[8–10] Furthermore, the combination of nucleotides with non-natural building blocks greatly enhances the number of possible constructs and their potential applications. Recently, we reported the synthesis of a non-nucleosidic, phenanthrene-derived building block and its incorporation into double stranded DNA.[11,12] This simple building block serves as a base surrogate without destabilizing the DNA duplex nor altering its overall B-DNA structure. Based on the data obtained, a model of interstrand-stacked phenanthrenes was proposed.[11] Consequently, replacement of the non-nucleosidic phenanthrenes by analogous pyrenes should then give rise to excimer formation.[13] Here, we report the synthesis, the hybridisation behaviour as well as the spectroscopic properties of duplex DNA containing non-nucleosidic pyrene building blocks.

The synthesis of the required pyrene building blocks is shown in Scheme 1. Thus, pyrene 1,8-dicarboxylic acid (**1**) was obtained according to a procedure reported in the literature.[14] Subsequent derivatisation with the corresponding linkers, followed by phosphitylation under standard conditions gave the desired pyrene phosphoramidites **2a–d**.† These building blocks were further used for the preparation of the oligomers **3a–d** and **4a–d** by automated oligonucleotide synthesis. Incorporation of the pyrene phosphoramidites proceeded without any difficulties, coupling yields being equal to those of unmodified nucleotide bases. In addition to the pyrene-containing oligomers, oligonucleotides **5** and **6** were prepared for control purposes. All oligomers were purified by reverse phase HPLC and characterized by *electrospray ionisation time-of-flight* (*ESI-TOF*) mass spectrometry (see ESI†).

Thermal denaturation experiments revealed a slight, but distinct, influence by the length of the non-nucleosidic linkers on the thermal stability of pyrene-modified duplexes (Table 1). The highest thermal stability was observed with pyrene building blocks having four methylene units in the linkers (duplex **3c*4c**). This hybrid is equally stable as the control duplex **5*6**, in which an AT-base pair substitutes for the two pyrenes. The building blocks with shorter (*i.e.* duplexes **3a*4a** and **3b*4b**) or longer (duplex **3d*4d**) linkers led to a decrease in the T_m-value in the range of 2–3 °C. Furthermore, circular dichroism (CD) spectra were typical for a B-DNA structure (see ESI†).

We next investigated the fluorescence properties of the different pyrene-containing oligomers (see Fig. 1). Upon irradiation at a wavelength of 354 nm, all hybrids showed emission at 398 nm, which is characteristic for pyrene monomer fluorescence. For the single stranded oligomer **3c**, emission was observed only at this wavelength. In contrast, all pyrene-containing duplexes showed a second fluorescence emission with a maximum value at 493 nm, typical for pyrene eximers. The degree of excimer formation is parallel to the thermal stability observed for the respective duplexes. Thus, the most stable duplex **3c*4c** showed the strongest excimer fluorescence. For the less stable duplex **3d*4d**, the monomer emission is dominant and only weak excimer fluorescence was observed. The other two hybrids, **3a*4a** and **3b*4b**, showed an intermediate behaviour. This trend is also illustrated by the excimer/monomer fluorescence ratio given in Table 1, which is highest for the most stable duplex **3c*4c** and least for **3d*4d**.

Like the structurally similar 3,6-disubstituted phenanthrene derivatives,[11] the non-nucleosidic pyrene derivatives described herein form stable DNA hybrids. With an optimal linker length, two pyrene building blocks contribute to duplex stability approximately as much as a natural AT-base pair. The observation of strong excimer fluorescence is clear evidence for interstrand-stacking interactions of the two pyrenes arranged in opposite



Scheme 1 Phosphoramidite building blocks and pyrene-modified oligonucleotides used in this study. Reagents and conditions: a) $H_2N(CH_2)_nOH$/$H_2N(CH_2)_nODMT$ (1 : 1), *Hünig's* base, BOP; b) 2-cyanoethyl diisopropylamidochloridophosphite, *Hünig's* base.

Table 1 T_m-values and fluorescence ratios of pyrene-modified double stranded DNAs[a]

Duplex	5*6	3a*4a	3b*4b	3c*4c	3d*4d
T_m/°C[b]	68.0	65.0	65.7	67.8	64.7
ΔT_m/°C[c]	—	−3.0	−2.3	−0.2	−3.3
Excimer : monomer ratio[d]	—	1.68	2.58	3.24	0.48

[a] Conditions: oligomer concentration 1.0 μM, 10 mM Tris–HCl, 100 mM NaCl, pH 7.4; temp. gradient: 0.5 °C min^{-1}. [b] Melting temperatures were determined from the maximum of the first derivative of the melting curve (A_{260} against temperature); exptl. error: ±0.5 °C. [c] Difference in T_m relative to **5*6**. [d] Emission wavelengths: excimer: 493 nm; monomer: 398 nm.

† Electronic supplementary information (ESI) available: experimental procedures and analytical data for all new compounds. See http://www.rsc.org/suppdata/cc/b4/b412831a/

DOI: 10.1039/b412831a

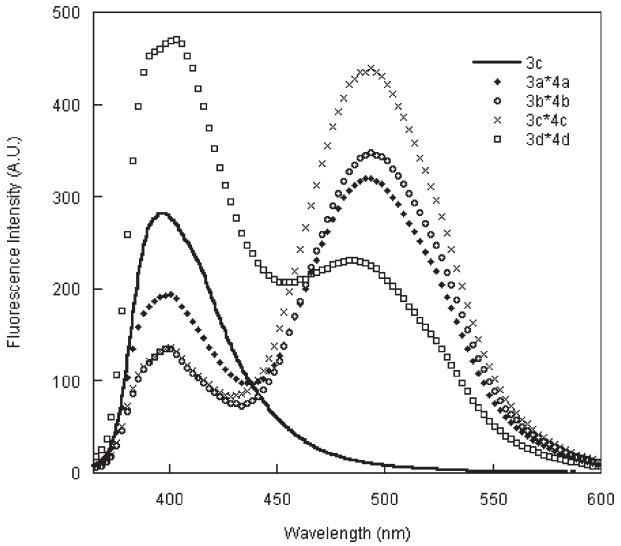


Fig. 1 Fluorescence spectra of pyrene-containing duplexes **3a–d*4a–d**, as well as of the single stranded oligonucleotide **3c**. Conditions: oligomer concentration 1.0 μM, 10 mM Tris–HCl, 100 mM NaCl, pH 7.4, room temperature. Excitation wavelength: 354 nm; excitation slit: 5 nm; emission slit: 7 nm.

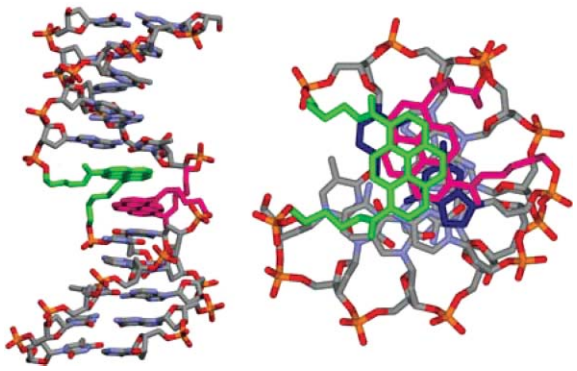


Fig. 2 Molecular model of the duplex **3c*4c** with two interstrand stacked pyrenes (HyperChem™ 7.0, minimised with *amber* force field).[15] The non-nucleosidic building blocks are highlighted in red and green. Left: view perpendicular to the helical axis. Right: view along the helical axis. showing the pyrenes stacked on the nucleotide bases; nucleotides on the viewers side have been omitted for clarity; the GC-base pair adjacent to the pyrenes is shown in blue.

positions within the DNA duplex. Furthermore, duplex stability and excimer formation are both influenced by the linker in the same way. This observation is well in agreement with a model of interstrand stacked pyrenes, since stacking interactions should favour the formation of the excimer[13] and, at the same time, also the interaction between the two strands.

Formation of excimers has been observed in pyrene-modified, single as well as double stranded DNAs.[16–23] Due to the many potential diagnostic applications, hybridisation-induced excimer formation is of particular interest. Thus, attachment of pyrene derivatives to the backbone[20,21,23] as well as to terminal positions[18,19] of oligonucleotides have been reported in this context. Hybridisation-induced pyrene excimer formation was observed by Korshun and coworkers[20] in the major groove of double stranded DNA and, very recently, also in the minor groove of DNA containing pyrene–LNA residues by Wengel and coworkers.[21] The results described here show, for the first time, that excimer formation can also take place through *interstrand stacking interactions*. This observation is different to the findings of Malakhov *et al.*, who reported that two pyrenes placed in opposite positions in double stranded DNA did not form an excimer.[24] Fig. 2 shows a molecular model of the hybrid **3c*4c** with the two pyrenes embedded within the aromatic core of the duplex. A similar arrangement of two intercalating pyrenes in a DNA duplex was recently reported by Nielsen and colleagues.[25] Furthermore, the model shows that the non-nucleosidic linkers do not disturb the overall continuity of the helical backbone.

In conclusion, we have shown that complementary DNA strands containing simple, non-nucleosidic pyrene building blocks in opposite positions form stable hybrids. The pyrene residues contribute to the overall stability of the duplex through interstrand stacking interactions. Strong excimer fluorescence is observed upon hybridisation of two modified strands.

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