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A DNA Mimic Made of Non-Nucleosidic Phenanthrene Building Blocks

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Dedicated to Prof. Peter B. Dervan on the occasion of his 60th birthday.

Modified oligonucleotides have found widespread applications as diagnostic and research tools. Furthermore, the generation of defined molecular architectures by using nucleotide-like building blocks is a research topic of increasing interest.[1–6] The repetitive and well-defined structural features of nucleic acids and related types of oligomers renders them valuable

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building blocks for the generation of nanometre-sized structures.[7] In addition, the combination of nucleotides with non-natural building blocks leads to a large increase in the number of possible constructs and applications. Recently, we reported the synthesis and properties of non-nucleosidic, phenanthrene-based building blocks and their incorporation into DNA.[8] These building blocks can serve as base surrogates without destabilising the DNA duplex or altering its overall B-DNA structure. Based on spectroscopic data, a model of interstrand stacked polyaromatic building blocks was proposed (Figure 1), which was further supported by the observation of excimer formation by pyrenes placed in opposite positions.[9] Replacement of two base pairs by non-nucleosidic phenanthrene building blocks was well tolerated and had almost no influence on duplex stability.[8] We subsequently investigated the effect of further replacements of base pairs by phenanthrene building blocks. Such DNA mimics are also important in view of the recent discovery of a natural, peptide-based DNA mimic used by *Mycobacterium tuberculosis* to evade the effect of antibiotic agents.[10] Here, we report that DNA containing extended stretches of phenanthrene building blocks forms stable hybrids and that the obtained data are in agreement with the model of interstrand stacked phenanthrenes.

The required phenanthrene phosphoramidite was prepared as described previously.[8] All oligomers were assembled by standard automated oligonucleotide synthesis and purified by reversed-phase HPLC. The correct identities of the purified oligomers were verified by electrospray ionisation time-of-flight (ESI-TOF) mass spectrometry (see Supporting Information). The effect on the DNA duplex stability of replacing base pairs with the phenanthrene building blocks was analysed by thermal-denaturation experiments. All oligomers investigated in this study showed a single, cooperative transition. The melting temperatures (T_m) are given in Table 1. As described previously, the replacement of one or two base pairs with phenenathrene has either no or only a slight stabilising effect (entries 2 and 3).[8] Subsequent replacement of a third and a fourth base pair is still well tolerated. The corresponding hybrids show only a small reduction in thermal stability. For the hybrid in which seven base pairs were replaced with phenanthrenes, a decrease of 8.3 °C was observed. This translates into a ΔT_m of −1.2 °C per modification. The middle part, which represents a considerable fraction of this duplex, is stabilised merely by interstrand stacking interactions. Molecular modelling suggests that the part of the phenanthrene stretch has a slightly smaller diameter than the regular B-DNA parts on both sides. It is possible that the stability can be further influenced by choosing a different type of linker to connect the phenanthrenes. Nevertheless, replacement of seven natural base pairs by the same number of flexible phenanthrene residues of this type results in the relatively small destabilisation of approximately 8 °C.

Table 1. Influence of the replacement of natural base pairs by non-nucleosidic phenanthrene building blocks on the thermal stability of duplex DNA.

	Duplex	T_m [°C][a,b]	ΔT_m[c]	ΔT_m per modification[d]
1	(5′) AGCTCGGTCATCGAGAGTGCA (3′) TCGAGCCAGTAGCTCTCACGT	68.0	–	–
2	(5′) AGCTCGGTCA**P**CGAGAGTGCA (3′) TCGAGCCAGT**P**GCTCTCACGT	68.3	0.3	0.3
3	(5′) AGCTCGGTC**PP**CGAGAGTGCA (3′) TCGAGCCAG**PP**GCTCTCACGT	69.3	1.3	0.6
4	(5′) AGCTCGGT**PPP**CGAGAGTGCA (3′) TCGAGCCA**PPP**GCTCTCACGT	67.7	−0.3	−0.1
5	(5′) AGCTCGG**PPPP**CGAGAGTGCA (3′) TCGAGCC**PPPP**GCTCTCACGT	66.0	−2.0	−0.5
6	(5′) AGCTC**PPPPPPP**GAGAGTGCA (3′) TCGAG**PPPPPPP**CTCTCACGT	59.7	−8.3	−1.2

[a] Conditions: oligomer concentration 1.0 μM, 10 mM Tris-HCl, 100 mM NaCl, pH 7.4; temperature 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); each T_m is the average of three independent experiments; experimental error: ±0.5 °C. [c] Difference in T_m relative to the control duplex (entry 1). [d] Difference in T_m relative to the control duplex (entry 1) divided by the number of modifications per single strand.

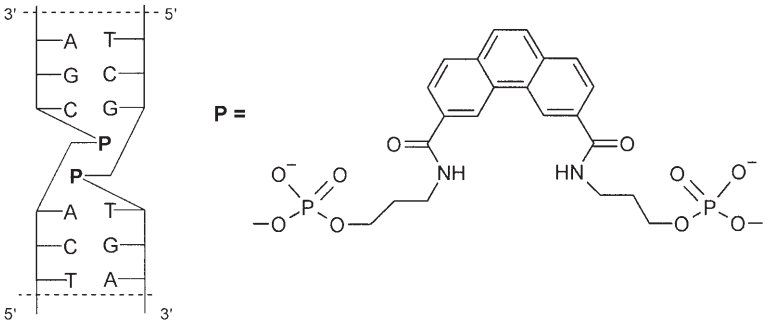

Figure 1. Schematic illustration of a duplex containing non-nucleosidic, interstrand stacked phenanthrene residues.

The circular dichroism (CD) spectra are in good overall agreement with that of a typical B-DNA structure (Figure 2). The minima and maxima of the modified duplexes correlate very well with the ones of the unmodified DNA duplex (250 nm and 280 nm, respectively). An additional shoulder is apparent in the spectra of the modified duplexes in the range of 240–250 nm. This corresponds to the region of the absorption of the phenanthrene building block (240–260 nm). The magnitude of this shoulder increases with the number of phenanthrene residues present in the hybrid.

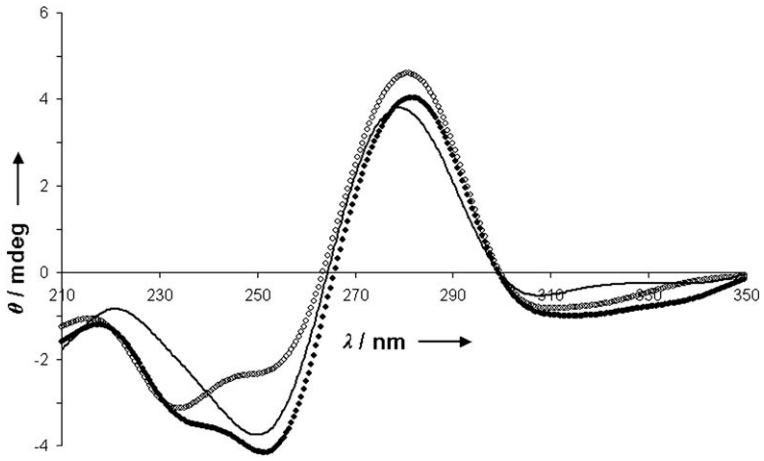

Figure 2. Circular dichroism spectra of a duplex containing three (◆; entry 4 in Table 1) or seven (◇; entry 6 in Table 1) phenanthrene "pairs" in comparison to the unmodified DNA duplex (——).

The model of interstrand stacked phenanthrenes predicts a lengthening of the duplex by approximately 3.4 Å for each replacement of a natural base pair by a pair of phenanthrene-derived building blocks. To illustrate this point, an amber-minimised structure[11] of the hybrid containing seven phenanthrene pairs is shown in Figure 3. In comparison to the unmodified duplex—containing the same overall number of residues—the interstrand stacked arrangement of the phenanthrene moieties leads to a significant extension of the DNA. The charge of the hybrids, on the other hand, remains the same throughout the whole series since the modified building blocks are, like the natural nucleotides, connected through phosphodiester bonds. To investigate this theoretical length dependence on the number of phenanthrenes, the relative gel mobilities of the different hybrids were examined. Figure 4 shows a gradual reduction in the gel mobility with an increasing number of phenanthrenes. As expected, the largest effect is observed upon replacement of seven natural base pairs by seven interstrand stacking phenanthrene pairs (lane 6). According to the interstrand stacked model, this part of the hybrid should possess a length that is comparable to a segment of an unmodified duplex containing 14 base pairs (Figure 3). In the context of the hybrid under discussion, this accounts for half of the overall length of the duplex.

Similar models of hybrids containing stretches of interstrand stacked building blocks with non-nucleosidic linkers have been proposed by other groups. Iverson and co-workers showed that interstrand stacking forces favour duplex formation of alternating electron-rich and electron-deficient aromatic units with flexible backbones.[12;13] Similarly, Zhou et al. reported the formation of a molecular duplexes driven by cooperative donor–acceptor interactions.[14] Interstrand stacking of two non-nucleosidic pyrene derivatives placed within a DNA duplex in opposite positions was shown by Nielsen et al.[15] Furthermore, the formation of stretches of interstrand stacked, 2′-deoxyribose-based building blocks has been reported elsewhere in the literature.[16,17] The data presented here show that also non-nucleosidic building blocks form extended stretches of interstrand stacked residues if embedded within a DNA duplex. While gel-mobility experiments show a lengthening of the duplex, the phenanthrene stretch does not alter the B-form structure of the flanking DNA parts significantly according to CD spectroscopy. The non-nucleosidic phenanthrene described here thus serves as an additional building block for the

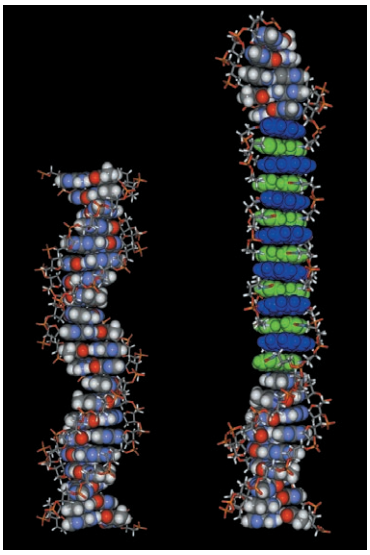

Figure 3. Amber-minimised models of a 21-mer DNA duplex (left) and a 21-mer DNA mimic in which seven base pairs have been replaced with phenanthrenes (see entry 6, Table 1). The phenanthrene residues of the two strands are shown in blue and green. The interstrand stacked arrangement of the phenanthrenes leads to an increase in the length of the duplex.

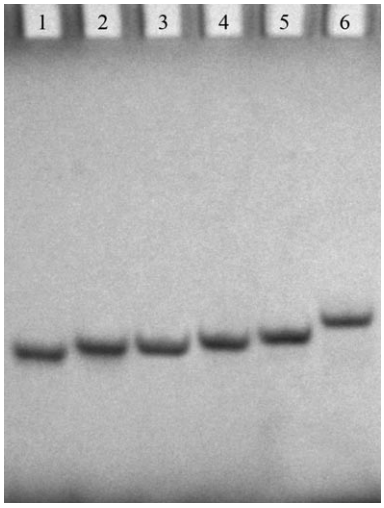

Figure 4. Nondenaturing 20% polyacrylamide gel of phenanthrene-containing hybrids. The line numbers correspond to the entry numbers from Table 1, that is, the number of phenanthrenes per strand increases from left (0) to right (7). Bands were visualised by UV light (260 nm).

modular assembly of nanometre-sized structures with nucleic acids.

In conclusion, we have shown that complementary DNA strands containing extended stretches of non-nucleosidic phenanthrene building blocks in opposite positions form stable hybrids. Up to seven phenanthrenes per strand are well tolerated in a 21-mer oligomer. The phenanthrene residues contribute to the overall stability of the duplex through interstrand stacking interactions. Continuous replacement of the bases by the phenanthrene moieties results in a proportional lengthening of the duplex, which is a consequence of the interstrand stacked arrangement of the phenanthrenes. Non-nucleosidic aromatic derivatives such as the ones described here can, thus, serve as building blocks for the construction of DNA mimics.

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Keywords: DNA · non-nucleosidic base surrogates · oligonucleotides · phenanthrene · stacking interactions

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