Duplex and hairpin dimer structures for perylene diimide–oligonucleotide conjugates†

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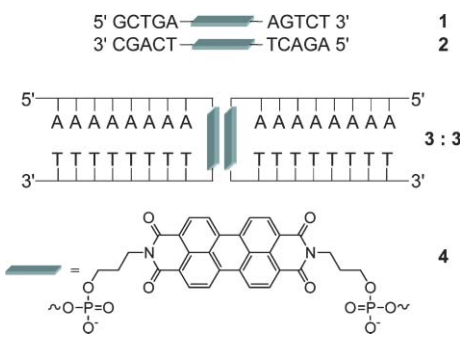
Perylene diimide–oligonucleotide conjugates can form either duplex or hairpin dimer structures, depending upon the choice of oligonucleotide base sequence; we have used a combination of optical spectroscopy and molecular modeling to investigate the structures of the duplex and hairpin dimer.

Perylene diimide (PDI) derivatives have an unusual propensity to form self-assembled dimers and aggregates and thus have been widely used as building blocks for supramolecular architectures.[1,2] Bis(oligonucleotide) conjugates possessing PDI linkers have been reported to form a variety of structures, including duplexes, triplexes, and capped hairpins, as well as novel structures possessing several PDI chromophores connected by disordered oligonucleotides.[3–5] Our interest in the structure and properties of oligonucleotide conjugates[6,7] led us to investigate the simple PDI-linked conjugates **1–3** (Scheme 1). The single stranded conjugates **1** and **2** do not form PDI aggregates in dilute aqueous solution. However, hybridization of **1** and **2** results in the formation of an exceptionally stable duplex **1:2**, in which the orientation of the two PDI chromophores is controlled by the duplex structure. The self-complementary conjugate **3** forms a novel hairpin dimer structure **3:3**, in which the PDI chromophores adopt a parallel structure similar to that favored by unconstrained PDI dimers and aggregates.[1,2]

The PDI conjugates **1–3** were prepared following the procedure of Letsinger and Wu[8] as modified by Rahe *et al.*[4] for the incorporation of the PDI linker **4**. Conjugates were purified by HPLC and characterized by MALDI-TOF mass spectrometry.†



Scheme 1 Structures of the duplex **1:2**, the hairpin dimer **3:3**, and the PDI linker **4**.

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† Electronic supplementary information (ESI) available: mass spectra and melting curves and derivatives and video clip of duplex molecular dynamics. See http://dx.doi.org/10.1039/b509754a

Conjugates **1–3** are non-fluorescent, in accord with previous observations of quenching of the PDI singlet state by nucleobases.[3] The UV spectra of conjugate **1**, an equimolar mixture of **1** and **2** (**1:2**), and **3** are shown in Fig. 1. The 260 nm bands are dominated by absorption of the nucleobases. The long wavelength band of **1** (Fig. 1a) is characteristic of the monomeric PDI chromophore, having a well-defined vibronic progression with $A^{0-0}/A^{0-1} \sim 1.4$.[1] The vibronic band intensities of **1:2** and **3:3** (Fig. 1b,c) are inverted at room temperature ($A^{0-0}/A^{0-1} \sim 0.6$), consistent with the formation of PDI dimers or aggregates in which the PDI chromophores are in close contact.[9]

Upon heating, the 260 nm bands of **1:2** and **3:3** increase in intensity, indicative of base pair melting.[10] Their 541 nm bands also increase in intensity and the 541/504 nm band ratio approaches a value of 1.6, characteristic of the PDI monomer. The melting temperature (T_M) for 1.8 μM **1:2**, obtained at either 260 nm or 541 nm is 70 ± 1 °C. The T_M value decreases with decreasing conjugate concentration (0.6–1.8 μM), as expected for duplex formation.† The 260 nm T_M value for **3:3** is independent of concentration (60 ± 1 °C),† as is the case for other hairpin-forming bis(oligonucleotide) conjugates.[8] The temperature dependence of the 541 nm band for **3** is concentration dependent, but does not have a well-defined first derivative. This information is sufficient to support the assignment of the duplex structure **1:2** and the hairpin dimer structure **3:3** (Scheme 1).

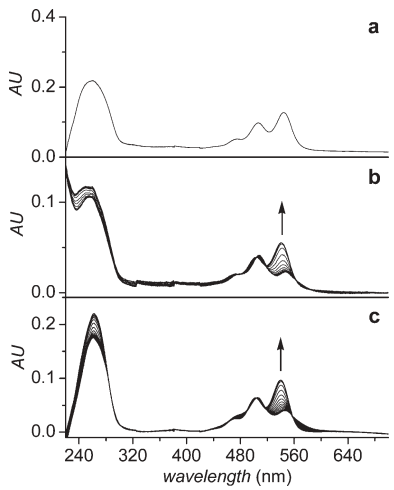

Fig. 1 Ultraviolet absorption spectra of (a) conjugate **1** (2.5 μM), (b) temperature dependent spectra of the duplex **1:2** (0.9 μM), and (c) temperature dependent spectra of the dimer **3:3** (1.3 μM). Arrows indicate increasing temperature (10–90 °C). All solutions contain 10 mM phosphate, 100 mM NaCl, pH 7.4.

The T_M value for 1.8 μM **1:2** is significantly higher than that for a 5.0 μM solution of either the analogous duplex possessing stilbenedicarboxamide linkers or a reference duplex with the same base sequence but lacking the PDI linkers (T_M = 70 ± 1 °C *vs.* 40 ± 1 °C).[6] Thus PDI dimer formation significantly enhances duplex stability. The value of T_M for **3:3** is similar to that for a stilbenedicarboxamide-linked hairpin possessing a 6-mer poly(dA)–poly(dT) base pair domain.[8] Evidently, the enthalpy of PDI dimer formation is sufficient to overcome the unfavorable entropy of hairpin dimerization. However, it is not sufficient to convert the hairpin structure, which is universally observed for bis(oligonucleotide) conjugates with complementary poly(dT) and poly(dA) arms,[8,11] to a duplex structure.

More detailed information about the structure of the duplex **1:2** and hairpin dimer **3:3** is provided by their CD spectra (Fig. 2). The CD spectrum of **1:2** at 20 °C resembles that of the duplex poly[d(A-G)]:poly[d(C-T)] in the base-pair region (200–300 nm), in accord with the presence of several d(A–G) steps.[12] The spectrum of **3:3** at 20 °C in the base pair region resembles that of poly(dA)–poly(dT).[12] The CD spectrum of **1:2** also displays a strong negative band at 560 nm and a positive band at 500 nm. Much weaker long-wavelength bands are observed for **3:3** at 20 °C. These long-wavelength CD bands are attributed to exciton coupling (EC-CD) between the two PDI chromophores.[13] CD melting profiles for 1.8 μM **1:2** (265 nm or 563 nm) and 6.0 μM **3:3** (245 nm) provide values of T_M similar to those obtained from the UV data in Fig. 1.

The intensity of the positive and negative bands of the EC-CD spectrum for two identical parallel chromophores can be described by Eq. 1,

$$\Delta\varepsilon \approx \pm \frac{\pi}{4\lambda} \mu_a \mu_d R_{da}^{-2} \sin(2\theta) \quad (1)$$

where μ_d and μ_a are the electronic transition dipole moments of the two chromophores, R_{da} is their center-to-center distance, and θ is the angle between their transition dipoles.[10,14] The $\sin(2\theta)$ dependence results in zero intensity when θ = 0, 90, or 180° and maximum intensity when θ = 45 or 135°. The very low CD intensity for the hairpin dimer **3:3** is consistent with a dimer geometry in which the PDI long axes are aligned, the preferred geometry for unconstrained PDI dimers and aggregates.[1,2] The much larger CD intensity for the duplex **1:2** is indicative of a non-aligned PDI dimer geometry.

Geometries for **1:2** and **3:3** were calculated using the AMBER force field by adopting the canonical B-form DNA structure for their base-pair domains, which were truncated to three base-pairs on either side of the PDI dimer.[15] The Amber 7 program suite was used to run molecular dynamics simulations for 2 ns with a step length of 2 fs.[16] A video clip of the dynamics simulation for **1:2** is provided as Electronic Supplementary Information.† Averaged structures obtained from the MD simulations for **1:2** and for one of the two minima for **3:3** are shown in Fig. 3. MD snapshots provide values of θ = 38 ± 4° for **1:2** and θ = 4 ± 19° and 180 ± 22° for the two isomeric dimers **3:3**, which differ only in the dihedral angle between the two PDI chromophores.

In the averaged structure for **1:2** stacking of the PDI dimer with the adjacent base pairs presumably minimizes hydrophobic forces, but is achieved at the expense of distortion of the sugar–phosphate backbone, as well as the PDI alignment. The small fluctuations in θ for **1:2** are indicative of restricted motion in the duplex interior. The value of θ is close to the value required for maximum EC-CD intensity (38° *vs.* 45°) and a negative/positive splitting pattern. The smaller values of θ calculated for the isomeric dimers **3:3** are consistent with its weak EC-CD spectrum (Fig. 2b). The large fluctuations in MD simulated dihedral angles are indicative of a shallow potential energy surface for rotation about the PDI–PDI axis.

In summary, PDI dimer formation can be utilized both to enhance the stability of conventional DNA duplex structures and for the assembly of hairpin dimers, without the assistance of base pairing. This novel form of DNA assembly promises to provide access to new DNA structural motifs. Conventional temperature and concentration-dependent UV data are sufficient to establish the formation of a duplex structure upon hybridization of conjugates **1** and **2** and a hairpin dimer structure by conjugate **3**. Information about the PDI geometry in the duplex and hairpin dimer structures is provided by a combination of EC-CD spectroscopy and molecular modeling. We note that this information is not readily available from solution UV or ^{1}H NMR

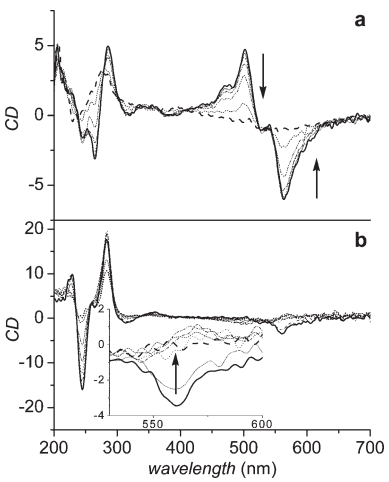

Fig. 2 Temperature dependent CD spectra of (a) the duplex **1:2** (1.8 μM) and (b) the hairpin dimer **3:3**. (6 μM monomer). Arrows indicate increasing temperature (20–80 °C).

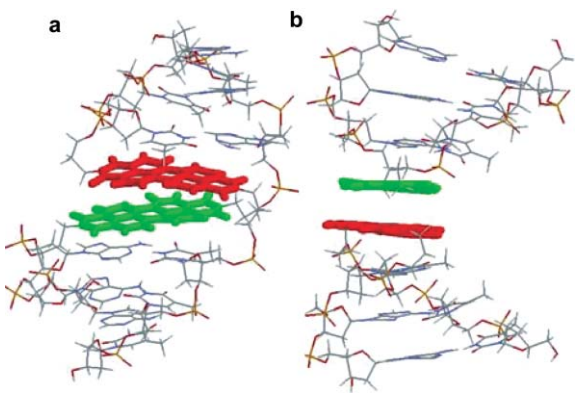

Fig. 3 Averaged structures for (a) the duplex **1:2** and (b) the hairpin dimer **3:3** (isomer with 4° dihedral angle between PDI hairpin linkers) obtained from MD simulations. Structures are truncated to three base pairs on either side of the PDI dimers which are in the middle of both structures.

spectroscopy. The CD spectra of the aggregates formed by chiral PDI derivatives have been previously reported.[17] However, this report describes the initial use of EC-CD spectroscopy to investigate the alignment of PDI dimers.

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