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PNA−DNA Duplexes, Triplexes, and Quadruplexes Are Stabilized with *trans*-Cyclopentane Units

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Peptide nucleic acids (PNAs) derived from an aminoethyl glycine (*aeg*) backbone can bind to oligonucleotides to form duplexes, triplexes, and quadruplexes.[1] While PNAs with the *aeg* backbone have been used in numerous applications, modified PNA derivatives that increase duplex stability have recently emerged.[2] Our research has demonstrated that incorporation of (*S*,*S*)-*trans*-cyclopentane (*t*cyp) units into the PNA backbone improves duplex stability and sequence specificity when binding DNA.[3] Previously, *t*cyp with the bases T, C, and A was incorporated into PNA. The increase in PNA−DNA stability obtained by *t*cyp incorporation can improve DNA detection systems, as demonstrated with nanoparticle-based scanometric DNA detection.[4] In addition to duplex structures, *aeg*PNA can form triplexes and quadruplexes with oligonucleotides, and modifications that improve the stability of these structures could be similarly useful.[5] We report in this Communication the development of chemistry to make a *t*cypPNA bearing guanine (Supporting Information) and the associated improvement in stability of PNA−DNA duplexes, bisPNA_2−DNA triplexes and PNA_2DNA_2 quadruplexes (Figure 1).

Consistent with our previous data,[3] one *t*cyp-G residue (**G***) improves the stability of a PNA−DNA duplex by ∼5 °C (Table 1, entries 1 and 2). To test the effects on triplexes, *t*cyp residues were incorporated into a bisPNA.[6] A bisPNA contains two PNA sequences, connected by a flexible linker. One PNA strand (antiparallel) binds the complementary DNA by Watson−Crick (W−C) hydrogen bonding while the other strand (parallel) recognizes the Hoogsteen face, thus forming a PNA_2DNA triplex. In the parallel strand, N7-guanine has been incorporated as a protonated cytosine mimic.[7] Unlike cytosine or the commonly used pseudo-isocytosine,[1b] N7-guanine cannot participate in W−C hydrogen bonding. Thus, any additional thermal stability conferred by $\mathbf{G}^{\dagger}$ can be confidently attributed to the *t*cypPNA residue participating in Hoogsteen bonding.

To confirm that a bisPNA with N7-guanine would behave similarly to the cytosine analogue, the triplex thermal stability of PNA 4 (Table 1) was compared to PNA 3 at pH 7. The similarity of the melting transitions demonstrates that both form triplexes under the same conditions, consistent with previous studies.[7] To test the effects of *t*cyp incorporation, a bisPNA was constructed that contained **T*** residues directly across from each other (Table 1, PNA 5). The binding of PNA 5 to DNA demonstrates that *t*cyp residues increase the binding affinity. Incorporating four $\mathbf{G}^{\dagger}$ residues into the Hoogsteen-binding (parallel) segment also increased the thermal stability of a bisPNA−DNA triplex (Table 1, PNA 6). Although the increase in stability per *t*cypPNA residue is modest compared to its duplex counterparts, this disparity is not surprising considering that the Hoogsteen strand binding affinity is usually lower than W−C hydrogen bonding at pH 7.[8] These results indicate that *t*cyp groups stabilize both W−C and Hoogsteen binding of PNA to DNA in triplex-forming complexes and should be fully compatible with associated applications.

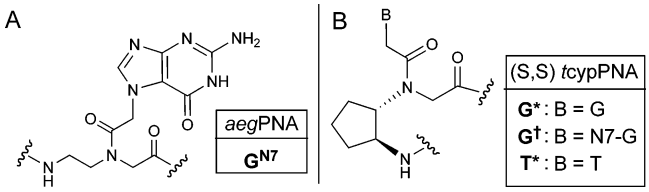

Figure 1. PNA residues incorporated into oligomers: (A) *aeg*PNA with an N7-guanine as its nucleobase; (B) *t*cypPNA residues examined in this communication.

Table 1. T_m Data for PNA−DNA Duplex and Triplexes

PNA[a]	sequence	T_m (°C)
	Duplexes[b]	
1	Ac-GTAGATCACT-Lys-NH_2	52.0
2	Ac-GTA**G***ATCACT-Lys-NH_2	56.8
	Triplexes[c]	
3	H-$(egl)_2$-TCTCTCTC-$(egl)_3$-CTCTCTCT−NH_2	54.3
4	H-$(egl)_2$-TCTCTCTC-$(egl)_3$-$G^{N7}TG^{N7}TG^{N7}TG^{N7}T$−$NH_2$	53.1
5	H-$(egl)_2$-TCTC**T***CTC-$(egl)_3$-$G^{N7}TG^{N7}\mathbf{T^*}G^{N7}TG^{N7}T$−$NH_2$	58.5
6	H-$(egl)_2$-TCTCTCTC-$(egl)_3$-$\mathbf{G^{\dagger}}T\mathbf{G^{\dagger}}T\mathbf{G^{\dagger}}T\mathbf{G^{\dagger}}T$−$NH_2$	57.0

[a] Structures of T*, G*, G^{N7}, $G^{\dagger}$ are defined in Figure 1. Residues designated egl represent 8-amino-3,6-dioxaoctanoic acid. Ac denotes PNA with an acetylated N terminus. Conditions for T_m measurement: 150 mM NaCl, 10 mM sodium phosphate buffer, pH 7.0, 0.1 mM EDTA, UV measured at 260 nm from 10 to 90 °C in 1 °C increments. [PNA] are all 5 μM. All values are averages from three or more experiments. Approximate error for T_m values is ±0.6 °C. [b] Duplex data obtained using DNA: 5′-AGTGTACTAC-3′. [c] Triplex data obtained using DNA: 5′-GAGAGAGA-3′.

The effect of a *t*cyp group on the stability of PNA-derived quadruplexes was examined by incorporating **G*** into a previously studied "G_4-PNA" sequence (H-$G_4T_4G_4$-NH_2).[1c,d] The *aeg* version of G_4-PNA interacts with itself to form a quadruplex composed of two PNAs (a homodimer),[1d] and also forms a quadruplex with the homologous G_4-DNA sequence in which the stoichiometry of PNA−DNA is 2:2 (a heterotetramer).[1c] Melting temperatures for the *aeg*G_4-PNA quadruplexes were consistent with reported values (Table 2, PNA 7). With the introduction of one **G*** residue and four **G*** residues in nonadjacent positions, the thermal stability of the quadruplexes, with and without DNA, increased (Table 2, PNAs 8 and 9). Furthermore, when four **G*** residues were adjacent to each other (PNA 10), the ability of this PNA to form a quadruplex with itself was lower compared to other G_4-PNAs. However, the quadruplex formed with PNA 10 and DNA was exceptionally stable (∼82 °C). These results indicate that careful *t*cyp incorporation into quadruplex-forming PNAs can be used to modulate binding preferences to DNA over PNA.

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Table 2. T_m Data for PNA and PNA−DNA Quadruplexes

PNA[a]	sequence	T_m (no DNA)	T_m[b] (+G_4-DNA)	ΔT_m[c] 1	ΔT_m[d] 2
7	Ac-GGGGT_4GGGG-Lys-NH_2	48.4	62.7		+14.3
8	Ac-GG**G***GT_4GGGG-Lys-NH_2	51.9	67.0	+4.3	+15.1
9	Ac-**G***G**G***GT_4**G***G**G***G-Lys-NH_2	61.0	72.9	+10.2	+11.9
10	Ac-**G***G**G***G***T_4GGGG-Lys-NH_2	41.0	81.6	+18.9	+40.6

[a] Structure of G* is defined in Scheme 2. Experiments with DNA performed with (5′-GGGGTTTTGGGG-3′) at 1:1 stoichiometry and with a total strand concentration of 20 μM. Conditions for T_m measurement: 150 mM NaCl, 10 mM sodium phosphate buffer, pH 7.0, 0.1 mM EDTA, UV measured at 295 nm from 90 to 10 °C, then heated from 10 to 90 °C, both in 1 °C increments. All values are averages from two or more experiments, and are reported in °C. Approximate error for T_m values is ±1.0 °C. [b] Minimal hysteresis was observed in complexes with G_4-DNA (∼1 °C) and the T_m listed is the average of heating and cooling experiments. [c] The difference between the *tc*ypPNA and *aeg*PNA G_4 sequences in the presence of G_4-DNA. [d] The difference between the G_4-PNA−DNA complex and the quadruplex containing only G_4-PNA.

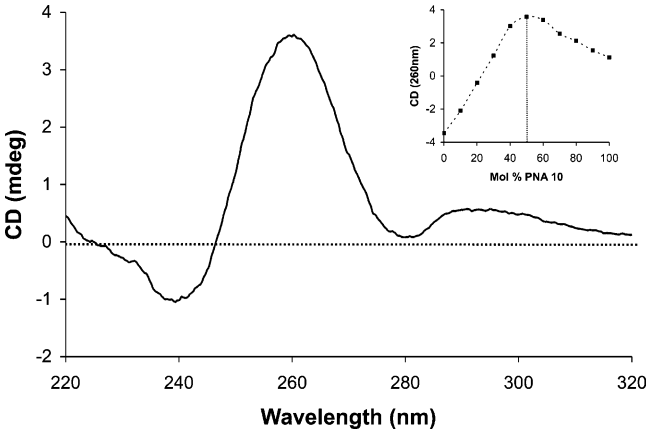

Figure 2. CD spectrum of PNA 10 annealed with G_4-DNA (5 μM each strand). The spectrum represents eight scans that were collected at a rate of 100 nm/min and then averaged. The inset shows the continuous variation experiment of PNA 10 with G_4-DNA, 10 μM total strand concentration.

To further characterize PNA quadruplexes formed with *tc*yp incorporation, circular dichroism (CD) spectra were acquired (Figure 2. The CD spectra of the quadruplex formed between DNA and PNAs 9 and 10 matched previously characterized *aeg*PNA_2−DNA_2 quadruplexes, with distinctive maxima at 260 and 295 nm (Figure 2 and Supporting Information). A continuous variation experiment (Job plot)[1c] confirmed that PNAs 9 and 10 form a quadruplex with G_4-DNA that has a 1:1 stoichiometry (Figure 2 and Supporting Information). These results indicate that *tc*yp-modified G_4-PNA probably forms PNA_2−DNA_2 quadruplexes, which is consistent with the previous results of the *aeg* analogues.[1c]

Because *aeg*PNA is achiral, quadruplexes formed with *aeg*G_4-PNA by itself show very little CD signal.[1c] However, PNAs 9 and 10 have defined CD spectra when annealed in the absence of DNA and in the presence of sodium salt (Figure 3). Furthermore, the CD spectra of these PNAs are significantly different when salt is omitted from solution, indicating a change in structure from a single strand to a quadruplex (Supporting Information).

The results of these studies indicate that *tc*ypPNA stabilizes

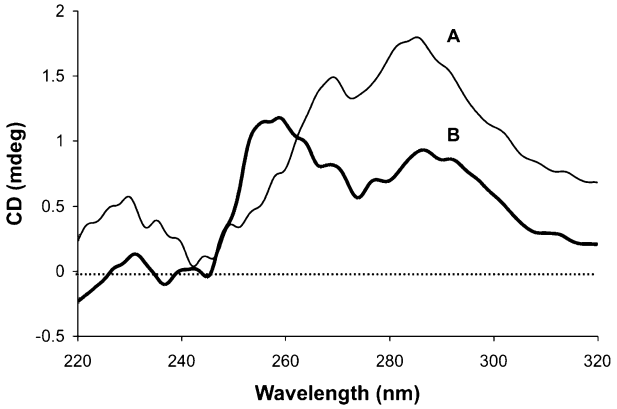

Figure 3. CD spectra of G_4 structures of PNA 9 (A), and PNA 10 (B) at 25 °C. All spectra were taken at 10 μM concentration and represent the average of eight scans collected at 100 nm/min.

multiple types of PNA−DNA structures. The ability to fine-tune the stability of PNA−oligonucleotide complexes can lead to better DNA detection strategies[4] and in vivo imaging techniques.[9] The general benefits of introducing *tc*yp groups should prove useful to researchers that study the basic properties of PNA and to those that employ PNA as a research tool. We will continue to further characterize the influences that *tc*yp has on PNA-based structures and apply them to diagnostic techniques.

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Supporting Information Available: Procedures for the synthesis of all PNA monomers and oligomers; melting curve data; additional CD data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Porcheddu, A.; Giacomelli, G. *Curr. Med. Chem.* **2005**, *12*, 2561−2599. (b) Egholm, M.; Christensen, L.; Dueholm, K. L.; Buchardt, O.; Coull, J.; Nielsen, P. E. *Nucleic Acids Res.* **1995**, *23*, 217−222. (c) Datta, B.; Schmitt, C.; Armitage, B. A. *J. Am. Chem. Soc.* **2003**, *125*, 4111−4118. (d) Datta, B.; Bier, M. E.; Roy, S.; Armitage, B. A. *J. Am. Chem. Soc.* **2005**, *127*, 4199−4207. (e) Marin, V. L.; Armitage, B. A. *J. Am. Chem. Soc.* **2005**, *127*, 8032−8033.
(2) (a) Kumar, V. A.; Ganesh, K. N. *Acc. Chem. Res.* **2005**, 404−412. (b) Lonkar, P. S.; Kumar, V. A. *J. Org. Chem.* **2005**, *70*, 6956−6959. (c) Govindaraju, T.; Madhuri, V.; Kumar, V. A.; Ganesh, K. N. *J. Org. Chem.* **2006**, *71*, 14−21.
(3) Pokorski, J. K.; Witschi, M. A.; Purnell, B. L.; Appella, D. H. *J. Am. Chem. Soc.* **2004**, *126*, 15067−15073.
(4) Pokorski, J. K.; Nam, J. M.; Vega, R. A.; Mirkin, C. A.; Appella, D. H. *Chem. Commun.* **2005**, 2101−2103.
(5) (a) Hillery, E.; Munkonge, F. M.; Xenarious, S.; Dean, D. A.; Alton, E. W. F. W. *Anal. Biochem.* **2006**, *352*, 169−175. (b) Forman, S. L.; Fettinger, J. C.; Pieraccini, S.; Gottarelli, G.; Davis, J. T. *J. Am. Chem. Soc.* **2000**, *122*, 4060−4067.
(6) (a) Griffith, M. C.; Risen, L. M.; Greig, M. J.; Lesnik, E. A.; Sprankle, K. G.; Griffey, R. H.; Kiely, J. S.; Freier, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 831−832. (b) Kuhn, H.; Demidov, V. V.; Frank-Kamenetski, M. D.; Nielsen, P. E. *Nucleic Acids Res.* **1998**, *26*, 582−587.
(7) D'Costa, M.; Kumar, V. A.; Ganesh, K. N. *J. Org. Chem.* **2003**, *68*, 4439−4445.
(8) (a) Hashem, G. M.; Wen, J.-D.; Do, Q.; Gray, D. M. *Nucleic Acids Res.* **1999**, *27*, 3371−3379. (b) Sugimoto, N.; Wu, P.; Hara, H.; Kawamoto, Y. *Biochemistry* **2001**, *40*, 9396−9405.
(9) Gasiorowski, J. Z.; Dean, D. A. *Mol. Ther.* **2005**, *12*, 460−467.

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