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Nucleic Acids Symposium Series No. 51 17–18
doi:10.1093/nass/nrm009

Property editing of peptide nucleic acids (PNA): gem-dimethyl, cyanuryl and 8-aminoadenine PNAs

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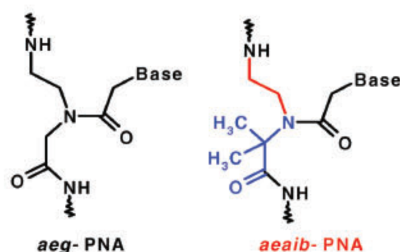
ABSTRACT

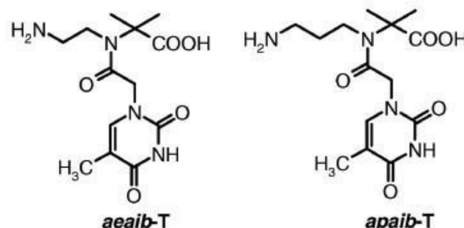
We herein describe the introduction of gem-dimethyl substitution into the aminoethylglycyl backbone of PNA to impart steric constraint and pre-organise PNA for selective recognition of nucleic acids. Introduction of cyanuric acid and 8-aminoadenine as pyrimidine and purine analogs that can form base pairing from either face is also described to overcome the rotameric problems in PNA sidechain orientations and thereby enhance the statistical probability for base pairing. The UV-thermal melting studies of the derived triplexes with complementary DNA provide support for this rationale.

INTRODUCTION

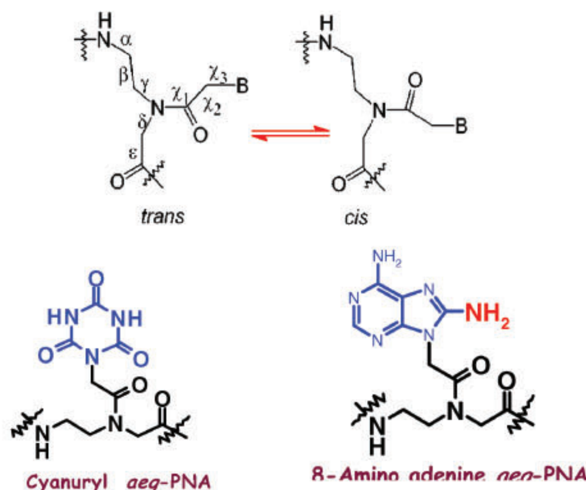
Peptide Nucleic Acid (aeg-PNA)[1] is one of the most elegant examples of the backbone modified DNA mimics for potential antisense therapeutics and biomolecular diagnostics. The repeating aminoethylglycyl (*aeg*) units that carry the nucleobases through tertiary acetamide linkages completely replace the sugar-phosphate backbone leading to uncharged achiral DNA analogues. PNA exhibited binding with both DNA and RNA targets in either parallel or antiparallel orientation with unprecedented stability. This promiscuous nature of PNA is not desirable for its application in therapeutics and needed approaches to its structure and property editing[2] to make it exhibit selectivity for recognizing either DNA or RNA in either parallel or antiparallel manner. We[3] surmised that introduction of chirality into PNA backbone could induce selectivity for parallel versus antiparallel binding with nucleic acids. To some extent, this rationale was a success, but not in a predictable way.[4] We describe herein the synthesis and study of achiral PNA analogues containing.



sterically constrained gem-dimethyl groups at different positions on backbone to introduce conformational rigidity into backbone to attempt at pre-organization of PNA backbone for selective hybridization properties.



Another feature of PNA which has not received considerable attention is the rotameric problem at the tertiary amide bond which locks the nucleobase carrying side chain in two conformations which are not easily inter-convertible at ambient temperatures, leading to a possibility of multitude of rotameric populations. This may affect the efficiency of hybridization with complementary DNA/RNA as the side chains on a statistical basis, may not be in different orientations not conducive for base pairing. We attempt the introduction of unnatural nucleobases such as cyanuric acid and 8-aminoadenine which have potential to form hydrogen bonds from either side, to provide a higher statistical probability for base pairing.



RESULTS AND DISCUSSION

Synthesis: The target gem-dimethyl substituted PNA monomers (aeaib-T, apaib-T) were synthesized by procedures analogous to standard PNA monomers, by alkylation of the corresponding amines with bromoalkyl derivatives. The amino components carrying the *gem*-dimethyl substituents gave low yields in coupling and hence reverse alkylation was done by using the bromo

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components having *gem*-dimethyl substituents. In addition to the aminoethyl analogues, the aminopropyl analogue (apaib-T) was also made.

The synthesis of cyanuric acid PNA and 8-aminoadenyl PNAs required some modifications of standard procedures. The monomers were incorporated into the desired sites in the PNA oligomer by solid phase assembly. For incorporating 8-aminoadenine, the 8-amino group requires another protecting group that may be removed under normal deprotecting conditions. To avoid this additional step, 8-bromoadenine PNA monomer was incorporated into the PNA oligomer, followed by its conversion to into 8-aminoadenine achieved straight on the resin. The conversion was followed by HPLC (Figure 1) which indicated the disappearance of peak at 7.34 min due to 8-bromoadenine PNA oligomer and the appearance of a peak at 9.2 min due to 8-aminoadenine PNA oligomer. All modified PNA oligomer products were unambiguously characterised by MALDI-TOF.

Figure 1: Crude HPLC profiles of 8-bromoadeninyl PNA oligomer (7.34 min) and its corresponding 8-aminoadeinyl PNA oligomer (9.2 min).

Biophysical studies: The PNA-T_n oligomers containing the modified units were annealed with the complementary DNA/RNA and the thermal stability of the resulting hybrids were monitored by temperature dependent UV absorbance studies. Some of the interesting data are shown in the Table 1. The incorporation of gem-dimethyl substituents into PNA backbone significantly stabilized the DNA/RNA hybrids (data not shown).

Among the 8-aminoadeninyl PNA oligomers, PNA with C-terminus modification (entry 4) and PNA with middle modification (entry 6) stabilize the derived triplexes over corresponding controls (entry 3 and 5) by 9.3°C and 30.8°C respectively, while PNA with N-terminus modification (entry 2) was destabilised compared to control (entry 1). Though it is difficult to explain the differences between N and C-terminus modifications, it is interesting to note that the middle modification overwhelmingly leads to significant stabilization. This property perhaps arises from the ability of 8-aminoadenine to base pair both sides, better than adenine itself.

The cyanuryl PNA oligomers showed similar effects depending on the site of substitution. The modification in the centre (entry 10) again showed stabilization suggesting the dual face hydrogen bonding ability of cyanuric acid to be the cause.

Table 1: UV_T_m of Cy-PNA:DNA_2 and 8-aminoadenine PNA:DNA_2 triplexes*

	PNA	Tm	ΔTm (°C)
1	H-**A**TTTTTTT-Lys-NH_2	65.0	------
2	H-**a**TTTTTTT-Lys-NH_2	33.8	-31.2
3	H-TTTTTT**A**-Lys-NH_2	70.0	-------
4	H-TTTTTT**a**-Lys-NH_2	75.5	+5.5
5	H-TTT**A**TTTT-Lys-NH_2	44.4	------
6	H-TTT**a**TTTT-Lys-NH_2	75.2	+30.8
7	H-TTTTTTTT-Lys-NH_2	44.6	------
8	H-**Cy**TTTTTTT-Lys-NH_2	34.2	-10.4
9	H-TTTTTTT**Cy**-Lys-NH_2	67.9	+23.3
10	H-TTTT**Cy**TTT-Lys-NH_2	66.5	+19.5

*A=adenine PNA, **a**=8-aminoadenine, **Cy**=cyanuryl PNA

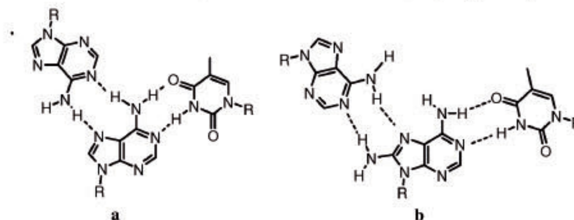

Figure 2: Hydrogen bonding in (a) adenine-adenine-thymine base triad and (b) adenine: 8-aminoadenine:

CONCLUSION

In conclusion, it is demonstrated that the triplexing properties of PNA can be enhanced by employing nucleobases endowed with ability to form hydrogen bonding from either face, which overcomes the rotameric limitations to increase the statistical efficiency of base pairing. PNAs with sterically constrained gem-dimethyl substituents were prepared and shown that conformational tuning of backbone may also result in increasing the hybridization efficiency. Future efforts are concentrated on examining the duplex stability of derived PNA:DNA complexes.

ACKNOWLEDGMENT

AG, RVB and PB thank CSIR, New Delhi for fellowship. KNG acknowledges DST for JC Bose Fellowship

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