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# Thermal melting studies with PNA and PNA–DNA chimera

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**Abstract**

We have performed thermal melting studies toward developing an empirical formula for PNA–DNA duplexes. Our results indicate the identity and sequence of the junction between the phosphodiester and 2-aminoethylglycine backbones are critical to the overall stability of the duplex and an important design consideration. © 1999 Elsevier Science Ltd. All rights reserved.

The biochemical applications of PNA (2-aminoethylglycine backbone) are limited due to self-aggregation and poor water solubility.[1] PNA–RNA heteroduplexes are not recognized by RNase H, an enzyme that cleaves the RNA strand in DNA–RNA duplexes, and these further limit applications of PNA involving antisense inhibition of translation. PNA–DNA chimera may overcome these limitations as they combine the high binding specificity and nuclease stability of PNA with the ability of DNA to promote RNase H cleavage. Furthermore, attachment of PNA to both ends of DNA could prevent unwanted exonucleolytic cleavage of the DNA portion of the chimera. Therefore, PNA–DNA–PNA chimeras may have potentially useful biological applications.[2] The high stability of PNA–DNA duplexes combined with pronounced sequence discrimination also make these molecules potentially useful as hybridization probes and primers. PNA–DNA chimera are highly water-soluble and can be analyzed and purified by standard reverse-phase and anion-exchange HPLC.[2] In this communication we report thermal melting studies of PNA, PNA–DNA and PNA–DNA–PNA chimera with complementary DNA and RNA sequences in an effort to understand their hybridization properties,[3] especially with regard to the junction sequence.

The uncharged nature of the PNA backbone has several important consequences. The most significant is the high thermal stability of PNA/DNA; PNA/RNA and PNA/PNA duplexes compared to DNA/DNA and DNA/RNA duplexes (Table 1). The stronger binding is attributed to absence of charge repulsion between the PNA and the DNA/RNA strand. Another important consequence of the neutral backbone is that the melting temperatures of PNA/DNA duplexes are essentially independent of salt concentration, in contrast to the melting of natural DNA/DNA duplexes which are highly dependent on ionic strength.

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Table 1
Thermal melting studies with PNA {PNA sequences, including the complementary sequences (not shown) have glycine at the *C*-terminus}

| | *Tm (°C)* | |
|---|---|---|
| ***Duplex*** | **5' tcta tcta tcta tcta- Gly 3'** | ***δ(°C)*** |
| DNA / DNA | 40.9 | |
| **PNA** / DNA | 50.5 | +9.6 |
| **PNA** / PNA | 78.6 | +37.7 |
| DNA / RNA | 46.0 | |
| **PNA** / RNA | 62.3 | +16.3 |
| DNA / 2'O-Me RNA | 46.6 | |
| **PNA** / 2'O-Me RNA | 65.4 | +18.8 |
| | **5' cgg act aag tcc att gc- Gly 3'** | |
| DNA / DNA | 55.9 | |
| **PNA** / DNA | 64.7 | +8.8 |
| | **5' cac ctt ggt cta cct - Gly 3'** | |
| DNA / DNA | 50.8 | |
| **PNA** / DNA | 59.1 | +8.3 |

It is also known that PNA can hybridize to target sequences at temperatures where DNA hybridization is strongly disfavored.[2]

We have investigated the binding affinity of PNA–DNA chimera to their complementary nucleic acid sequences (Table 2). The results indicate that the duplexes formed were generally less stable than the corresponding DNA/DNA duplex. The incorporation of PNA at both ends of DNA led to significant destabilization of the duplex, resulting in a 15°C lower $T_m$. This decrease may be attributed to the speculation that PNA/DNA nucleobases are positioned with less favorable H-bond distances relative to DNA/DNA nucleobases, or that the PNA–DNA duplex has a narrower minor groove than the DNA–DNA duplex which imparts more strain in the helix. The DNA–PNA chimera also hybridized more weakly to complementary DNA than the corresponding control, indicating that the junction between the phosphodiester and 2-aminoethylglycine backbones may not be structurally optimal and that a thermodynamic cost is therefore incurred in forming the junction.

Brown and co-workers have shown that the PNA–DNA chimera (Table 3, sequence 1) had a much higher melting temperature than the corresponding DNA/DNA duplex.[4] Replacement of PNA nucleobase '$\underline{t}$' in sequence 1 with '$\underline{c}$' from sequence 5 significantly destabilized the PNA/DNA duplex confirming that the nucleobase junction is an important aspect to be considered during design of a PNA/DNA chimera. In an effort to investigate the general determinants of affinity ($T_m$) and specificity (°C $T_m$), we made substitutions to the first base after the crossover (in the 5′PNA–DNA3′ chimera) in the PNA strand. Replacement of '$\underline{c}$' nucleobase in sequence 2 (Table 3) with a '$\underline{t}$' nucleobase in sequence 3 (Table 3) resulted in a stabilizing effect of the PNA–DNA/DNA duplex, suggesting that the nucleobase junction is also involved in the cooperative binding to the complementary DNA. Inversion of configuration from '$\underline{c}$' (sequence 2) to '$\underline{g}$' (sequence 4) again resulted in the lowering of the melting temperature.

A mismatch in the complementary DNA region of a PNA–DNA/DNA duplex at the junction also results in the lowering of the $T_m$. These results are summarized in Table 4. The biggest destabilizing effect was observed when the DNA nucleotide 'G' (in the complementary sequence) was replaced by a

Table 2
Thermal melting studies with PNA–DNA chimera. The complimentary sequence in all cases is a perfect match DNA. PNA bases are denoted in small, bold letters[5,6]

| | 5' tac cta ac T* AGA CGG T 3' | |
|---|---|---|
| ***Duplex*** | ***Tm (°C)*** | ***δ (°C)*** |
| DNA / DNA | 53.6 | |
| **pna**-DNA / DNA | 50.9 | -2.7 |
| | **5' CAC CTT GG t* cta cct - Gly 3'** | |
| DNA / DNA | 53.5 | |
| DNA / RNA | 59.6 | |
| DNA-**pna** / DNA | 50.3 | -3.2 |
| | **5' cacct T* GGTC t* a cct - Gly 3'** | |
| **pna**-DNA-**pna** / DNA | 38.5 | -15.0 |
| **pna**-DNA-**pna** / RNA | 52.6 | -7.0 |

Table 3
Thermal melting studies with 5'PNA–DNA3' chimera: Effect of change at the junction. The data is for a perfect match duplex, showing the effect of different bases at the junction. Sequence 5 differs from Sequence 1 at the junction site (underlined). Sequences 2, 3 and 4 differ at junction site (*underlined and italic*)

| ***Duplex*** | ***Tm (°C)*** | ***δ (°C)*** |
|---|---|---|
| 1) | **5' ttt ctt T* GCCAT 3'** | |
| DNA / DNA | 44.8 | |
| **pna**-DNA / DNA | 55.0 | + 10.2 |
| 2) | **5' tac cta ac T* AG ACG GT3'** | |
| DNA/DNA | 53.6 | |
| **pna**-DNA /DNA | 50.9 | -2.7 |
| 3) | **5' tac cta at T* AG ACG GT3'** | |
| DNA/DNA | 46.5 | |
| **pna**-DNA /DNA | 51.2 | +4.7 |
| 4) | **5' tac cta ag T* AG ACG GT3'** | |
| DNA/DNA | 54.8 | |
| **pna**-DNA /DNA | 52.2 | -2.6 |
| 5) | **5' ttt ctc T* GCCAT 3'** | |
| DNA / DNA | 43.0 | |
| **pna**-DNA / DNA | 28.0 | -15 |

'T' (Table 4). Similar observations were made with several other sequences with the largest $T_m$ difference observed with the first base over the junction (in the complementary DNA sequence).

In another study to investigate the influence on the proportion of PNA in a PNA–DNA chimera we synthesized several PNA–DNA sequences as described in Table 5. The results suggest that at least 60% of the strand must be PNA to have a higher $T_m$ than the parent DNA sequence to accommodate the thermodynamic penalty associated with forming the chimera junction.

For diagnostic and antisense applications these results are of significant interest due to the potential of the PNA–DNA chimera for enhanced exonuclease stability, ability to induce RNase H duplex cleavage and strong Watson–Crick base-pairing with complementary DNA and RNA strands. We are actively

Table 4
The effect on the melting temperature of PNA–DNA/DNA duplex by incorporating a mismatch at sites adjacent to the junction in the complementary DNA sequence

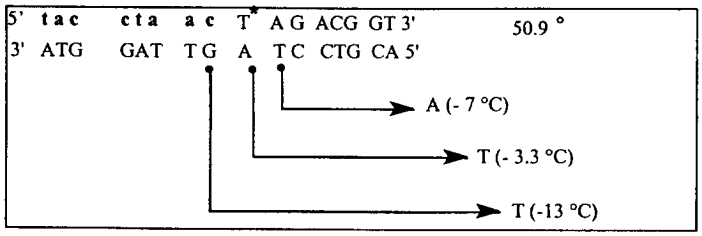


Table 5
Effect of increase in the proportion of PNA in a PNA–DNA chimera

| | *Tm (°C)* | *δ (°C)* |
|---|---|---|
| TTT TTG TAT TTC ATG T GT ACA TTC G 3' | 57.2 | |
| ttt ttg taT* TTC ATG T GT ACA TTC G 3' | 55.7 | (-1.5 °C) |
| ttt ttg tat ttc a T*G T GT ACA TTC G 3' | 56.5 | (-0.7 °C) |
| ttt ttg tat ttc a t g T* GT ACA TTC G 3' | 59.2 | (+2°C) |

pursuing the $T_m$ studies with these and other types of chimera in pursuit of arriving at an empirical formula for the $T_m$ prediction for PNA–DNA/DNA duplexes.[7]

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5. PNA and PNA–DNA chimera were synthesized on an Applied Biosystems 394 DNA/RNA synthesizer as described previously.[6] The chimera were purified by reverse-phase HPLC on an ABI Aquapore RP-300, 7 μm column. Gradient elution was performed at ambient temp. by building a gradient starting with buffer A (0.1 M TEAA/$H_2O$) and applying buffer B ($CH_3CN$) with a flow rate of 1.0 mL/min. The used gradient was: 0% B to 50% B in 32 min. The thermal melting studies were performed on a Perkin–Elmer Lambda 12 UV–vis spectrophotometer outfitted with a peltier heating unit and controlled with UV TempLab software. The following experimental conditions were employed: 10 mM $NaH_2PO_4$, 100 mM NaCl, 0.1 mM EDTA, pH 7 (0.5°C/min): Abbreviations: T*: 3′-*O*-phosphoramidite of 5′-*N*-(4-methoxytrityl)amino-5′deoxythymidine;[6] t*: tetrabutylammonium salt of *N*-(2-hydroxyethyl)-*N*-(thymin-1-yl-acetyl)glycine.
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