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A Novel Pyrrolidinyl PNA Showing High Sequence Specificity and Preferential Binding to DNA over RNA

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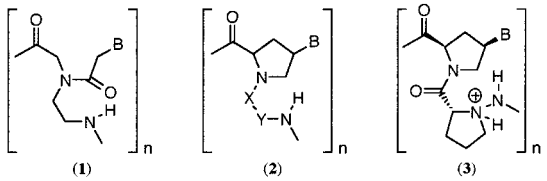
PNA (**1**) is a DNA analogue with the deoxyribose phosphate replaced by a polyamide backbone derived from *N*-aminoethylglycine.[1] Inspite of such a dramatic structural change, PNA shows a high affinity toward DNA and RNA in a sequence-specific fashion. Because of the great potential as tools in many biological applications, analogues of PNA became an attractive research target soon after PNA was reported by Nielsen and co-workers.[2,3]

As part of our continuing investigation into conformationally constrained chiral analogues of peptide nucleic acids (PNA) based on the pyrrolidine core structure (**2**),[4] we have recently reported that a pyrrolidinyl PNA (**3**), derived from alternating 4′*R*-thymin-1-ylpyrrolidine-2′*R*-carboxylic acid and aminopyrrolidine-2*R*-carboxylic acid (D-Apc) (Chart 1), could bind to its complementary oligodeoxynucleotide as shown by gel electrophoresis.[5] We have further investigated the interaction between **3** and nucleic acids by UV and CD spectroscopy and have discovered that **3** displays a remarkable preferential affinity for complementary DNA over RNA.

Chart 1. Structure of PNA[a]



[a] **3a**: B = thymine; n = 10; C-terminal lysine amide-capped.

A mixture of **3a** and poly(dA) at 1:1 T:A ratio showed a temperature dependence of the UV absorption at 260 nm (Figure 1). Only a single transition was observed from which the T_m was estimated to be >85 °C. Under the same conditions, the mixture of **3a** and $(dA)_{10}$ has a slightly lower T_m value (76 °C). In contrast, a 1:1 mixture of **3a** and poly(rA) showed a much lower T_m (32 °C) (Figure 1). In all cases the melting was reversible on a cooling/reheating cycle, and no hysteresis was observed at the heating/cooling rate of 0.5 °C/min. No binding to poly(rU) was observed under the same conditions. To further demonstrate the specific recognition of adenine in DNA by thymine in the PNA **3a**, T_m of hybrids formed between **3a** and $(dA)_{10}$, $d(A_4TA_5)$, $d(A_3TATA_4)$, and $(dT)_{10}$ were determined under low-salt conditions (Table 1, entries 1−4). Introduction of a mismatch base resulted in a large decrease of the T_m (ΔT_m > 20 °C per mismatch). The decrease in T_m observed for a single mismatch is significantly greater than for PNA (**1**) ($\Delta T_m \approx 13$ °C) and many other PNA reported.

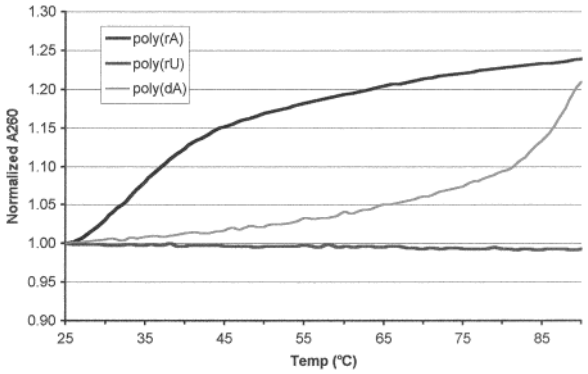


Figure 1. Melting curve of 1:1 mixture between **3a** (2 μM) and poly(rA), poly(rU), and poly(dA) in 150 mM NaCl, 10 mM Na phosphate buffer, pH 7.0.

Table 1. T_m of Homopolymeric PNA−Nucleic Acid Hybrids

entry	PNA	oligonucleotide (conditions)[a]	T_m, °C[b] (% hyperchromicity)
1	**3a**	$(dA)_{10}$	80 (18)
2	**3a**	$d(A_4TA_5)$	57 (15)
3	**3a**	$d(A_3TATA_4)$	<30 (6)[c]
4	**3a**	$(dT)_{10}$	<20
5	**3a**	$(dA)_{10}$ (pH 5.5)	80 (18)
6	**3a**	$(dA)_{10}$ (pH 8.0)	71 (17)
7	**3a**	$(dA)_{10}$ (1 mM salt)	82 (22)
8	**3a**	$(dA)_{10}$ (100 mM NaCl)	76 (17)

[a] The T_m was measured at a ratio of PNA:DNA = 1:1, concentration of PNA strand = 2 μM, 10 mM sodium phosphate buffer, pH 7.0, heating rate 0.5 °C/min. [b] T_m was determined from first derivative plot. [c] Partial melting was observed at the lowest temperature used for melting curve determination (25 °C).

The hybridization properties of **3a** with poly(dA) was also studied by CD spectroscopy. By following the CD signal of the mixture at different ratios of the reactants, the 1:1 stoichiometry of the PNA:DNA hybrid was established (Figure 2). Even at high ionic strength (1 M NaCl) there was no evidence of triplex formation, suggesting the 1:2 complex is structurally precluded. Addition of **3a** to poly(rA) also induced a dramatic change in the CD spectrum although saturation was not observed even at a 4:1 PNA:RNA ratio. This is not surprising considering that the T_m of the PNA·RNA hybrid is not much above the temperature at which the study was carried out (20 °C). The CD spectrum of the 1:1 hybrid formed between **3a** and poly(dA) showed ellipticity minima at 205, 248, and 267 nm and ellipticity maxima at 216, 260, and 284 nm which are similar to those of the B-type double helix formed between poly(dT) and poly(dA).[6] UV titration provides further support that **3a** and $(dA)_{10}$ form a 1:1 hybrid. From the specificity observed and the stoichiometry data, it is very likely that the complex is formed by Watson−Crick base pairing.

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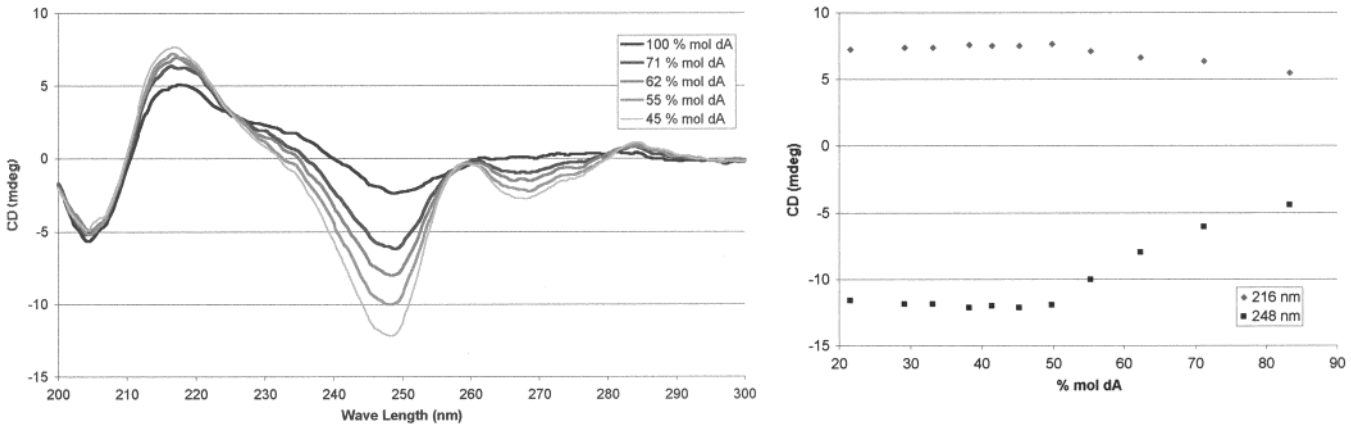

Figure 2. (Left) CD titration curve of PNA (**3a**) and poly(dA). Conditions: concentration of poly(dA) was constant at 8.0 μM dA, 10 mM sodium phosphate buffer, pH 7.0, 20 °C. For clarity not all the CD spectra are shown, in particular, below 45% mol dA spectra are identical. (Right) Plot between mol % A and the ellipticity at 216 and 248 nm.

Table 2. T_m of PNA H-T_5AT_4-LysNH_2 (**3b**) and H-T_3ATAT_4-LysNH_2 (**3c**) with Oligonucleotides

entry	PNA	oligonucleotide (conditions)[a]	T_m, °C[b] (% hyperchromicity)	note
1	**3b**	d(A_4GA_5)	36 (8)	single mismatch
2	**3b**	d(A_4CA_5)	48 (12)	single mismatch
3	**3b**	$(dA)_{10}$	51 (9)	single mismatch
4	**3b**	d(A_4TA_5)	71 (15)	perfect match
5	**3c**	$(dA)_{10}$	<20	double mismatch
6	**3c**	d(A_4TA_5)	44 (15)	single mismatch
7	**3c**	d(A_4TATA_3)	69 (18)	perfect match

[a] The T_m was measured at a ratio of PNA:DNA = 1:1, concentration of PNA strand = 1 μM, 10 mM sodium phosphate buffer, pH 7.0, heating rate 0.5 °C/min. [b] T_m was determined from first derivative plot.

To determine whether the tight binding of the pyrrolidinyl PNA was restricted to complementary homopolymers of DNA, two mixed sequences of PNA (**3**) were synthesized, H-T_5AT_4LysNH_2 (**3b**, MALDI-TOF *m*/*z* found 3485.0, calcd for M 3485.5) and H-T_3ATAT_4 LysNH_2 (**3c**, MALDI-TOF *m*/*z* found 3493.6, calcd for M 3494.6)) and their T_m determined with complementary oligodeoxyribonucleotides, assuming antiparallel binding (Table 2). Entries 4 and 7 show that the mixed-sequence PNA bind to complementary oligonucleotides with similar $T_m = 70 \pm 1$ °C. A single mismatch can, however, cause a fall in T_m of 20−35 °C (entries 1−4), and a double mismatch (entry 5) prevents measurable binding at 20 °C. Thus, this novel class of pyrrolidinyl PNA shows powerful discrimination for DNA.

The unusual stability of the hybrids formed between pyrrolidinyl PNA and oligodeoxyribonucleotides could at least partly be attributed to the electrostatic attraction between the positively charged protonated hydrazine nitrogen atom on PNA to the negatively charged phosphate group of the DNA. The above proposal was supported by the fact that T_m of the hybrid is sensitive to pH, being greater at lower pH (Table 1, entries 5, 6). Furthermore, the T_m is dependent on ionic strength (Table 1, entries 7, 8), being decreased at higher ionic strength similar to other positively charged oligonucleotide analogues.[7] However, the presence of the structurally rigid Apc linker with appropriate geometry is probably the main factor that contributes to the strong binding properties of the pyrrolidinyl PNA. The corresponding PNA bearing L-Apc spacer showed no observable binding according to gel-binding shift assay[5] and T_m experiments.

The different behavior toward poly(dA) and poly(rA) of PNA **3a** is perhaps the most striking feature of this β-amino acid containing PNA. Although the basis of this selectivity is not yet established, it presumably arises from the steric requirement of the 2′-hydroxyl group in ribonucleotides. It is expected that this unique property will render this PNA system potentially useful when such selectivity is desired as in antigene research and diagnostic applications where high sequence specificity and affinity are required.

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Supporting Information Available: Experimental details of the synthesis of PNA **3a**, UV melting curves, and CD spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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