Enhancement of αPNA Binding Affinity and Specificity through Hydrophobic Interactions

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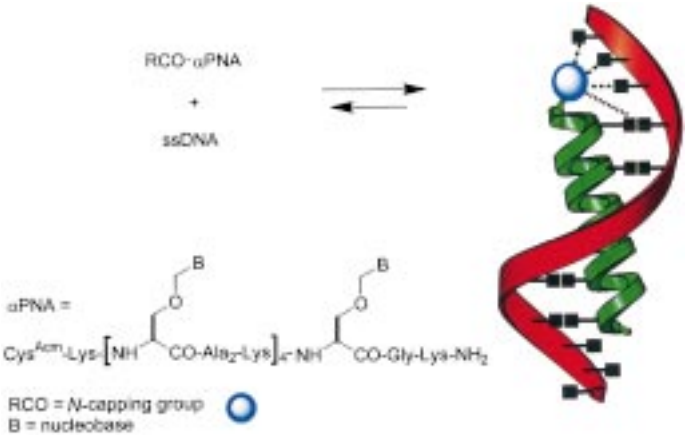
KEYWORDS:

DNA recognition · hydrophobic effect · peptide nucleic acids · stacking interactions · supramolecular chemistry



Scheme 1. *Proposed model for binding of hydrophobic α-helical peptide nucleic acids (αPNAs; shown in green) to single-stranded DNA (red).*

We recently disclosed that α-helical peptide nucleic acids (αPNAs), hybrid molecules that combine structural properties of peptides with the codified molecular recognition elements of nucleic acids, bind to complementary tracks of single-stranded DNA (ssDNA) with high affinity and sequence specificity.[1] Under equivalent hybridization conditions, αPNA·DNA complexes exhibit considerably higher melting temperatures than the corresponding DNA duplexes. An interesting facet of αPNA·DNA complexation was the preferred directionality of binding, with the "parallel" (N/5′) orientation being favored both thermodynamically and kinetically over the "antiparallel" (N/3′) orientation. This is analogous to the well-known antiparallel (5′/3′) strand orientation which is generally observed in nucleic acid duplexes. Evidence that the parallel complex Ac-CTCCT·d(A_3-GAGGAA_3) and its antiparallel counterpart Ac-CTCCT·d(A_3AG-GAGA_3) (noncomplementary "dangling" bases are italicized) possessed different secondary structures could be gleaned from their circular dichroism (CD) profiles, especially in the 230–290-nm region where the interaction of asymmetrically disposed nucleobases is expected to dominate the CD spectrum.[2] The spectrum of the complex formed from an αPNA with a symmetrical base sequence (Ac-CCTCC), capable of forming either a parallel or antiparallel complex, was consistent with the former orientation.

To gain a better understanding of (and control over) this remarkable platform for nucleic acid recognition, we set out to examine the binding properties of a series of αPNAs that incorporated a variety of arenyl (π-stacking and hydrophophic effects) as well as aliphatic (hydrophobic effect only) *N*-caps (Scheme 1). (Experimental details for the synthesis, purification, and characterization of *N*-capped αPNAs, purification protocols for DNAs, thermal denaturation profiles, and CD spectra for αPNA·DNA complexes can be found in the Supporting Information.) Our design reasoning was based on the assumption that tighter binding would result if "end-fraying" were minimized by incorporating stabilizing interactions at one or both ends of the αPNA chain. This could be important since our αPNA modules rely on only five base-pair interactions and end-fraying could adversely affect up to 50% (in CTTTC) of the interstrand Watson–Crick hydrogen bonds. The enhancement of duplex stability through such π–π interactions had been demonstrated with unpaired "dangling" nucleobases at the 5′ ends of RNA,[3] and analogous effects were observed when nucleobases as well as their unnatural aromatic counterparts were incorporated at the 5′ ends of DNA.[4, 5] A stabilizing effect on oligonucleotide duplexes has also been noted when they were 5′-capped with aliphatic steroids.[6–8] We now report that *N*-capped αPNAs lead to both enhanced affinity as well as orientational specificity with complementary ssDNA targets. Unexpectedly, the observed *N*-cap effects in the preferred parallel series were found to be more strongly correlated to *N*-cap hydrophobicity rather than π-stacking potential.

The *N*-capped αPNAs were synthesized following our usual solid-phase peptide synthesis (SPPS) protocol, but substituting the appropriate carboxylic acid in the *N*-capping step.[9] The *N*-caps investigated consisted of a series of commercially available arenyl-substituted and aliphatic carboxylic acids (Scheme 1). Both sets of *N*-caps included a flexible methylene linker to allow for the anticipated cap–base interaction without significantly disrupting the complex structure. Thermal UV denaturation (given in form of the melting temperature T_m) data for the *N*-cap-CTCCT·d(A_3GAGGAA_3) (N/5′ or parallel orientation) and *N*-cap-CTCCT·d(A_3AGGAGA_3) (N/3′ or antiparallel orientation) complexes are collected in Table 1.[10, 11] A number of interesting trends emerge from these data. First, all of the *N*-cap modifications resulted in higher T_m values for the parallel αPNA·DNA complexes relative to the "parent" *N*-acetylated compounds. However, contrary to our expectations, the most striking enhancement occurred in the parallel aliphatic *N*-cap series, with the 4-(cyclohexyl)butyryl-*N*-cap resulting in the highest T_m value (47.9 °C). This represented an increase of 11 °C compared to the parent *N*-acetylated αPNA. We also prepared a cholic-acid-capped αPNA to make a comparison with the nucleic acid literature data[8] and found that parallel cholyl-CTCCT·d(A_3GAGGAA_3) exhibited a T_m of 45.6 °C. Aliphatic *N*-caps would

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Table 1. *UV melting data for parallel and antiparallel* N*-capped αPNA·DNA complexes.*

Cap-CTCCT cap =	d(A_3-GAGGA-A_3) T_m [°C] (parallel)	d(A_3-AGGAG-A_3) T_m [°C] (antiparallel)[a]
CH_3CO	37.0	32.0
$CH_3CH_2CH_2CO$	40.0	30.2
$CH_3CH_2CH_2CH_2CH_2CO$	41.0	33.4
$C_5H_9CH_2CH_2CO$	43.7	33.8
$C_6H_{11}CH_2CH_2CH_2CO$	47.9	33.4
cholyl	45.6	34.6
$C_6H_5(CH_2)_3CO$	41.8	33.6
p-$MeOC_6H_4(CH_2)_3CO$	41.3	34.2
p-$NO_2C_6H_4(CH_2)_3CO$	41.6	34.4
2-naphthyl-$(CH_2)_3CO$	42.7	36.3
1-pyrenyl-$(CH_2)_3CO$	42.6	39.0

[a] No cooperative transition was observed during the cooling cycle.

not be expected to have a greater effect than arenyl *N*-caps if π stacking were responsible for the additional stabilization of these αPNA·DNA complexes. The relative insensitivity of the melting temperatures of 4-(aryl)butyryl-*N*-capped compounds to substitutions with electron-donating and electron-withdrawing groups is consistent with this interpretation.[12]

Analogous T_m enhancements were observed (Table 2) with the symmetrical sequences *N*-4-(cyclohexyl)butyryl-CCTCC·d(A_3GGAGGA_3) (60.8 °C) and *N*-4-(cyclohexyl)butyryl-CCCCC·d(TA_3GGGGGA_3T) (64.9 °C), thus excluding the possibility that these trends were related solely to the base sequence. The

Table 2. *UV melting data for symmetrical* N*-4-(cyclohexyl)butyryl-αPNA·DNA complexes.*

αPNA·DNA complex	T_m [°C]
$C_6H_{11}(CH_2)_3CO$-CCTCC 5′-d(A_3-GGAGG-A_3)-3′	60
$C_6H_{11}(CH_2)_3CO$-CCCCC 5′-d(TA_3-GGGGG-A_3T)-3′	65
$C_6H_{11}(CH_2)_3CO$-CCTCC 5′-d(TA_3-GGGGG-A_3T)-3′	50

incorporation of a single base-pair mismatch (*N*-4-(cyclohexyl)-butyryl-CCTCC·d(TA_3GGGGGA_3T)) lowered the T_m value by 15 °C, which is consistent with our previous observations in the *N*-acetyl series and indicates that the *N*-cap effect is decoupled from base pairing. This reduction in melting temperature presumably arises from residual hydration of the mismatched nucleobases and provides additional support for our proposed Watson – Crick binding model.

In keeping with our earlier studies, the antiparallel *N*-capped αPNA·DNA complexes exhibited lower T_m values than their parallel counterparts. However, contrary to our results in the parallel series, the highest antiparallel T_m values were obtained with the 4-(2-naphthyl)butyryl and 4-(1-pyrenyl)butyryl *N*-caps (36.3 and 39.0 °C, respectively). In no case was cooperative binding observed during the cooling cycle of these antiparallel melting experiments, indicating relatively slow annealing kinetics. Except for the complexes that incorporate the pyrene chromophore, the CD spectra of the parallel *N*-capped αPNA·DNA complexes are nearly superimposable on the CD spectra of the corresponding *N*-acetyl-substituted complexes (see Figure 1 and Supporting Information), suggesting that the *N*-caps are not changing the overall structure of the parallel αPNA·DNA complexes. Significantly, the *N*-4-(cyclohexyl)butyryl *N*-cap led to enhanced *orientational* specificity, with ΔT_m (parallel T_m − antiparallel T_m) increasing from 5 to 15 °C.

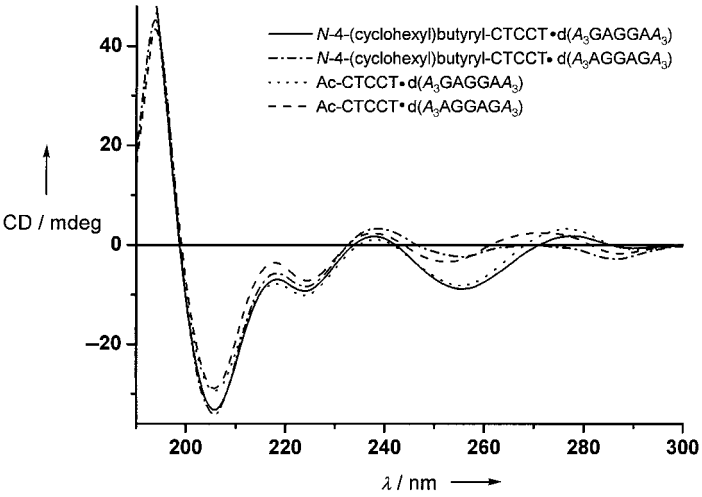

Figure 1. *Comparative CD spectra of parallel and antiparallel αPNA·DNA complexes. Samples were made by combining 5 μM solutions of each component in HPLC-grade water in a stoppered optical quartz cell (1 cm path length); the spectra were recorded at 5 °C. Nitrogen gas was purged through the sample compartment. Each data point is the average of eight (baseline-corrected) points.*

A plot of T_m versus calculated octanol/H_2O partition coefficients (log *P* values) for each *N*-cap (Figure 2) clearly shows that the melting temperatures of αPNA·DNA complexes is proportional to *N*-cap hydrophobicity.[13] We hypothesize that the strong dependence of T_m on log *P* in the parallel aliphatic *N*-cap series is due to a combination of "classical" (entropically driven) desolvation and "nonclassical" (enthalpically driven) packing hydrophobic effects.[14] For the antiparallel complexes, which exhibit uniformly lower T_m values than their parallel counterparts, a weaker dependence is found for the arenyl *N*-caps. This suggests the existence of compensating π-stacking effects superimposed on the hydrophobic effects and is probably related to different overall structures (see CD data, Table 1), but this is still conjecture that will have to await the completion of αPNA·DNA structural studies. On the other hand, melting temperature is essentially independent of *N*-cap hydrophobicity in both the parallel/aromatic and antiparallel/aliphatic series.

By demonstrating that strategically placed aliphatic groups can be used to modulate the affinity and orientational specificity of αPNA complexation to DNA, we have expanded the repertoire of binding interactions available to these novel nucleic acid surrogates. These studies provide a direct comparison between aliphatic and aromatic end caps showing that—at least for αPNAs—the former can have a stronger effect on hybridization than the latter.

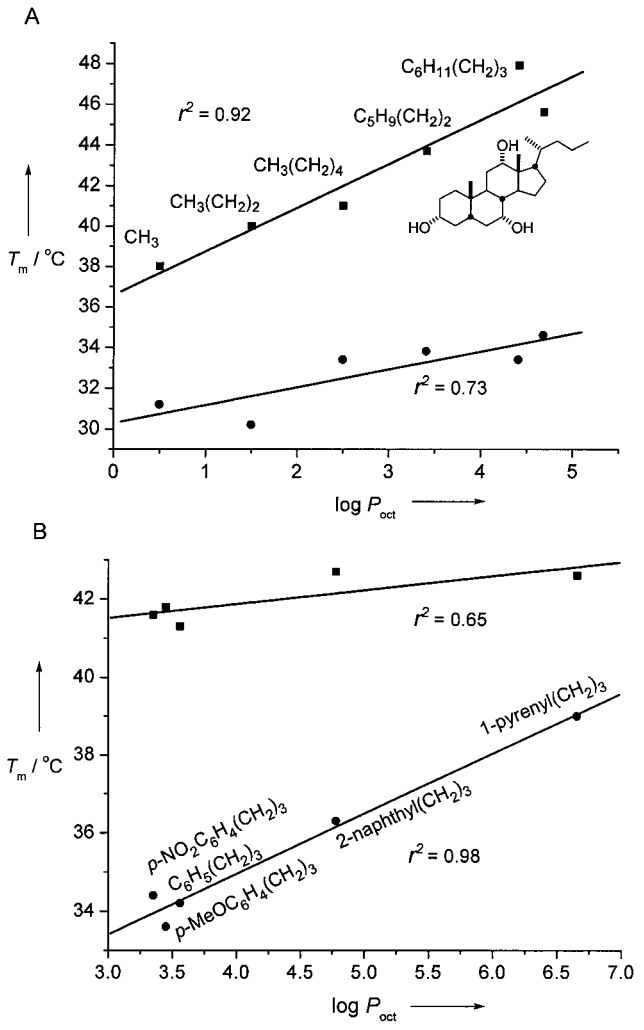

Figure 2. *Plots of* T_m *versus calculated log* P *values for parallel* N*-capped CTCCT·d(*A_3*GAGGAA*$_3$*) (upper data points, ■) and antiparallel* N*-capped CTCCT·d(*A_3*AGGAGA*$_3$*) (lower data points, ●). A: Aliphatic* N*-caps. B: Arenyl* N*-caps. The correlation coefficient* r^2 *(Pearson's correlation) is shown for each data set (see Supporting Information for details).*

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[9] All of the αPNAs reported here possess the backbone shown in Scheme 1 and are described by the *N*-cap followed by the nucleobase sequence (N to C terminus from left to right). Electrospray ionization mass spectral (ESI^+-MS) characterization data for *N*-capped αPNAs: *m/z*: 4-phenylbutyryl-CTCCT: calcd 2811.41, found 2812.61 ± 0.41; 4-(*p*-methoxyphenyl)butyryl-CTCCT: calcd 2841.42, found 2842.36 ± 0.13; 4-(*p*-nitrophenyl)butyryl-CTCCT: calcd 2856.40, found 2857.55 ± 0.24; 4-(2-naphthyl)-butyryl-CTCCT: calcd 2861.43, found 2862.68 ± 0.40; 4-(1-pyrenyl)butyryl-CTCCT: calcd 2935.44, found 2937.02 ± 0.39; *N*-butyryl-CTCCT: calcd 2735.38, found 2736.52 ± 0.54; *N*-hexanoyl-CTCCT: calcd 2763.41, found 2766.01 ± 1.19; 3-cyclopentylpropionyl-CTCCT: calcd 2789.43, found 2792.10 ± 1.01; 4-cyclohexylbutyryl-CTCCT: calcd 2817.46, found 2818.41 ± 0.74; 4-cyclohexylbutyryl-CCTCC: calcd 2802.46, found 2803.12 ± 0.39; 4-cyclohexylbutyryl-CCCCC: calcd 2787.46, found 2787.83 ± 0.06; cholyl-CTCCT: calcd 3055.61, found 3055.69 ± 1.67.
[10] Thermal UV denaturation curves were obtained with sample solutions made by combining the *N*-capped αPNA and ssDNA components (5 μM each) in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7). These solutions were then incubated overnight at 4 °C. All measurements were conducted in a quartz cell (1 cm pathlength) equipped with a temperature probe. The absorbance at 260 nm was recorded as a function of temperature by using a Perkin – Elmer Lambda 20 UV/Vis spectrophotometer equipped with a PTP-1 Peltier System thermocontroller with heating/cooling rates of 1 °C min^{-1}. Below 20 °C, dry N_2 gas was passed through the spectrophotometer sample chamber to prevent moisture condensation. Data was collected and evaluated by using the TempLab sofware package (version 1.5, Perkin – Elmer Corp., Norwalk, CT, USA). The melting temperature T_m (defined as the temperature at which 50% of a complex is dissociated into its constituent components) was determined from the inflection point maximum of the first derivative of the melting curves and is the average of two separate runs.
[11] For a given DNA target, d(A_3-NNNNN-A_3) (N = purine nucleobase), the level of hysteresis (difference between heating and cooling transitions) appears to be inversely proportional to G·C content and, for the most part, independent of *N*-cap structure. Thus, for 4-(cyclohexyl)butyryl-CTCCT·d(A_3-GAGGA-A_3), ΔT_m (heating – cooling) is about 3 °C, whereas no hysteresis is observed for 4-(cyclohexyl)butyryl-CCCCC·d(A_3-GGGGG-A_3). The only exception is the pyrene-containing *N*-cap, which shows no hysteresis and may be accelerating binding kinetics through a favorable intermolecular π – π interaction prior to structural reorganization to give the final base-paired αPNA·DNA complex.
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