α-Helical Peptide Nucleic Acids (αPNAs): A New Paradigm for DNA-Binding Molecules

Philip Garner,* Subhakar Dey, and Yumei Huang

Department of Chemistry
Case Western Reserve University
Cleveland, Ohio 44106-7078

Received September 7, 1999

α-Helical peptide nucleic acids (αPNAs) are novel synthetic constructs that merge the α-helical peptide secondary structure (a structural feature found in many DNA-binding proteins) with established nucleobase molecular recognition patterns.[1] This is accomplished by attaching the nucleobases to regularly spaced serine residues so that they can form Watson−Crick base pairs with complementary single-stranded nucleic acid targets upon α-helix formation (Scheme 1). The main advantage of this αPNA design is its potential for modification of the peptide scaffold to optimize specific physical and/or chemical properties. We now report that αPNA modules with as few as five nucleobases bind with high affinity to complementary DNA strands in a sequence-specific manner. In contrast to Nielsen's prototype polyamide nucleic acids,[2] αPNAs exhibit excellent water solubility (up to 18 mM) with no evidence of self-aggregation.

To overcome the slow kinetics of annealing observed with our prototype αPNAs (see ref 1, Supporting Information), backbones b2 and b2′ were designed in which the Asp and Glu residues were replaced with Lys.[3] This modification resulted in αPNAs having a net positive charge at neutral pH and was expected to enhance nonspecific binding to negatively charged DNA prior to sequence-specific base-pairing.[4] The resulting T_5(b2) and T_5(b2′) disulfide dimers both exhibited well-defined melting curves in TE-buffer (10 mM Tris-HCl, 1 mM EDTA disodium salt, pH 7.0) after only an overnight incubation period (Table 1, entries 1 and 2). Even the T_5(b2) module itself hybridized with d(A_{10}) (entry 3)—albeit weakly as expected for a complex held together by only A·T base pairs. Backbone b2 αPNAs that contained cytosine resulted in remarkably stable αPNA·DNA complexes. For example, C_5(b2)·d(*TA*$_3$G$_5$*A*$_3$*T*)[5] exhibited a T_m of 54 °C in TE-buffer and no hysteresis was observed in the cooling curve. This T_m is 35 °C higher than the corresponding DNA·DNA duplex (in TE-buffer + 150 mM NaCl)! Indeed, faster and stronger hybridization was observed for all *matched* cytosine-containing complexes formed with backbone b2 αPNAs and complementary ssDNAs with equilibrium dissociation constants in the micromolar range.[6] Added salt appears to disrupt favorable charge−charge interactions between cationic αPNAs and anionic DNA (see Supporting Information). Incorporation of mismatches into the αPNA (entries 4 and 7) or DNA (entries 6 and 7) strands resulted in a marked lowering of T_m for the complexes, a result which is consistent with sequence-specific base recognition. Finally, the parallel orientation (N-terminus of αPNA adjacent to the 5′-end of DNA or N/5′) resulted in a higher T_m when compared to the antiparallel (N/3′) alternative (entries 8 and 9).

Scheme 1. α-Helical Peptide Nucleic Acid (αPNA) Design Concept: Proposed Mode of Binding between an αPNA Module and Its Single-Stranded DNA Complement

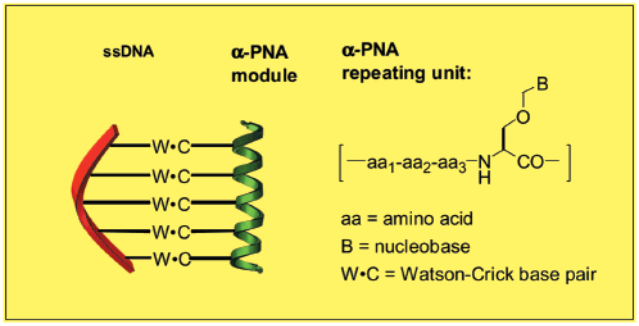

Table 1. UV Melting Data for αPNA·DNA Complexes

entry	αPNA (N→C)	DNA sequence (5′→3′)	T_m (°C)
1	T_5(b2)-dimer	d(A_{10})	46, 54[a]
2	T_5(b2′)-dimer	d(A_{10})	46, 57[a]
3	T_5(b2)	d(A_{10})	17
4	C_5(b2)	d(*TA*$_3$G$_5$*A*$_3$*T*)	54
5	C_5(b2)	d(G_5)	35
6	CCTCC(b2)	d(*A*$_3$GGAGG*A*$_3$)	49
7	CCTCC(b2)	d(*TA*$_3$G$_5$*A*$_3$*T*)	38
8	CTCCT(b2)	d(*A*$_3$AGGAG*A*$_3$)	32
9	CTCCT(b2)	d(*A*$_3$GAGGA*A*$_3$)	37

[a] Two-step melting was observed.

To investigate the stability of the αPNA·DNA hybrids as well as their binding stoichiometry and structure, both gel-shift mobility[7] and circular dichroism (CD) titration[8] studies were performed. For the complex between CCTCC(b2) and d(*A*$_3$-GGAGG*A*$_3$), a single new slower-migrating species corresponding to CCTCC(b2)·d(*A*$_3$GGAGG*A*$_3$) was detected in the gel shift assay (Figure 1A). The intensity of the d(*A*$_3$GGAGG*A*$_3$) band steadily decreased with increasing amounts of CCTCC(b2) until there was no unbound ssDNA at a αPNA/DNA ratio of 1.5. The formation of a triplex between two CCTCC(b2)s (net charge +6 each) and d(*A*$_3$GGAGG*A*$_3$) (net charge −10) is precluded on the grounds that the resultant ternary complex would have a net positive charge and therefore be expected to migrate toward the negative electrode. Evidence for duplex formation was also obtained from experiments on CTCCT(b2) + d(*A*$_3$GAGGA*A*$_3$) and CTCCT(b2) + d(*A*$_3$AGGAG*A*$_3$) (see Supporting Information). These gels also confirmed that the parallel (N/5′) orientation resulted in stronger binding. No binding was observed between an "abasic" αPNA(b2) peptide Ac-Cys^{Acm}-Lys-(Ser-Ala$_2$-Lys)$_4$-Ser-Gly-Lys-NH$_2$) and d(TA$_3$G$_5$A$_3$T), which underscores the role that nucleobases play in αPNA molecular recognition. Interestingly, the PAGE experiment with T_5(b2)-dimer (net charge +12) and d(*C*$_3$*T*(*TC*$_2$)$_2$A$_{10}$*C*-(*TC*$_2$)$_3$) (net charge −29) produced an additional slower-moving species at the expense of the initially formed species at αPNA/DNA ratios greater than 1 (Figure 1B). This result is consistent with the formation of a ternary complex (note 2-step melting of the dimers). Further support for the binding stoichiometries of

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(2) Recent PNA reviews: Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. *Angew. Chem., Int. Ed.* **1998**, *37*, 2796; Dueholm, K. L.; Nielsen, P. E. *New J. Chem.* **1997**, *21*, 19; Eriksson, M.; Nielsen, P. E. *Quart. Rev. Biophys.* **1996**, *29*, 369.
(3) αPNA backbone b2 = Ac-Cys^{Acm}-Lys-(Ser^B-Ala$_2$-Lys)$_4$-Ser^B-Gly-Lys-NH$_2$ and b2′ = Ac-Lys$_2$-(Ser^B-Ala$_2$-Lys)$_4$-Ser^B-Gly-Cys^{Acm}-NH$_2$. Amino acid abbreviations: Ala = L-alanine, Cys^{Acm} = *S*-acetamidomethyl-L-cysteine, Gly = glycine, Lys = L-lysine, Ser = L-serine, Ser^T = 1-[(Ser)methyl]thymine, Ser^C = 1-[(Ser)methyl]cytosine. Nucleobase abbreviations: A = adenine, C = cytosine, G = guanine, T = thymine, B = generic nucleobase.
(4) Cf. Corey, D. R. *J. Am. Chem. Soc.* **1995**, *117*, 9373.
(5) Non-complementary flanking nucleotides were incorporated into the DNA target to facilitate PAGE analysis of αPNA·DNA complexes. Flanking bases also resulted in stronger binding, greater hypochromicity, and less hysteresis. "Dangling" nucleobases are known to have an analogous effect on DNA duplex stability: Senior, M.; Jones, R. A.; Breslauer, K. J. *Biochemistry* **1988**, *27*, 3879.
(6) Marky, L. A.; Breslauer, K. J. *Biopolymers* **1987**, *26*, 1601.
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10.1021/ja993216d CCC: $19.00
Published on Web 02/24/2000

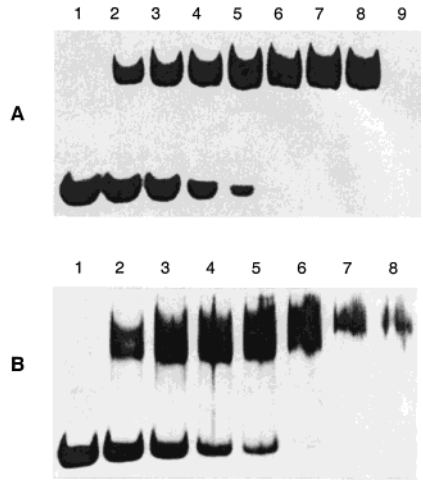

Figure 1. Binding of (A) CCTCC(b2) to d(A_3GGAGGA_3) and (B) T_5(b2)-dimer to d($C_3T(TC_2)_2A_{10}C(TC_2)_3$). Solutions were made up by combining DNA (40 μM) with varying amounts of αPNA in 7.5 μL of TE-buffer followed by heating at 80 °C for 5 min, then cooling and storage at 4 °C overnight. Before electrophoresis, 2.5 μL of loading buffer (0.01% xylene cyanol FF, 0.01% bromophenol blue solution, 60% (w/v) glycerol in running buffer) was added to the sample and mixed, and 3.5 μL of the sample was loaded onto the gel. Free and αPNA bound DNA were resolved by nondenaturing 14% polyacrylamide gel electrophoresis (PAGE) in 44 mM Tris-borate, pH 7.2 for 1 h at 14 V cm^{-1} at 4 °C. The ratios of αPNA to DNA in (**A**) for lanes 1−8 are 0/1, 1/2, 3/4, 1/1, 5/4, 3/2, 7/4, and 2/1. Lane 9 represents only αPNA. The ratios in (B) from lanes 1−8 are 0/1, 1/2, 1/1, 3/2, 2/1, 5/2, 3/1, and 4/1. Gels were developed using the PlusOne DNA silver staining kit (Pharmacia Biotech).

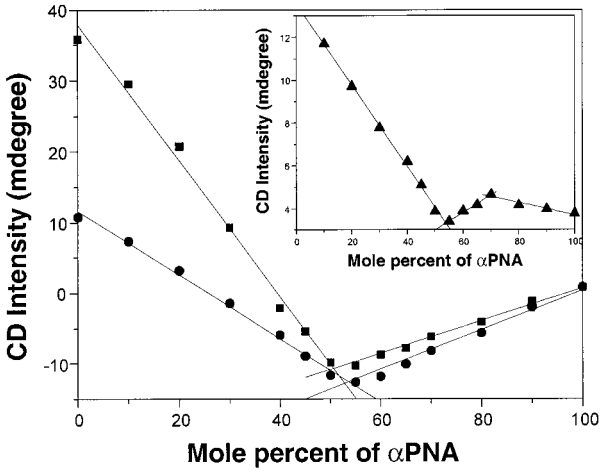

Figure 2. Job plots for CD intensities of C_5(b2) + d($TA_3G_5A_3T$) (at 258 nm, squares), CCTCC(b2) + d(A_3GGAGGA_3) (at 258 nm, circles), and T_5(b2)-dimer + d(A_{10}) (at 261 nm, triangles). Spectra were recorded at 5 °C using a JASCO J-600 CD spectropolarimeter. Samples having a total concentration ([αPNA] + [DNA]) of 12 μM were made up in doubly deionized water and placed in a stoppered optical quartz cell (1 cm path length). Dry air was purged through the sample compartment. Each data point represents the average of eight (baseline corrected) points.

the αPNA•DNA complexes comes from CD titration studies (Figure 2) on C_5(b2) + d($TA_3G_5A_3T$) and CCTCC(b2) + d(A_3-GGAGGA_3) which both showed intensity minima at 50 mol % αPNA as expected for a 1:1 binding stoichiometry. In addition to a duplex, T_5(b2)-dimer + d(A_{10}) also showed evidence of a 2:1 complex (Figure 3, inset). This is in line with the PAGE experiment with T_5(b2)-dimer and d($C_3T(TC_2)_2A_{10}C(TC_2)_3$). Finally, the CD spectrum (Figure 3)[9] of a solution containing equimolar amounts of CCTCC(b2) and d(A_3GGAGGA_3) shows the characteristic peptide CD signatures of an α-helix (minima at 220 and 206 nm, maximum at 196 nm).[10] The maximum at 280 and minimum at 255 nm are suggestive of an ordered right-handed DNA helix. Since control CD spectra show that the αPNA is disordered and the ssDNA possesses a different secondary structure, it appears that they are each acting as templates for hybridization. Analogous behavior has been noted previously for peptides corresponding to the basic regions of DNA-binding proteins.[11]

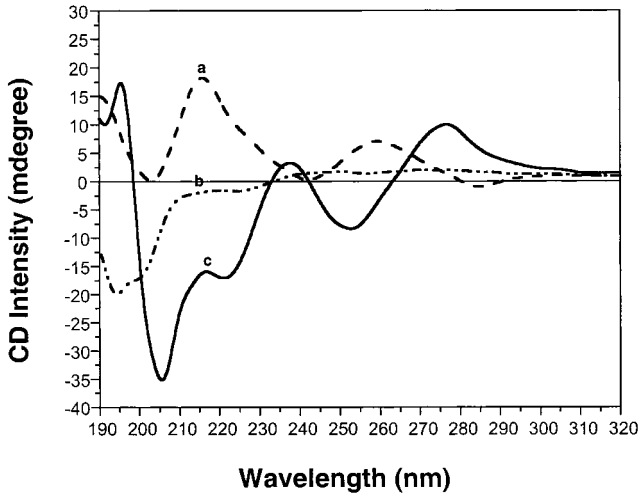

Figure 3. Comparative CD spectra of (a) DNA d(A_3GGAGGA_3) alone, (b) αPNA CCTCC(b2) alone, and (c) a 1:1 mixture of d(A_3GGAGGA_3) + CCTCC(b2) (6 μM each) in distilled H_2O. Spectra were recorded as described in Figure 2.

Acknowledgment. This work was supported by a grant from the National Institutes of Health (GM54796). Special thanks to Pieter DeHaseth for reading the manuscript and providing helpful comments. We also thank Tony Berdis, Irene Lee, and Gianina Panaghie for technical assistance and advice.

Supporting Information Available: Experimental details for synthesis, purification, and characterization of all αPNAs, purification protocols for DNAs, thermal denaturation profiles for αPNA·DNA complexes, and plot showing the effect of added salt (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) The relative intensities of the composite CD bands in the "peptide region" may be due to additional contributions of bound DNA, the αPNA nucleobase side chains, and/or an alternate PNA helix structure. Distinction between these possiblities will have to await the results of further structural studies on PNA·DNA complexes.

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