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Polycyclic aromatic hydrocarbons as universal bases in peptide nucleic acid

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Abstract—Polycyclic aromatic hydrocarbons were found to be promising universal bases in PNA·DNA double helices. Several of the arenes paired promiscuously with any of the four canonical bases. However, the stabilities of the duplexes depended on the size and shape of the arene.

Peptide nucleic acid (PNA) is a synthetic analog of DNA consisting of repeating *N*-(2-aminoethyl)glycine units with nucleobases attached by methylene carbonyl linkers.[1,2] PNA is able to hybridize to complementary DNA following canonical Watson–Crick base pairing rules. When forming PNA·DNA duplexes, the charge neutrality of the PNA backbone imparts numerous advantages including tighter binding to complementary DNA, higher sensitivity to base mismatches, and relative independence from solution salt concentration.[2] As such, PNAs have shown great potential as biotechnological and diagnostic tools and as medicinal agents.[2] Moreover, the clear relationship between the structures of PNA-containing complexes and their all-natural congeners makes PNA a potentially valuable system for the examination of the biophysical interactions within nucleic acid complexes. This prospect is further enhanced when the ease of the synthesis of novel PNA monomers is considered.[3–6]

One application of base-modified PNAs has been the development of universal bases.[4–8] Universal bases are residues that pair equally well with any of the four natural bases and, optimally, can be incorporated without destabilizing the double helical complex.[9] Universal bases can be introduced as wildcard residues into primers and probe molecules in cases when the target sequence is either ambiguous or unknown.[9] Universal bases also have potential utility for increasing sequence diversity in microarray-based sequencing assays.[10]

Studies of universal bases in PNA have utilized a variety of base replacements including simple structures such as benzene (phenyl) and hydrogen (abasic),[5] substituted aromatic residues such as nitroazoles[4,7] and fluoroarenes,[6] and fluorescent chromophores such as thiazole orange.[8] Despite the greater mismatch penalty seen in PNA-containing duplexes, PNA universal base residues have generally shown promiscuity when paired with any of the four natural bases in a complementary DNA strand. However, the incorporation of these residues results in varying degrees of destabilization of the corresponding double helical complex. The thiazole orange residue is the least destabilizing due to a positive charge that largely overcomes the loss of base pairing.[8] However, due to electrostatic repulsion, cationic residues are expected to perform poorly when incorporated at adjacent sites within a probe molecule. To date, no single PNA or DNA universal residue has demonstrated optimal overall properties.

Described herein is the examination of another series of universal base candidates: polycyclic aromatic hydrocarbons (PAHs). It has long been known that these molecules interact strongly with the DNA bases through intercalation into helical structures.[11] More recently, Kool and co-workers[12,13] have shown that nucleoside derivatives bearing hydrophobic aromatic moieties in the places of the nucleobases can significantly stabilize a DNA·DNA duplex by stacking onto the ends of the helices. Furthermore, the incorporation of an internal pyrene nucleoside provided stability comparable to an A–T pair when paired with an abasic residue and showed little pairing selectivity with any of the canonical bases.[14]

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Because of these results, polycyclic aromatic residues appeared to be promising candidates for PNA universal bases. Preliminary studies were performed using five candidate PAH PNA residues: 1-pyrene (**1-pyre**), 1-anthracene (**1-anth**), 2-anthracene (**2-anth**), 4-biphenyl (**4-biph**), and 5-acenaphthene (**5-acen**) (Fig. 1A). The synthesis of the Fmoc-protected **1-pyre** monomer was accomplished as described previously[3] by the reaction of 1-pyreneacetic acid with *tert*-butyl 2-*N*-Fmoc-2-aminoethyl glycinate;[15] similar treatment of the other arylacetic acids[16] produced the corresponding monomers.[17] Finally, brief treatment with trifluoroacetic acid prepared the monomers for PNA oligomer synthesis. Pentadecamer PNAs were prepared via standard Fmoc automated solid phase synthesis[18] with manual coupling of the novel residues. Following deprotection, the crude oligomers were purified by reversed phase HPLC, and the identities of the compounds were confirmed by MALDI-TOF mass spectrometry. Complementary DNA oligomers were synthesized containing each of the four natural nucleobases opposite to the modified site of the PNA (Fig. 1B).

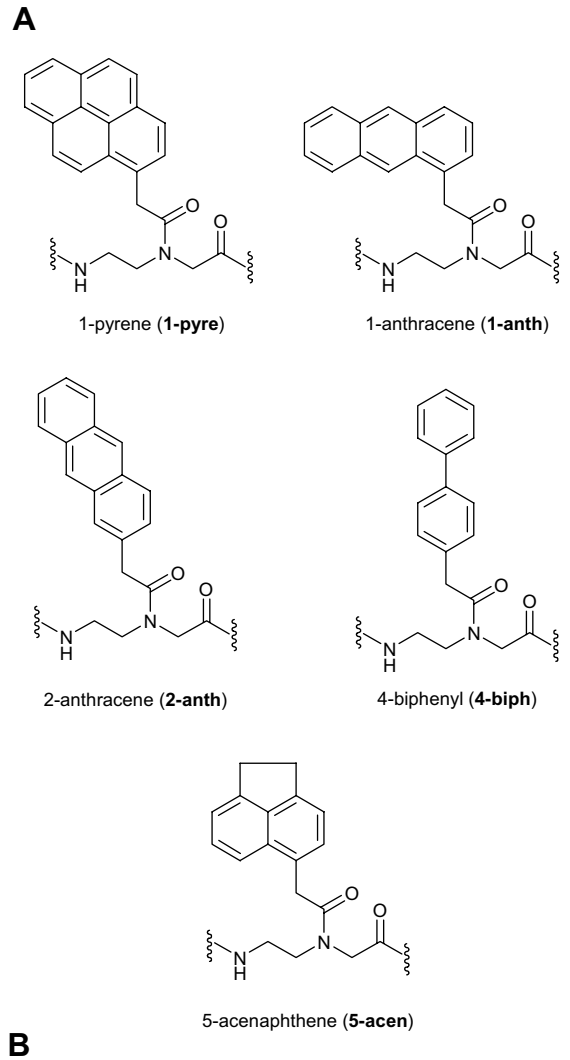

Figure 1. (A) Structures of arene PNA residues. (B) Sequences of PNA and DNA oligomers used in thermal denaturation experiments.

Hybridization of the PNAs with each of the four complementary DNAs was performed in PES buffer (10 mM phosphate, 0.1 mM EDTA, 100 mM NaCl, pH 7). Thermal denaturation of the PNA·DNA complexes was followed using absorbance spectroscopy at 260 nm. All of the absorbance versus temperature plots showed sigmoidal curves indicating cooperative transitions, and the data were fit to a two-state model using Meltwin 3.5 software.[19]

As expected, PNA·DNA complexes containing a pyrene residue showed considerable promiscuity when paired with each of the four natural nucleosides: the range of T_ms (ΔT_m) was 1.2 °C (Table 1). The acenaphthene residue showed even less specificity ($\Delta T_m = 0.3$ °C). This positions the **1-pyre** and **5-acen** residues among the most indiscriminate universal bases examined thus far. The **1-anth**, **2-anth**, and **4-biph** residues were somewhat less universal, showing ΔT_ms of 2.1, 4.2, and 2.8 °C, respectively. Interestingly, the **4-biph** residue showed modest selectivity when paired with cytosine, and the **2-anth** residue was selectively destabilized when paired with adenine. In both cases, the residues showed good promiscuities when paired with the remaining three bases.

Table 1. Data for the thermal denaturation of PNAs containing hydrophobic aromatic residues with complementary DNAs[a]

X	Y	T_m (°C)	
1-pyre	A	59.0	
	C	60.1	$\Delta T_m = 1.2$ °C
	G	59.2	Avg $T_m = 59.6$ °C
	T	60.2	
1-anth	A	60.9	
	C	58.8	$\Delta T_m = 2.1$ °C
	G	58.9	Avg $T_m = 59.7$ °C
	T	60.1	
2-anth	A	55.4	
	C	58.5	$\Delta T_m = 4.2$ °C
	G	59.6	Avg $T_m = 57.9$ °C
	T	58.1	
4-biph	A	54.2	
	G	57.0	$\Delta T_m = 2.8$ °C
	C	54.8	Avg $T_m = 55.5$ °C
	T	55.8	
5-acen	A	56.1	
	C	55.8	$\Delta T_m = 0.3$ °C
	G	56.1	Avg $T_m = 56.0$ °C
	T	55.8	
T	A	67.3[6]	

[a] Absorbance versus temperature curves were measured at 4.0 μM concentrations for each strand. T_ms were determined from Meltwin fits of triplicate runs, and the errors are estimated to be ±0.2 °C.

The **1-pyre** and **1-anth** residues showed similar average T_ms (59.6 °C versus 59.7 °C, respectively). These values are also similar to the T_ms seen in PNA·DNA duplexes of the same sequence containing the commonly investigated universal base 5-nitroindole.[4] The incorporation of these universal residues produces a moderate destabilization of the duplexes compared to a natural thymine–adenine base pair ($T_m = 67.3$ °C).[6] The **2-anth** residue was more destabilizing (avg $T_m = 57.9$ °C), but this value is depressed primarily by the destabilization of the **2-anth**/A pair. The **4-biph** (avg $T_m = 55.5$ °C) and **5-acen** (avg $T_m = 56.0$ °C) residues show stabilities between those of the larger arenes and a phenyl residue (avg $T_m = 54.7$ °C).[20]

The performance of the larger **1-pyre** and **1-anth** residues suggests that these moieties can be accommodated within the double helix without significant distortion or specificity. The sizes of these residues approach those of Watson–Crick base pairs (**1-pyre**: 213 Å^2, **1-anth**: 200 Å^2, A–T base pair: 266 Å^2);[21] therefore, the arenes must partly stack with the complementary base residue in an interleaved arrangement. This face-to-face arrangement may explain the lack of base specificity. Furthermore, internalization of hydrophobic residue within the duplex core should overcome any local structural distortion. These results agree with those observed for DNA·DNA double helices containing a pyrene nucleoside paired with natural nucleobases[14] or with 2-aminopurine (2-AP).[22]

The slightly reduced affinity and unusual selectivity of the **2-anth** residue compared to **1-anth** highlight the importance of shape in stacking. The melting transitions for duplexes containing this residue were broader than those of the other residues; the Meltwin fits indicated a greater importance of the entropy change in the transition free energy (data not shown). Good fits were obtained using the explicit two-state assumption in Meltwin; however, a deviation from this model cannot be completely discounted. In any case, model building suggests that **2-anth** extends into either the major or minor groove. The influence of this feature upon the stability of a PNA·DNA duplex is unclear and will require further study.

PNA·DNA duplexes containing the smaller **4-biph** (184 Å^2) and **5-acen** (178 Å^2) residues were approximately 4 °C less stable than those containing **1-pyre**. Moreover, these duplexes were only slightly more stable than those incorporating the smaller phenyl residue (109 Å^2). These results are similar to those reported by Singh et al.[22] for biphenyl and acenaphthene deoxynucleoside residues paired with 2-AP in a double helical DNA complex. The acenaphthene moiety was accommodated within the duplex without significant perturbation of 2-AP stacking, but the biphenyl residue caused an 'innerhelical unstacking' of the complementary residue.[22] In our experiments, the **5-acen** PNA residue is effectively universal, whereas the **4-biph** residue shows modest base pairing selectivity. Work is underway to examine the stacking of universal bases and their complementary nucleobases within the duplex structure.

The results observed in this laboratory indicate that large, fused, polycyclic aromatic hydrocarbon moieties have potential as universal base candidates. PNA·DNA duplexes incorporating either **1-pyre** or **1-anth** PNA residues paired opposite to any of the four natural bases show good thermal stability and excellent promiscuity. These results, along with the previously observed results reported for the β-heptafluoronaphthalene residue,[6] suggest that large, hydrophobic base surrogates may prove to be useful universal bases. Significantly, the T_ms for all of the PNA·DNA duplexes bearing PAH universal bases were higher than that of the analogous DNA·DNA 15-mer duplex with an A–T base pair at the variable position ($T_m = 53.3$ °C).[23] This illustrates the potential utility of PNA probe molecules containing non-natural residues for hybridization to DNA targets.

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17. *General procedure for coupling of areneacetic acids to PNA backbone segment*:[3] To a solution of areneacetic acid (1.26 mmol) in anhydrous DMF (10 mL) was added *tert*-

butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-glycinate[15] (1.05 mmol), HOBt (1.26 mmol), TBTU (1.26 mmol), and *N*,*N*-diisopropylethylamine (1.26 mmol). The resulting solution was stirred for 4–22 h under a nitrogen atmosphere; the progress of the reaction was monitored using TLC. The solvent was then removed under reduced pressure, and the residue was purified using silica gel flash column chromatography. *tert*-Butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-*N*-[(pyrene-1-yl)acetyl] glycinate (**1-pyre** monomer *t*-butyl ester): This compound was prepared and characterized as previously described.[3] *tert*-Butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-*N*-[(anthracene-1-yl)acetyl] glycinate (**1-anth** monomer *t*-butyl ester, 76% yield): ^{1}H NMR ($CDCl_3$) (two rotomers) δ 8.58–8.32 (m, 2H, Ar), 8.12–7.85 (m, 3H, Ar), 7.75 (d, $J = 7$ Hz, 2H, Ar), 7.48–7.27 (m, 8H, Ar), 5.96/5.58 (br, 1H, NH), 4.36–4.31 (m, 2H), 4.27 (s, 1H), 4.19 (m, 2H), 4.16 (s, 1H), 4.06/4.0 (s, rotomer, 2H), 3.66/3.59 (tt, $J = 5$, 5 Hz, 2H), 3.46/3.37 (qq, $J = 6$, 6 Hz, 2H), 1.49/1.48 (s, rotomer, 9H); MS (HR-FAB): Calcd for $[C_{39}H_{38}N_2O_5+H^+]$ *m*/*z*, 615.2859; found *m*/*z*, 615.2857. *tert*-Butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-*N*-[(anthracene-2-yl)acetyl] glycinate (**2-anth** monomer *t*-butyl ester, 79% yield): ^{1}H NMR ($CDCl_3$) (two rotomers) δ 8.33 (d, 2H, $J = 14.6$ Hz, Ar), 7.93–7.91 (m, 3H, Ar), 7.76–7.72 (m, 3H, Ar), 7.58–7.56 (m, 2H, Ar), 7.45–7.24 (m, 8H, Ar), 5.89/5.56 (br, 1H, NH), 4.36–4.31 (m, 2H), 4.03/3.49 (s, rotomer, 2H), 3.90/3.81 (s, rotomer, 2H), 3.62–3.56 (m, 2H), 3.44–3.34 (m, 2H), 1.49/1.43 (s, rotomer, 9H); MS (HR-FAB): Calcd for $[C_{39}H_{38}N_2O_5+H^+]$ *m*/*z*, 615.2859; found *m*/*z*, 615.2876. *tert*-Butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-*N*-[(4-biphenyl)acetyl] glycinate (**4-biph** monomer *t*-butyl ester, 65% yield): ^{1}H NMR ($CDCl_3$) δ 7.77–7.74 (m, 2H, Ar), 7.61–7.50 (m, 6H, Ar), 7.43–7.28 (m, 9H, Ar), 5.86/5.53 (br, rotomer, 1H, NH), 4.38 (dd, 2H, $J = 22.6$, 6.9 Hz), 4.22 (t, 1H, $J = 6.3$ Hz), 4.00/3.93 (s, rotomer, 2H), 3.75/3.66 (s, rotomer, 2H), 3.60–3.53 (m, 2H), 3.42–3.35 (m, 2H), 1.49/1.48 (s, rotomer, 9H); MS (HR-FAB): Calcd for $[C_{37}H_{38}N_2O_5+H^+]$ *m*/*z*, 591.2859; found *m*/*z*, 591.2886. *tert*-Butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-*N*-[(acenaphthene-5-yl)acetyl] glycinate (**5-acen** monomer *t*-butyl ester, 65.2% yield): ^{1}H NMR ($CDCl_3$) (two rotamers) δ7.76 (d, 2H), 7.65–7.57 (m, 3H), 7.49–7.37 (m, 3H), 7.32–7.26 (m, 4H), 7.19 (d, 1H), 4.39–3.93 (m, 7H), 3.62–3.30 (m, 8H), 1.49/1.46 (rotomer s, 9H); MS (FAB): 591 $(M+H)^+$.

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