ORGANIC LETTERS

1999
Vol. 1, No. 10
1639−1641

Nitroazole Universal Bases in Peptide Nucleic Acids

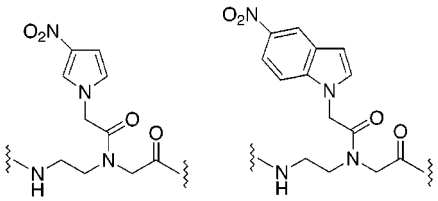
Hemavathi Challa, Melanie L. Styers, and Stephen A. Woski*

Department of Chemistry and Coalition for Biomolecular Products, The University of Alabama, Tuscaloosa, Alabama 35487-0336

swoski@bama.ua.edu

Received September 3, 1999

ABSTRACT



The syntheses of PNA oligomers containing potential ambiguous nucleobase analogues, namely 3-nitropyrrole and 5-nitroindole, have been accomplished. Hybridization properties of these PNAs with complementary oligodeoxynucleotides were evaluated by thermal denaturation experiments. Both novel residues exhibited little variation in T_m (≤1.5 °C) when positioned against any of the four nucleoside bases. The capability to incorporate degenerate sites should further expand the utility of PNA in applications where precise sequence information is not available.

With the pending completion of several genome projects, a wealth of genetic information for several organisms is rapidly becoming available. One key application of these data will be the elucidation of the biological function of genes by comparison of homologous sequences from different species. Oligonucleotide primers based upon a reference sequence from one organism can be used to identify orthologous sequences from another.[1] However, phenomena such as sequence differences between species can complicate such analyses by creating ambiguous sites for primer annealing. Similarly, the degeneracy of the genetic code can introduce numerous indeterminate base positions when designing oligonucleotide probes from the amino acid sequence of a protein (reverse genetics). To avoid the use of multiple probe molecules to account for each possible sequence, nucleoside analogues that lack base pairing specificity have been incorporated.[2] Ideally, such universal base residues will show complete degeneracy without reducing the overall affinity of the probe molecule for its complement. Such probes should be valuable for primer extensions, in situ hybridizations, and the production of mutagenesis libraries. A number of modified nucleosides have been studied as potential universal residues. These include naturally occurring residues such as inosine,[3] nonnatural deoxyribofuranosides such as 3-nitropyrrole,[4−6] 5-nitroindole,[7−10] and azole-4-carboxamides,[11−13] and residues with both modified bases and acyclic sugar portions.[14]

(1) Hacia, J. G.; Makalowski, W.; Edgemon, K.; Erdos, M. R.; Robbins, C. M.; Fodor, S. P.; Brody, L. C.; Collins, F. S. *Nat. Genet.* **1998**, *18*, 155−158.

(2) For a commentary, see: Burgess, K.; Welch, M. B. *Chemtracts: Org. Chem.* **1995**, *8*, 187−190.

(3) Ohtsuka, E.; Matsuki, S.; Ikehara, M.; Takahashi, Y.; Matsubara, K. *J. Biol. Chem.* **1985**, *260*, 2605−2608.

(4) Nichols, R.; Andrews, P. C.; Zhang, P.; Bergstrom, D. E. *Nature* **1994**, *369*, 492−493.

(5) Bergstrom, D. E.; Zhang, P.; Toma, P. H.; Andrews, P. C.; Nichols, R. *J. Am. Chem. Soc.* **1995**, *117*, 1201−1209.

(6) Amosova, O.; George, J.; Fresco, J. R. *Nucleic Acids Res.* **1997**, *25*, 1930−1934.

(7) Loakes, D.; Brown, D. M. *Nucleic Acids Res.* **1994**, *22*, 4039−4043.

(8) Loakes, D.; Hill, F.; Linde, S.; Brown, D. M. *Nucleosides Nucleotides* **1995**, *14*, 1001−1003.

(9) Loakes, D.; Brown, D. M.; Linde, S.; Hill, F. *Nucleic Acids Res.* **1995**, *23*, 2361−2366.

(10) Loakes, D.; Hill, F.; Brown, D. M.; Salisbury, S. A. *J. Mol. Biol.* **1997**, *270*, 426−435.

(11) Johnson, W. T.; Zhang, P.; Bergstrom, D. E. *Nucleic Acids Res.* **1997**, *25*, 559−567.

(12) Zhang, P.; Johnson, W. T.; Klewer, D.; Paul, N.; Hoops, G.; Davisson, V. J.; Bergstrom, D. E. *Nucleic Acids. Res.* **1998**, *26*, 2208−2215.

10.1021/ol990270q CCC: $18.00
Published on Web 09/29/1999

While the incorporation of universal bases into oligodeoxynucleotides has been extensively studied, little work has been performed on DNA analogues with completely modified backbones. Among the most important analogues are the peptide nucleic acids (PNAs).[15−17] PNAs are surrogates for natural oligonucleotides constructed on uncharged pseudopeptide backbones. Nucleobases are positioned on this backbone such that they can hybridize with complementary sequences of DNA and RNA to form double- and, depending upon sequence, triple-helical complexes. Moreover, PNA can readily hybridize with complementary DNA sequences with concurrent displacement of the DNA complement (strand invasion). Due to the uncharged nature of the backbone, binding is usually very tight and largely independent of salt concentration. PNA is generally more sensitive to the presence of mismatches than DNA probes.[16] Because of these characteristics, PNA has been viewed primarily as a potential antisense/antigene reagent.[15,17] Significantly, PNAs have been shown to specifically inhibit human telomerase in cells.[18] PNAs are also being used in hybridization assays and mutation analysis.[17] Furthermore, recent work has shown that PNAs[19] and PNA−DNA chimeras[20] can serve as primers for some polymerases. While new applications are being developed for PNA, the incorporation of novel residues has not received much attention. Described herein is the synthesis of PNAs bearing the potential universal bases and an examination of the hybridization properties of PNA oligomers containing these residues.

Because of the success of the nitroazole deoxynucleoside residues as universal bases,[4−10] it was decided to examine whether these heterocycles would behave similarly when incorporated into PNA. The synthesis of the nitroazole PNA monomers began with a two-step conversion of 3-nitropyrrole and 5-nitroindole into their corresponding acetic acid derivatives (synthetic details are provided in the Supporting Information). Condensation of these carboxylic acids with 2-*N*-Fmoc-2-aminoethylglycine *tert*-butyl ester[21] provided the fully blocked monomers in good yields. Removal of the C-terminal *tert*-butyl esters was accomplished by brief treatment with trifluoroacetic acid, producing monomers for PNA synthesis. PNA oligomers were made using the standard automated solid-phase synthetic method[22] except that the novel residues were coupled. The PNAs were purified using HPLC, and oligomers containing the novel residues produced satisfactory MALDI-TOF mass spectra.[23]

Hybridization of the 15-mer PNA oligomers with oligodeoxynucleotides was performed in PES buffer (10 mM phosphate, 0.1 mM EDTA, 100 mM NaCl, pH 7), and the stability of each of these complexes was evaluated by thermal denaturation experiments using absorbance spectroscopy (Figure 1). All of the absorbance vs temperature curves were

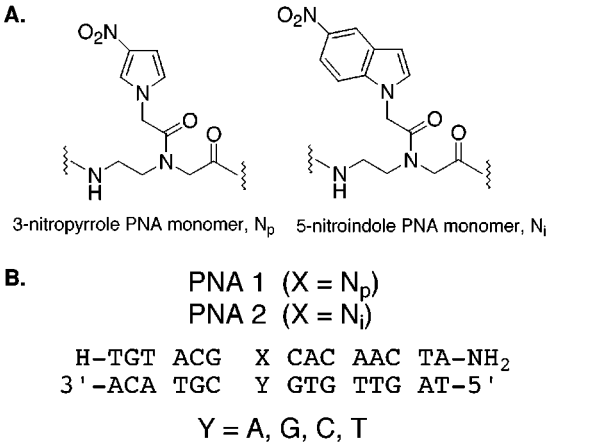


Figure 1. A. Structures of modified PNA residues. B. Sequences of PNA and DNA oligomers used in thermal denaturaion assays.

sigmoidal, indicating that double helix formation is cooperative (Figure 2). PNA **1**, which contains the 3-nitropyrrole residue, showed T_m values ranging from 54.7 °C (opposite

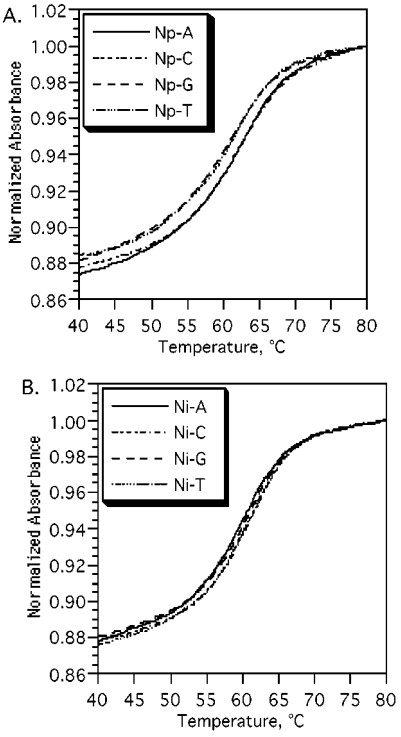


Figure 2. Thermal denaturation curves for PNA·DNA double helices. Absorbance was measured at 260 nm; the concentration of each strand was 4 μM. A. PNA **1** (3-nitropyrrole) + complementary DNAs. B. PNA **2** (5-nitroindole) + complementary DNAs.

dG) to 56.2 °C (opposite dA) (Table 1). This range of T_m values is smaller than that observed for oligodeoxynucleotides containing the 3-nitropyrrole deoxynucleoside residue

(13) Pochet, S.; Dugué, L. *Nucleosides Nucleotides* **1998**, *17*, 2003−2009.
(14) For example, see: Van Aerschot, A.; Rozenski, J.; Loakes, D.; Pillet, N.; Schepers, G.; Herdewijn, P. *Nucleic Acids Res.* **1995**, *23*, 4363−4370.
(15) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497−1500.
(16) Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. *Nature* **1993**, *365*, 566−568.

Table 1. Thermodynamic Parameters for the Hybridization of Nitroazole−PNAs with Complementary DNAs[a]

	Y	T_m (°C)	ΔH (kJ/mol)	ΔS (kJ/mol·K)
X = N_p	A	56.2	−317	−0.85
	G	54.7	−318	−0.86
	C	55.9	−309	−0.83
	T	55.1	−306	−0.82
X = N_i	A	58.4	−338	−0.91
	G	58.9	−344	−0.93
	C	59.7	−343	−0.97
	T	59.3	−350	−0.94
X = T	A	68.5[b]	−446[b]	−1.20[b]

[a] Absorbance vs temperature curves were measured at 260 nm in PES buffer. T_m values were determined by multiplying the maxima of the derivative plots (T_{max}) by 0.971. Thermodynamic parameters were calculated by the method of Gralla and Crothers.[24] [b] Data from Egholm et al. (ref 16).

(1.5 °C versus 3 °C).[4,5,7] The substitution of a natural base with 3-nitropyrrole is mildly destabilizing. A PNA·DNA duplex with the same sequence studied here but containing a normal A−T pair in the variable position was found by Egholm and co-workers[16] to have a T_m of 68.5 °C. Duplexes with nitropyrrole−nucleobase pairs have approximately equivalent stabilities when compared to mismatched duplexes containing a thymine residue in the PNA strand against dG, dC, or T in the DNA strand.[16]

The incorporation of the 5-nitroindole residue into the PNA strand produces a molecule (PNA **2**) with improved hybridization characteristics (Table 1). The range of T_m values for PNA **2** was narrower than that of PNA **1** (ΔT_m = 1.3 °C). More significantly, incorporation of the nitroindole residue was less destabilizing, only showing a drop in T_m of approximately 9 °C versus the matched duplex. These results correspond well with those seen in oligodeoxynucleotides with nitropyrrole and nitroindole bases.[4,5,7] Oligonucleotides with either of these residues showed little variation in T_m when positioned against any of the four natural bases (ΔT_m = 3 °C),[4,5,7] but duplexes containing the 5-nitroindole residues were more stable.[7] Presumably, this stabilization is due to the improved stacking ability of the indole moiety as compared to that of the smaller pyrrole.[10] The higher enthalpies calculated for duplexes with nitroindole versus duplexes with nitropyrrole also support the role of improved stacking (Table 1). Because PNA·DNA double helices maintain base stacking arrangements similar to those seen in DNA·DNA duplexes,[25] it is not surprising that the 5-nitroindole residue is less destabilizing.

The thermodynamic parameters for formation of PNA·DNA double helices containing nitroazole bases (Table 1) show similar trends to the analogous DNA·DNA system.[26] In both a large drop in ΔH from the unsubstituted system is seen, presumably arising from the loss of hydrogen bonding.[26,27] In both cases, this loss is partially counterbalanced by an increase in ΔS. This entropic effect has been attributed to the greater conformational freedom seen by the nucleobase positioned opposite to the non-hydrogen bonding universal base[25] or to solvation/desolvation effects.[26] Future work is needed to fully explore this phenomenon. Nevertheless, incorporation of the nitroazole universal base residues into both PNA·DNA and DNA·DNA double helices appears to behave in a qualitatively similar fashion. However, as would be expected given the greater mismatch sensitivity of PNA probes, substitution of PNA with the nitroazole residues is more destabilizing than in DNA probes.

Incorporation of the universal bases 3-nitropyrrole and 5-nitroindole into PNA oligomers can be accomplished easily and produces sites that are completely degenerate with respect to base-pairing preferences. The substitution of these residues in the PNA strand of a PNA·DNA double helix is slightly destabilizing; however, both of these complexes show significantly higher T_m values than the corresponding DNA·DNA double helix containing an A−T base pair (T_m = 53.3 °C).[16] To test the generality of these substitutions, experiments are currently underway to examine the effects of sequence context on hybridization. Overall, the ability to introduce completely degenerate sites into PNA probes should greatly enhance their utility as tools for molecular biology.

Acknowledgment. M.L.S. was supported by an REU grant from the NSF (CHE-9531496). The authors are grateful to Dr. Joseph Hacia for helpful discussions and to Greg Miller for measurement of high-resolution FAB mass spectra.

Supporting Information Available: Synthetic details for 3-nitropyrrole and 5-nitroindole PNA monomers and PNA oligomers, experimental details for thermal denaturation experiments, and discussion of calculations used to determine thermodynamic parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

OL990270Q

(17) For some recent reviews, see: Hyrup, B.; Nielsen, P. E. *Bioorg. Med. Chem.* **1996**, *4*, 5−23. Dueholm, K. L.; Nielsen, P. E. *New J. Chem.* **1997**, *21*, 19−31; Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. *Angew. Chem. Int. Ed.* **1998**, *37*, 2796−2823.

(18) Hamilton, S. E.; Simmons, C. G.; Kathiriya, I. S.; Corey, D. R. *Chem. Biol.* **1999**, *6*, 343−351.

(19) Lutz, M. J.; Benner, S. A.; Hein, S.; Breipohl, G.; Uhlmann, E. *J. Am. Chem. Soc.* **1997**, *119*, 3177−3178.

(20) Misra, H. S.; Pandey, P. K.; Modak, M. J.; Vinayak, R.; Pandey, V. N. *Biochemistry* **1998**, *37*, 1917−1925.

(21) Thomson, S. A.; Josey, J. A.; Cadilla, R.; Gaul, M. D.; Hassman, C. F.; Luzzio, M. J.; Pipe, A. J.; Reed, K. L.; Ricca, D. J.; Wiethe, R. W.; Noble, S. A. *Tetrahedron* **1995**, *51*, 6179−6194.

(22) *PNA Chemistry for the Expedite Nucleic Acid Synthesis System*; PerSeptive Biosystems, Inc.: Framingham, MA, 1998.

(23) PNA **1** (H-TGT ACG N_p CAC AAC TA-NH_2) *m/z* 4033.5 $[M + H]^+$, calcd 4033.6. PNA **2** (H-TGT ACG N_i CAC AAC TA-NH_2) *m/z* 4083.9 $[M + H]^+$, calcd 4084.3.

(24) Gralla, J.; Crothers, D. M. *J. Mol. Biol.* **1973**, *78*, 301−319.

(25) Leijon, M.; Graeslund, A.; Nielsen, P. E.; Buchardt, O.; Norden, B.; Kristensen, S. M.; Eriksson, M. *Biochemistry* **1994**, *33*, 9820−9825.

(26) Bergstrom, D. E.; Zhang, P.; Johnson, W. T. *Nucleic Acids Res.* **1997**, *25*, 1935−1942.

(27) Seela, F.; Bourgeois, W.; Rosemeyer, H.; Wenzel, T. *Helv. Chim. Acta* **1996**, *79*, 488−498.