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DNA sequence selectivity of hairpin polyamide turn units

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Abstract

A class of hairpin polyamides linked by 3,4-diaminobutyric acid, resulting in a β-amine residue at the turn unit, showed improved binding affinities relative to their α-amino-γ-turn analogs for particular sequences. We incorporated β-amino-γ-turns in six-ring polyamides and determined whether there are any sequence preferences under the turn unit by quantitative footprinting titrations. Although there was an energetic penalty for G·C and C·G base pairs, we found little preference for T·A over A·T at the β-amino-γ-turn position. Fluorine and hydroxyl substituted α-amino-γ-turns were synthesized for comparison. Their binding affinities and specificities in the context of six-ring polyamides demonstrated overall diminished affinity and no additional specificity at the turn position. We anticipate that this study will be a baseline for further investigation of the turn subunit as a recognition element for the DNA minor groove.

Keywords

Molecular recognition; DNA minor groove; Foldamer

Hairpin pyrrole-imidazole (Py/Im) polyamides are a class of programmable synthetic ligands able to bind a broad repertoire of DNA sequences with affinities and specificities comparable to those of DNA-binding proteins.[1,2] They have been shown to localize to the nuclei of living cells[3,4] and regulate endogenous gene expression by interfering with transcription factor/DNA interfaces.[5–10] Discrimination of the four Watson–Crick base pairs is dependent upon Py/Im ring pairings in the minor groove. Pairing rules have been established whereby *N*-methyl imidazole/*N*-methylpyrrole (Im/Py) pairs target G·C, the reverse (Py/Im) target C·G, and Py/Py pairs target A·T and T·A.[11–13]

The turn unit in the hairpin is a recognition element, favoring T·A/A·T over G·C/C·G.[14] There is an energetic penalty for unfavorable steric interaction with the exocyclic amine present at the edge of the G·C base pair (Fig. 1). The question arises whether discrimination between T·A and A·T with the turn unit can be achieved. Previous efforts toward the improvement of hairpin binding affinity have involved modifications of the turn unit. Early studies showed the optimal length of the turn element to be three methylene units, resulting in the use of γ-aminobutyric acid (γ-turn).[15] Modification of the α-position of the parent γ-turn with (*R*)-2,4-diaminobutyric acid (α-amino-γ-turn) results in an approximately 15-fold increase in DNA-binding affinity.[16] In contrast, hairpins containing the opposite enantiomer, (*S*)-α-amino-γ-turn, bind DNA with diminished affinities likely due to a steric clash of the amine with the wall of the minor groove. Polyamides containing α-hydroxy-γ-turns[17] and α-

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Supplementary data
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diaminobutyric acid[18] turns have been reported to impart additional elements of specificity, but with the cost of diminished binding affinities.

In a formal sense, a DNA minor groove binding hairpin Py/Im polyamide is an early example of a class of oligomers encoded by the order of monomer units that fold to a desired shape with a specific function, referred to as 'foldamers.' [19–21] The ring order of Py/Im polyamides codes in a programmable manner for a specific, contiguous sequence of Watson–Crick base pairs. The turn unit is both a shape element as well as a DNA recognition element, allowing the molecule to fold in a U-conformation. The turn unit deserves more attention and this Letter represents an effort to create a baseline for the field.

Recently, we introduced a new class of hairpin polyamides linked by 3,4-diaminobutyric acid, resulting in a β-amino-γ-turn.[22] These molecules showed improved binding affinities relative to their α-amino-γ-turn analogs for A/T-rich sequences. Additionally, polyamides containing the β-amino-γ-turn were found to have improved tolerance for synthetic modification at the amine position presumably due to their more central location on the floor of the minor groove. Due to limitations of quantitative DNase I footprinting titrations[23–25] for compounds where $K_a \geq 10^{10}$ M^{-1}, relative binding affinities for high affinity molecules are compared by using their thermal stabilization of DNA duplexes. It has been shown previously that increases in melting temperatures (ΔT_m) of DNA duplexes bound by hairpin polyamides correlate with DNA-binding affinity.[26,27]

We report herein a comparison of the sequence specificities of hairpin Py/Im polyamides containing the α-amino-γ-turn and the β-amino-γ-turn. Additionally, we have synthesized both hydroxyl and fluoro-substituted γ-turns and determined their affinities and specificities in polyamides with analogous core ring pairs. By employing six-ring polyamides, which have lower binding affinities compared to eight-ring polyamides,[28] we are able to determine reliable equilibrium association constants (K_a) via quantitative DNase I footprinting titrations, and compare them with DNA duplex thermal stabilizations.

Polyamide synthesis

Six-ring hairpin polyamides (ImImPy-turn-PyPyPy) targeting the DNA sequence 5′-WWGGWW-3′ were synthesized by solid phase methods on Pam resin (Fig. 2, Supplementary data 1).[22,29] In addition to the parent molecule containing an unsubstituted γ-aminobutyric acid hairpin (**1**), oligomers containing an amine moiety in the α and β turn positions (**2R**, **4R**, **4S**) and two polyamides with acetylated amines (**3R, 5R**) were also synthesized. The acetylated, or capped, molecules were used to determine tolerance for modifications at the turn. Acetylated turn units have been shown to improve nuclear uptake of polyamides.[4]

Thermal stabilization of DNA duplexes

Spectroscopic analyses were performed on the 11mer DNA duplex shown in Table 1.[22] All hairpins analyzed provided an increase in melting temperature, confirming the formation of DNA/polyamide complexes. The ΔT_m values obtained for polyamides containing a free amine were within error of each other. However, acetylated polyamide **3R** (α-amino turn) showed a greater decrease in affinity than **5R** (β-amino-γ-turn). As had been demonstrated with eight-ring hairpin molecules,[22] improvements in binding affinities for β-over α-amino-γ-turn six-ring polyamides are more pronounced with decreasing imidazole content (Supplementary Fig. 1, Table 1).

DNA binding affinity and sequence selectivity

The plasmid pCDMF6 was prepared to characterize polyamides targeting the sequence 5′-WWGGWW-3′ (Fig. 3). The designed insert contains four binding sites, varying the nucleotide base pair present under the turn unit. Quantitative DNase I footprinting titrations were performed with the polyamides in order to measure their binding site affinities and specificities, as previously described.[25] As expected, the parent hairpin containing the γ-turn retained the lowest binding affinity, while experiments for **2R, 4R**, and **4S** (Fig. 4, Table 2) corroborated the similar ΔT_m values obtained for duplex stabilization. None of these molecules bound the G·C base pair, and binding to C·G was greatly diminished. Polyamide **4R** revealed only a ~2-fold specificity for T·A over A·T (Table 3). Study of an additional polyamide series revealed similar trends (Supplementary Fig. 2, Table 2). Analysis of polyamides **3R** and **5R** (Fig. 4, Table 2) revealed a greater decrease in binding affinities for the α-amino-γ-turn molecule than the β. Between **2R** and **3R** there is a five-fold decrease over T·A, and an eight-fold decrease over A·T. For β-amino-γ-turn polyamides **4R** and **5R**, there are 1.7 and 3.2-fold decreases for T·A and A·T, respectively. **5R** shows ~3-fold preference for binding T·A versus A·T (Table 3).

Fluoro and hydroxyl substituted turn units

Fluorine and hydroxyl substituted hairpin turns were synthesized (Supplementary Figs. 3 and 4, Supplementary data 1[30,31]) and incorporated in six-ring hairpin polyamides targeting the DNA sequence 5′-WWGGWW-3′ (Fig. 5). Polyamides were synthesized on Pam resin using standard solid phase methods.

Thermal stabilization analysis of the polyamides on an 11mer DNA duplex revealed that fluoro and hydroxyl substituted hairpins resulted in lower stabilizations than the corresponding amine-substituted and acetylated polyamides (Table 4, Supplementary Table 3). DNase I footprinting titrations on the plasmid pCDMF6 (Fig. 3, Supplementary Fig. 5) showed that both enantiomers of the hydroxyl and fluoro-hairpin turns resulted in decreased polyamide binding affinities relative to their amine substituted counterparts (Supplementary Table 4). Additionally, none of these subunits resulted in increased elements of specificity at the turn position of the molecule (Table 5).

By utilizing six-ring hairpin polyamides, we were able to combine DNase I footprinting titration and duplex stabilization analyses in order to fully characterize the binding preferences of various hairpin turn subunits. Although the hairpin turns investigated herein show modest DNA binding specificities, we anticipate that further study will yield moieties enabling discrimination amongst all four Watson–Crick base pairs and add an additional element for DNA recognition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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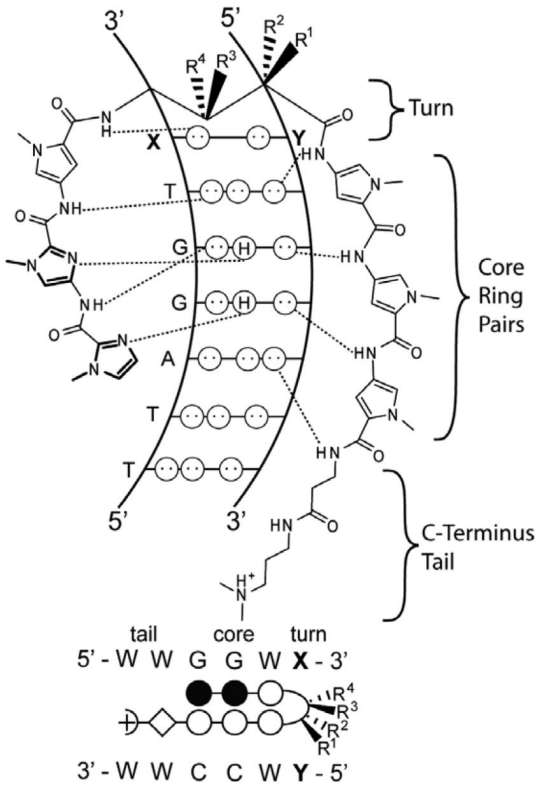


Figure 1.
Schematic diagram of six-ring hairpin polyamide (ImImPy-turn-PyPyPy) targeting the DNA sequence 5′-WWGGWW-3′. Dashed lines indicate hydrogen bonds between the polyamide and DNA base pairs.

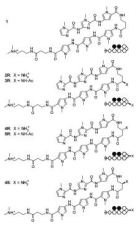


Figure 2.
Chemical and ball-and-stick structures of polyamides containing free and acetylated amines. Ball and stick symbols are defined as follows: pyrrole is denoted by an open circle, imidazole is denoted by a filled circle, and β-alanine is denoted by a diamond shape.

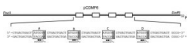


Figure 3.
Illustration of the EcoRI/PvuII restriction fragment derived from plasmid pCDMF6, used to characterize polyamides. The designed polyamide binding sites are indicated by boxes; single base-pair mismatches are indicated by shaded regions.

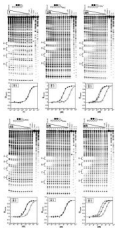


Figure 4.

Quantitative DNase I footprinting titration experiments for polyamides **1**, **2R**, and **4R** (top, left to right) and **4S**, **3R**, and **5R** (bottom, left to right) on the 5′ end labeled PCR product of plasmid pCDMF6: lanes 1–11, 100 nM, 30 nM, 10 nM, 3 nM, 1 nM, 300 pM, 100 pM, 30 pM, 10 pM, 3 pM, and 1 pM polyamide, respectively; lane 12, DNase I standard; lane 13, intact DNA; lane 14, A reaction; lane 15, G reaction. Respective isotherms are shown below.

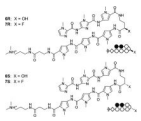
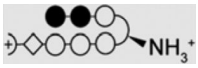
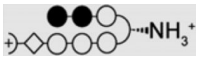
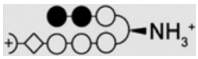
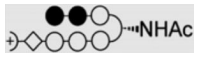


Figure 5.

Chemical and ball-and-stick structures of polyamides containing fluoro and hydroxyl substituted hairpin turns. Ball and stick symbols are defined as follows: pyrrole is denoted by an open circle, imidazole is denoted by a filled circle, and β-alanine is denoted by a diamond shape.

Table 1

Melting temperatures of DNA/polyamide complexes

DNA = 5′-CTATGGTA GAC-3′			
	Polyamides	T_m (°C)	ΔT_m (°C)
	–	45.1 (±0.1)	–
1		54.3 (±0.2)	9.2
2R		59.8 (±0.3)	14.7
4R		60.0 (±0.2)	14.9
4S		59.8 (±0.2)	14.7
3R		54.9 (±0.1)	10.1
5R		57.0 (±0.2)	12.2

All values are derived from at least three melting temperature experiments, with standard deviations indicated in parentheses. ΔT_m values are given as T_m (DNA/poiyamide) – T_m (DNA).

Table 2

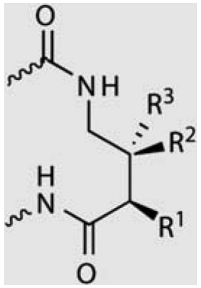
Binding affinities (M^{-1}) for polyamides

	5′-ATGGTT-3′	5′-ATGGTA-3′	5′-ATGGTC-3′	5′-ATGGTG-3′
1	2.1 (±0.6)× 10^8	2.0 (±0.5) × 10^8	<10^7	<10^7
2R	1.6 (±0.7) × 10^9	1.7 (±0.9) × 10^9	3.9 (±2.0) × 10^7	<10^7
4R	3.3 (±0.1} × 10^9	1.8 (±0.2) × 10^9	5.0 (±1.1) × 10^7	<10^7
4S	1.1 (±0.1) × 10^9	1.3 (±0.2) × 10^9	6.0 (± 0,4) × 10^7	<10^7
3R	3.2 (±0.4) × 10^8	2.1 {±0.3) × 10^8	<10^7	<10^7
5R	2.0 (±0.4) × 10^9	5.2 (±1.6) × 10^8	8.9 (±2.8) × 10^7	<10^7

Equilibrium association constants are reported as mean values from three DNase I footprinting titration experiments. Standard deviations are shown in parentheses.

Table 3

Relative binding affinities for polyamides

	R^1,R^2,R^3	T·A	A·T	C·G	G·C
	H, H, H	1.0	1.0	<0.01	<0.01
	NH_3^+, H, H	1.0	0.9	0.02	<0.01
	H, H, NH_3^+	1.0	0.5	0.02	<0.01
	H, NH_3^+, H	1.0	1.2	0.05	<0.01
	NH-Ac, H, H	1.0	0.7	<0.01	<0.01
	H, H, NH-Ac	1.0	0.3	0.04	<0.01

Relative binding affinities are reported as ratios of binding affinities (K_a) as determined by DNase I footprinting titration experiments for polyamides targeting 5′-WGGWW-3′.

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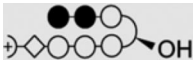
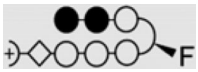
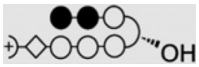
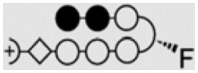
Table 4

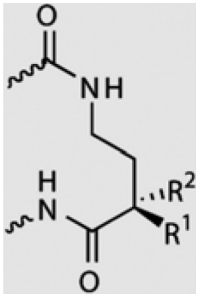
Melting temperatures of DNA/ polyamide complexes

DNA = 5′-CTATGGTA GAC-3′			
	Polyamide	T_m (°C)	ΔT_m (°C)
	–	45.1 (±0.1)	–
6R		55.1 (±0.2)	10.0
7R		53.4 (±0.2)	8.3
6S		50.9 (+0.2)	5.8
7S		53.0 (±0.2)	7.9

All values are derived from at least three melting temperature experiments, with standard deviations indicated in parentheses. ΔT_m values are given as T_m (DNA/polyamide) – T_m (DNA).

Table 5

Relative binding affinities for polyamides

	R^1,R^2	T·A	A·T	C·G	G·C
	OH, H	1.0	1.0	<0.01	<0.01
	F,H	1.0	0.8	<0.01	<0.01
	H, OH	1.0	1.3	1.4	0.8
	H, F	1.0	0.6	0.6	<0.01

Relative binding affinities are reported as ratios of binding affinities (K_a) as determined by DNase I footprinting titration experiments for polyamides targeting 5′-WGGWW-3′ Sites containing G·C at the hairpin position have relative affinities <0.01 (not shown).