Long-Range Cooperativity Due to C5-Propynylation of Oligopyrimidines Enhances Specific Recognition by Uridine of ribo-Adenosine over ribo-Guanosine

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In theory, nucleic acid-based molecular recognition can be extremely potent and specific for antisense-based drugs,[1−3] microarray screens,[4−7] molecular beacon probes,[8−10] and self-assembling nanostructures.[7,11−13] The many functions of RNA in cells make targeting RNA particularly important for both therapeutics and diagnostics. One limitation on the specificity of targeting RNA with natural nucleotides is that A:U and G:U pairs have similar stabilities. This promiscuity may be crucial to evolution since G:U wobble pairs are often important for RNA function[14−16] and account for 50% of known non-Watson−Crick pairs.[17−19] Replacing the O2 of rU with sulfur can increase the specificity for pairing with rA over rG by 10-fold in the binding constant.[20] We report that total C5-(1-propynyl)ation of oligopyrimidine sequences[21,22] can enhance the specificity of dU for rA over rG roughly 100-fold, while also increasing binding by roughly 10^5-fold, without changing the hydrogen bonding groups of the bases.

Consecutive C5-(1-propynyl)-2′-deoxyribo-pyrimidines, Y^p's (Figure 1), within a fully propynylated oligodeoxynucleotide, or PODN, exhibit long-range, highly cooperative interactions when bound to RNA.[23] This phenomenon is very sensitive to helix composition and can be eliminated by removing only a single amino or propynyl group from the minor or major groove, respectively, of a PODN:RNA duplex. Here, we show that this sensitivity enhances discrimination between formation of rA:dU^p and rG:dU^p pairs (Figure 1).

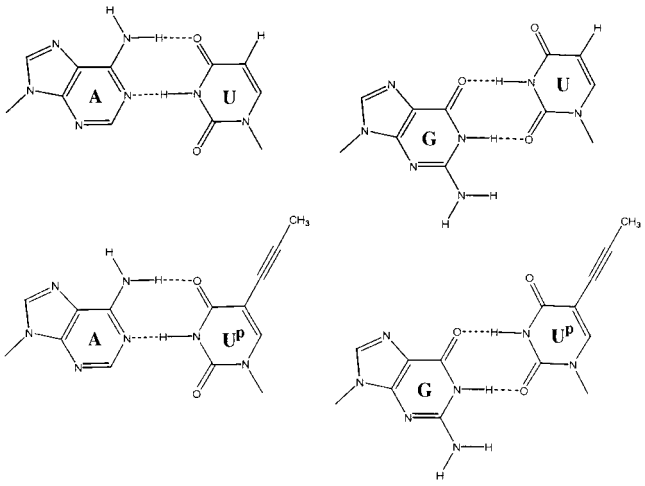

Figure 1. (a) A:U and A:U^p Watson−Crick pairs. (b) G:U and G:U^p wobble pairs.

(i) C5-(1-Propynyl)ation Enhances Discrimination. Thermodynamic parameters from UV melting studies are listed in Table 1 for a series of DNA and PODN heptamers hybridized to RNA strands containing 5′ and 3′ terminal unpaired nucleotides. The unpaired dangling nucleotides simulate RNA targets, which are typically longer than DNA probe strands. These duplexes are denoted as (A:U)-*n* and (A:U^p)-*n*, where *n* is the entry number in Table 1. Single rA→rG substitutions were made in each RNA strand, producing single rG:dU or rG:dU^p pairs. These duplexes are denoted (G:U)-*n* and (G:U^p)-*n*. Representative melting curves of all four duplex types are shown in Figure 2. Subtracting the free energy of (A:U)-*n* or (A:U^p)-*n* duplexes from that of their respective (G:U)-*n* or (G:U^p)-*n* duplexes provides the thermodynamic impact, $\Delta\Delta G^\circ_{37}$, of a single rG:dU wobble on hybrid duplex formation (Table 1).

The $\Delta\Delta G^\circ_{37}$ for dU^p ranges from 2.6 to 4.2 kcal/mol and averages 3.3 kcal/mol compared with a range of 0.1−0.9 and an average of 0.5 kcal/mol for dU. Thus, dU^p in propynylated oligopyrimidines provides a roughly 100-fold greater discrimination in the relative binding constants than that observed with dU in unmodified oligopyrimidines. Discrimination against G:U formation occurs in all nearest neighbors and positions tested, in contrast with results for 2-thio-rU.[20] The range of PODN:RNA duplex $\Delta\Delta G^\circ_{37}$'s suggests that the magnitude of rG:dU^p discrimination is somewhat nearest neighbor dependent. The largest discriminations are seen in the context of d(5′C^{p}U^{p}U^{p}3′)/r(3′GGA5′).

The average $\Delta\Delta G^\circ_{37}$ for unmodified dU of 0.5 kcal/mol is the same value as the average $\Delta\Delta G^\circ_{37}$ expected for rU within RNA:RNA duplexes.[24,25] Furthermore, the average $\Delta\Delta G^\circ_{37}$ for dU reported here corresponds well with that expected for dU (0.3 kcal/mol) in DNA:RNA hybrids.[26,27]

(ii) Enhanced Discrimination Is Coupled to Long-Range Cooperative Interactions between Y^p's. Single propynyl deletions were made within PODN entries (A:U^p)-3, -4, and -5 from Table 1. These oligonucleotides are referred to as s-PODNs. Their

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Table 1. Thermodynamic Parameters of PODN:RNA, DNA:RNA, and s-PODN:RNA Duplexes Containing A:U vs G:U Pairs[a]

Entry (n)	DNA	Matched RNA (A:U)-n	T_m(°C)	$-\Delta H^o$ (kcal/mol)	$-\Delta S^o$ (eu)	$-\Delta G^o_{37}$ (kcal/mol)	Mismatched RNA[c] (G:U)-n	T_m(°C)	$-\Delta H^o$ (kcal/mol)	$-\Delta S^o$ (eu)	$-\Delta G^o_{37}$ (kcal/mol)	$\Delta\Delta G^o_{37}$ (kcal/mol)
1	d(5'CCUUCUU3')	r(3'GA**GGAAGAA**AU5')	38.2	57.6	163.8	6.8	r(3'GA**GGAGGAA**AU5')	35.8	62.8	182.4	6.3	0.5
2	d(5'CUUCCUU3')	r(3'GA**GAAGGAA**AU5')	38.2	57.5	163.6	6.8	r(3'GA**GAAGGAG**AU5')	35.1	54.6	156.2	6.2	0.6
3	d(5'CUUCCUU3')	r(3'GA**GAAGGAA**AU5')	38.2	57.5	163.6	6.8	r(3'GA**GGAGGAA**AU5')	33.5	53.1	152.2	5.9	0.9
4	d(5'UCUCCUU3')	r(3'GA**AGAGGAA**AU5')	39.8	54.1	151.7	7.0	r(3'GA**GGAGGAA**AU5')	38.7	62.1	178.2	6.9	0.1
5	d(5'CCUCUUU3')	r(3'GA**GGAGAAA**AU5')	38.7	56.4	159.7	6.8	r(3'GA**GGAGGAA**AU5')	35.2	62.0	180.1	6.2	0.6
	PODN[b]	**(A:U^p)-n**					**(G:U^p)-n**					
1	d(5'**CCUUCUU**3')^p	r(3'GA**GGAAGAA**AU5')	70.8	84.8	225.6	14.9	r(3'GA**GGAGGAA**AU5')	64.4	70.8	188.7	12.3	2.6
2	d(5'**CUUCCUU**3')^p	r(3'GA**GAAGGAA**AU5')	75.6	79.9	208.1	15.4	r(3'GA**GAAGGAG**AU5')	69.4	63.0	162.9	12.5	2.9
3	d(5'**CUUCCUU**3')^p	r(3'GA**GAAGGAA**AU5')	75.6	79.9	208.1	15.4	r(3'GA**GGAGGAA**AU5')	64.4	57.2	148.5	11.2	4.2
4	d(5'**UCUCCUU**3')^p	r(3'GA**AGAGGAA**AU5')	73.7	77.2	201.5	14.7	r(3'GA**GGAGGAA**AU5')	66.8	59.6	154.3	11.8	2.9
5	d(5'**CCUCUUU**3')^p	r(3'GA**GGAGAAA**AU5')	70.7	78.8	208.3	14.2	r(3'GA**GGAGGAA**AU5')	58.2	61.9	165.7	10.5	3.7
	s-PODN[b]	**(A:U^p)-s*n*[d]**					**(G:U^p)-s*n*[d]**					
s3	d(5'**CUUCCUU**3')^p	r(3'GA**GAAGGAA**AU5')	63.7	70.2	187.3	12.1	r(3'GA**GGAGGAA**AU5')	56.9	81.9	227.1	11.5	0.6
s4	d(5'**UCUCCUU**3')^p	r(3'GA**AGAGGAA**AU5')	67.9	66.2	173.0	12.5	r(3'GA**GGAGGAA**AU5')	62.3	68.6	183.5	11.7	0.8
s5	d(5'**CCUCUUU**3')^p	r(3'GA**GGAGAAA**AU5')	62.8	71.8	192.6	12.0	r(3'GA**GGAGGAA**AU5')	57.9	61.9	165.8	10.5	1.5

[a] All thermodynamic parameters determined by $1/T_M$ vs $\ln(C_T)$ plots. Buffer is 1.0 M NaCl, 0.5 mM Na_2EDTA, 20 mM sodium cacodylate, pH 7.0. T_M is for 0.1 mM total strand concentration. The left half lists results for duplexes where the DNA forms only Watson−Crick matched pairs with the bold nucleotides of the RNA. The right half lists results for duplexes containing a single GU pair, which is underlined. Note that the two terminal nucleotides on each end of the RNA do not form base pairs. [b] Every bold C and U is propynylated. [c] Underline denotes the position of the G:U pair. [d] Unbold rG's within the recognition sequence denote that they are paired to unmodified dC's.

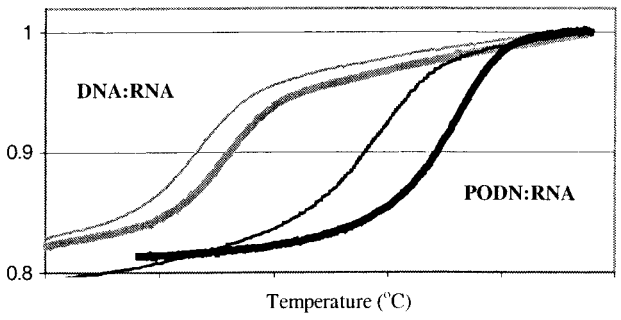

Figure 2. Representative UV melting curves at about 10 μM total strand concentration for DNA:RNA duplexes (A:U)-3 (gray-thick) and (G:U)-3 (gray-thin) at A_{260} and PODN:RNA duplexes (A:U^p)-3 (black-thick) and (G:U^p)-3 (black-thin) duplexes at A_{280}. Relative absorbance is plotted vs temperature from 0−100 °C.

duplexes with RNA sequences having an A:U or G:U pair are denoted (A:U^p)-s*n* and (G:U^p)-s*n*, respectively. The thermodynamics of these duplexes are in Table 1. All propynyl deletions within s-PODNs occur at least two base pairs away from the position of an rA:dU^p→rG:dU^p modification. Thus, nearest neighbor pairs directly adjacent to each position of a rA→rG modification are not changed by eliminating the propynyl group.

Comparison of the stabilities of (A:U^p)-s*n* and parent (A:U^p)-*n* duplexes in Table 1 indicates that the thermodynamic contribution of a single propynyl group to the overall stability of each PODN:RNA duplex ranges from 2.2 to 3.3 kcal/mol, and averages to 2.6 kcal/mol. This is within experimental error of average reported thermodynamic contributions of single propynyl groups (3.1 kcal/mol) to overall PODN:RNA duplex stability.[23] By subtracting the free energy of (A:U^p)-s*n* duplexes from that of their respective (G:U^p)-s*n* duplexes in Table 1, the thermodynamic impact, $\Delta\Delta G^o_{37}$, of a single G:U^p pair on s-PODN:RNA duplex formation is found to range from 0.6 to 1.5 kcal/mol. These values are more similar to those of DNA:RNA than PODN:RNA duplexes with the same sequences in Table 1.

The data in Table 1 show that elimination of a single propynyl group reduces discrimination by 2.1−3.6 kcal/mol. The difference in the average $\Delta\Delta G^o_{37}$'s is 2.6 kcal/mol. This is remarkably similar to the 2.6 kcal/mol attributed to highly cooperative long-range interactions between seven consecutive Y^p's in a different PODN:RNA duplex.[23] This suggests that enhanced discrimination against G:U^p is lost due to loss of long-range cooperative interactions between Y^p's when a single propynyl group is removed.

The results in Table 1 show that full propynylation of an oligopyrimidine DNA strand participating in PODN:RNA hybridization enhances discrimination of rG:dU^p pair formation, thus increasing specificity 100-fold. It is likely that highly cooperative interactions between Y^p's can also enhance discrimination against less stable mismatches in PODN:RNA duplexes. These results show that modifying positions not directly involved in molecular recognition can enhance specificity. An oligopyrimidine heptamer has been used to specifically target the SV40 Tag mRNA in a cell culture assay,[28] but general applicability will require mixed pyrimidine−purine sequences. It will be interesting to see if modifications can be found to extend specificity to mixed pyrimidine−purine sequences of RNA and DNA, as well as to other backbones suitable for designing therapeutics, diagnostics, and structures for nanotechnology.

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Supporting Information Available: Purification procedures of oligonucleotides, methods of UV melting, $1/T_M$ vs $\ln(C_T)$ plots, and comparisons of thermodynamic parameters from averaging melting curves with those from $1/T_M$ vs $\ln(C_T)$ plots (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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