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2-Ureidoquinoline: a useful molecular element for stabilizing single cytosine and thymine bulges

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Abstract—We have demonstrated that aromatic heterocycles having hydrogen-bonding surfaces complementary to those of nucleotide bases are effective molecular elements for the binding to single nucleotide bulges and base mismatches. We here report that a new molecule, 2-ureidoquinoline having an alignment of hydrogen-bonding groups in the order of acceptor–donor–donor stabilizes single cytosine and thymine bulges in duplex DNAs. Furthermore, a dimeric form of 2-ureidoquinoline stabilizes cytosine–cytosine and cytosine–thymine mismatches.

Having completed a sequence analysis of human genome, effective detections of genetic mutations became an indispensable technology in many research fields of molecular biology, genetics, and chemical biology.[1–3] Binding of small molecular probes to single nucleotide bulges and base mismatches in duplex DNA provides an innovative method in heteroduplex analysis of deletion and insertion mutations as well as single nucleotide polymorphisms (SNPs).[4–6] We have demonstrated that aromatic heterocycles having hydrogen-bonding surfaces complementary to those of nucleotide bases are effective molecular elements for the binding to single nucleotide bulges and base mismatches.[7–15] *N*-Acyl-2-amino-1,8-naphthyridine in which the hydrogen-bonding groups are aligned in the order of acceptor–acceptor–donor stabilized a duplex containing a guanine bulge.[7] Guanine–guanine mismatches were strongly stabilized by a form of covalently linked dimer.[8] In addition to the strong preferences of *N*-acyl-2-amino-1,8-naphthyridine to a guanine base, a modest stabilization of the cytosine bulge was also observed. Since the hydrogen-bonding surface of the molecule was partially matched to that of a cytosine, these observations prompted us to modulate the hydrogen-bonding surface of the molecule to be fully complementary to a cytosine.

We here report that a new molecule, 2-ureidoquinoline having an alignment of hydrogen-bonding groups in the order of acceptor–donor–donor stabilizes single cytosine and thymine bulges in duplex DNAs. Furthermore, a dimeric form of 2-ureidoquinoline stabilizes cytosine–cytosine and cytosine–thymine mismatches (Figs. 1 and 2).

We have synthesized a series of molecules **1a**–**d** having different aromatic heterocycles with a side chain containing a urea structure. These molecules were obtained by a coupling of corresponding amino-substituted heterocycles with *N*-Boc-4-isocyanatobutylamine, that was prepared by the reaction of *N*-Boc-5-aminovaleric acid and diphenylphosphoryl azide. The effect of these compounds having urea structures on the stabilization

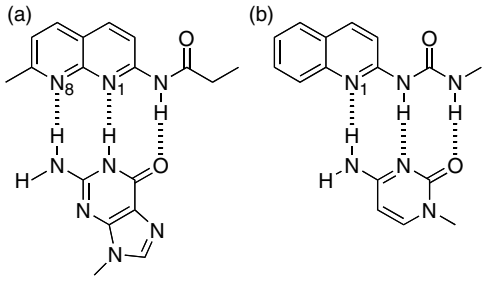

Figure 1. Possible hydrogen bonding between (a) *N*-acyl-2-amino-1,8-naphthyridine and guanine, and (b) 2-ureidoquinoline and cytosine.

Keywords: Ureidoquinoline; Bulge; Mismatch.
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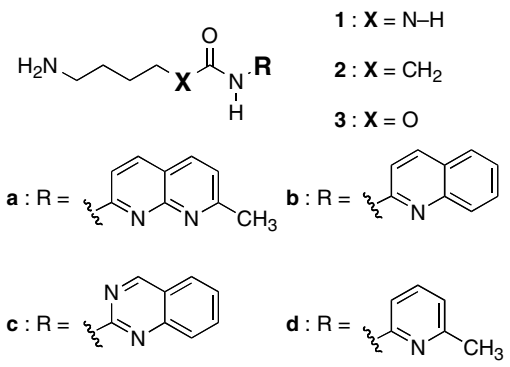


Figure 2. Molecules examined in these studies.

of a single nucleotide bulge in duplex DNA was compared with corresponding amide compounds **2a** and **2b** as well as quinolinylcarbamate **3b**. Amide compounds were synthesized by the coupling of amino heterocycles with pentafluorophenyl *N*-Boc-6-aminohexanoate, whereas **3b** was obtained by Curtius rearrangement of quinaldic acid followed by a coupling with *N*-Boc-aminobutanol. Deprotection of the Boc group furnished the synthesis of all molecules. The number of atoms between the carbonyl carbon to the terminal amino group in the linker was kept constant for all molecules. The effects of these molecules on a stabilization of a single nucleotide bulge were investigated by measuring a melting temperature (T_m) of duplexes 5′-d(TCC AG GCA AC)-3′/3′-d(AGG TC**X** CGT TG)-5′ containing a single nucleotide bulge at the position of **X**. The difference of the melting temperature (ΔT_m) in the absence and presence of the molecule (100 μM) in sodium cacodylate buffer (10 mM, pH 7.0) was summarized in Table 1.

In the presence of **1a** consisting of a naphthyridine ring and a urea linker, T_m was increased for all duplexes-containing single nucleotide bulges, but not for the fully matched 11-mer duplex. ΔT_m for cytosine, thymine, guanine, and adenine bulges were 4.8, 3.7, 2.4, and 3.1 °C, respectively. Ureidoquinoline **1b** that was lacked by one ring nitrogen compared to **1a** showed a comparable ΔT_m for cytosine and thymine bulges, but a decreased ΔT_m for guanine and adenine bulges. Ureidoquinazoline **1c**, where only one of two ring nitrogens can simultaneously participate in the hydrogen bonding to the bulged nucleotide, showed much lower ΔT_m for all bulges than **1b**. Truncation of naphthyridine and quinoline rings to a pyridine ring as in **1d** resulted in a complete loss of stabilizing effects of bulged duplexes. The significance of a urea structure of **1a** and **1b** for the stabilization of single nucleotide bulges was clearly demonstrated by comparing the ΔT_m values with those obtained by the corresponding amide compounds **2a** and **2b**. The ΔT_m obtained for the cytosine, thymine, and adenine bulges in the presence of **2a** was decreased by 3.8, 3.7, and 3.2 °C, respectively, from those obtained in the presence of **1a**, but the ΔT_m for the guanine bulge was slightly increased instead. A similar propensity of ΔT_m values was observed for **2b**. Quinolinylcarbamate **3b** where one hydrogen donor in a urea structure was replaced by hydrogen acceptor exhibited a median effect on the stabilization of single nucleotide bulges.

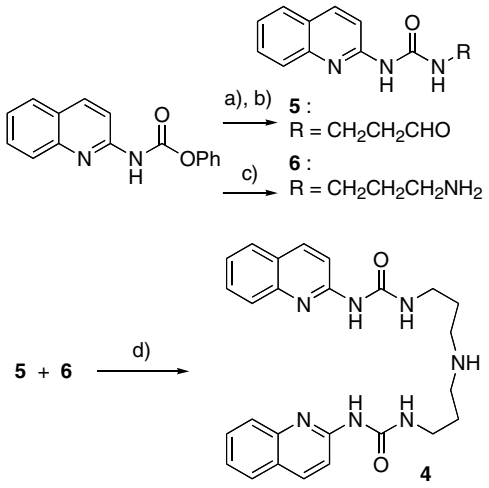
Comparing **1a** with **1b** and also **1b** with **2b**, the nitrogen at a position 8 of 1,8-naphthyridine was not necessary for the stabilization of cytosine, thymine, and adenine bulges, but hydrogen-bonding donor of a urea group was essential. The low ability of **1c** for the stabilization of single nucleotide bulges is most likely due to a conformational variety regarding the urea group. Conformational calculations of **1c** indicated that hydrogen bonding between N3 of quinazoline and N–H in urea would be stable by forming six-membered ring.[16] In that conformation, hydrogen-bonding groups aligned in the order of acceptor–donor–acceptor. Preorganization of hydrogen groups was reported an important factor for producing a stable hydrogen-bonded complex.[17–20] Marked modulations of the ΔT_m observed by changing N–H in **1b** into methylene and oxygen in **2b** and **3b**, respectively, clearly demonstrated the effect of hydrogen-bonding interactions on the stabilization of single nucleotide bulges.

Having found that ureidoquinoline **1b** effectively stabilized the cytosine and thymine bulges, the potential for the stabilization of a base mismatch by a dimeric form of **1b** was investigated. Ureidoquinoline dimer **4** was synthesized by a reductive amination of aldehyde **5** with primary amine **6**, which were obtained by a common precursor of phenylcarbamate (Scheme 1).[21] ΔT_m values for the base mismatches were obtained from the melting temperatures of 11-mer duplex containing a single base mismatch (**X**–**Y**) in the middle of the sequence (Table 2).

Table 1. Increased T_m (°C) of bulge-containing duplexes in the presence of testing molecules[a]

Drug	5′-d(TCCAG_GCAAC)-3′ 3′-d(AGGTC**X**CGTTG)-5′				Match (11-mer)
	X: C	T	G	A	
1a	4.8	3.7	2.4	3.1	−0.3
1b	4.9	4.2	1.8	2.6	−0.4
1c	1.1	1.4	−1.9	−0.1	−0.6
1d	0.8	0.0	−0.1	0.1	−0.7
2a	1.0	0.0	2.9	−0.1	0.2
2b	1.0	0.2	0.6	0.1	−0.1
3b	2.2	1.9	1.0	0.9	0.0

[a] [DNA base] = 100 μM, [ligand] = 100 μM, [NaCl] = 100 mM, [sodium cacodylate] = 10 mM (pH 7.0).



Scheme 1. Reagents and conditions: (a) 3,3-diethoxypropylamine, (b) AcOH, H_2O, 99% (two steps), (c) propylenediamine, 79%, (d) **5**+**6**, $NaBH_3CN$, AcOH, MeOH, 54%.

Table 2. Increased T_m (°C) of mismatch-containing duplexes in the presence of **4**[a]

5′-d(CTAAC**X**GAATG)-3′ 3′-d(GATTG**Y**CTTAC)-5′			
X–Y	ΔT_m	X–Y	ΔT_m
C–C	6.8	G–T	1.0
C–T	6.1	T–T	0.5
C–A	2.4	A–A	−0.2
G–G	2.4	G–C	0.0
G–A	2.0	A–T	−0.8

[a] [DNA base] = 100 μM, [ligand] = 100 μM, [NaCI] = 100 mM, [sodium cacodylate] = 10 mM (pH 7.0).

The ΔT_m of 6.8 °C was observed for the C–C mismatch, whereas the fully matched duplexes where **X**–**Y** were G–C and A–T were not stabilized at all under the same conditions. In marked contrast to dimer **4**, little increase of the T_m was observed for the C–C mismatch in the presence of ureidoquinoline **1b**, demonstrating that a covalent connection of two ureidoquinolines is quite effective for the stabilization of the C–C mismatch.[22] The ΔT_m of 6.1 °C was also observed for the C–T mismatch. Other mismatches including C–A, G–G, G–A, G–T, A–A, and T–T showed a small increase of their T_m values. Stabilization of C–C and C–T mismatches by **4** is consistent with that a monomeric form **1b** stabilize cytosine and thymine bulges. A complete failure of stabilizing the thymine–thymine mismatch by **4** implies that the stabilization of thymine–thymine mismatch needs a different strategy from that used for the G–G and C–C mismatches, although we have so far succeeded in stabilizing base mismatches by utilizing a dimeric form of bulge-stabilizing molecules.

In conclusion, ureidoquinolines were found a good molecular element for stabilizing single cytosine and thymine bulges. Integration of this binding element into its dimeric form provides a molecule stabilizing cytosine–cytosine and cytosine–thymine mismatches.

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