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Oligo-2′-deoxyribonucleotides Containing Uracil Modified at the 5-Position with Linkers Ending with Guanidinium Groups

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The possibility of using modified oligonucleotides (ODNs) for diagnostic and therapeutic applications is now well established.[1−3] Numerous modifications have been developed to improve cell-specific delivery, cellular-uptake efficiency, intracellular distribution, stability against nuclease degradation, binding affinity and specificity of ODNs, and finally target inactivation. One way to increase duplex and triplex stabilities is to reduce the overall negative charge number of modified ODNs to reduce the negative charge-to-charge repulsion between the two or three strands of the complexes. Several approaches have included the introduction of either positive permanent charges or those that can be generated by the protonation of amino groups at suitable pH.[4] Among them, the use of a guanidinium group ($pK_a \approx 12.5$) introduces a positive charge over a wide pH range. ODNs containing internucleotidic guanidinium linkages in place of phosphate groups form duplex and triplex structures with DNA or RNA that are much more stable at physiological ionic strength than the corresponding unmodified structures.[5] Since the guanidinium group is planar and thus capable of inducing directionality in H-bonding interactions, nucleobase analogues 4-guanidinium-pyrimidines and 4-guanidinium-2-pyrimidinone, were designed to mimic the double hydrogen bonding of protonated cytosines.[6−7] More recently, it was shown that guanidinylation of the aminoethoxy linker of the tricyclic analogue G-clamp provided the CG base pair with a fifth hydrogen bond.[8]

We report here duplex and triplex stabilization using ODNs containing 2′-deoxyuridines, modified at the 5-position by linkers ending with either two or one guanidinium groups. One or two modified 2′-deoxyuridines, involving either two (dU^{BAG}) or one (dU^{PG}) guanidinium groups, were incorporated into pyrimidine strands, and their influence on the stability of the duplex (with both DNA and RNA targets) and triplex structures was studied. The effect of the presence of modified nucleosides containing guanidinium groups (dU^{BAG}) and (dU^{PG}) was also compared to that of modified nucleosides involving either two (dU^{BA}) or one (dU^{p}) primary amino functions at the same positions inside the sequences. The structures of the modified nucleosides are given in Chart 1, and the ODN sequences are listed in Chart 2. 5-[(*N*,*N*-bis-Aminoethyl)-3-amino-propynyl]2′-deoxyuridine (dU^{BA}) was obtained by a Sonogashira reaction between 5-iodo-2′-deoxyuridine and bis-trifluoroacetylated bis-aminoethyl-3-aminopropyne. 5-(1-Propargylamino)-2′-deoxyuridine (dU^{p}) was synthesized as described in the literature. Then (dU^{BA}) and (dU^{p}) were incorporated, at one or two positions, into the ODNs via phosphoramidite chemistry. The modified ODNs containing the guanidinium derivatives (dU^{PG}) and (dU^{BAG}) were obtained by treatment of the ODNs containing (dU^{p}) and (dU^{BA}) with 1H-pyrazole-1-carboxamidine hydrochloride.[9] All of the modified ODNs **1**−**8** were purified by chromatography and characterized by ESI mass spectrometry.

ODNs **1**−**9** were hybridized with the complementary single-stranded DNA target **10** or RNA target **11** and the target duplex **12** + **13** (see Chart 2 and Table 1). The thermal stability of the resulting duplexes and triplexes was studied by absorption spectroscopy. When modified ODNs were hybridized with the single-stranded DNA **10** and RNA **11** targets, in all cases, an increase in stability was observed as compared to that of the unmodified duplexes **9** + **10** and **9** + **11** and the T_m values observed with the RNA target were higher than those obtained with the DNA target. Studies with DNA single-stranded target **10** and ODNs involving one or two modified 2′-deoxyuridines showed that stability was dependent on the number of modifications included in the sequences. The binding specificity of modified ODNs **1**, **3**, **5**, and **7** involving a single modified nucleobase located near the 3′-end of the sequence was verified by hybridization with ODNs **14** and **15** involving C and G, respectively, in place of A present in the fully complementary sequence **10** (Table 4 in Supporting Information). A comparison of the T_m values obtained for modified ODNs **2**, **4**, **6**, and **8** involving two modified 2′-deoxyuridines and either the single-stranded DNA or RNA targets gave different results, depending on the modified nucleosides present in the ODNs. When ODN **6** containing one amino group per modified nucleoside was used, nearly equivalent stabilization was observed with each target.

Chart 1. Structures of the Modified 2′-Deoxyuridines

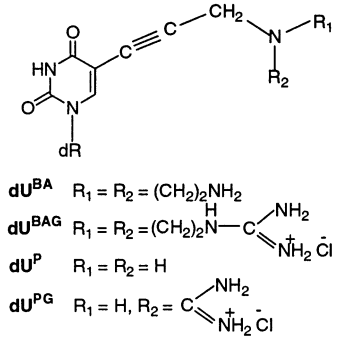

Chart 2. Oligonucleotides Used for These Studies

1 d-5′TTCTTTTTCTTCTCTU^{BA}T3′
2 d-5′TTCTTU^{BA}TTCTTCTCTU^{BA}T3′
3 d-5′TTCTTTTTCTTCTCTU^{BAG}T3′
4 d-5′TTCTTU^{BAG}TTCTTCTCTU^{BAG}T3′
5 d-5′TTCTTTTTCTTCTCTU^{P}T3′
6 d-5′TTCTTU^{P}TTCTTCTCTU^{P}T3′
7 d-5′TTCTTTTTCTTCTCTU^{PG}T3′
8 d-5′TTCTTU^{PG}TTCTTCTCTU^{PG}T3′
9 d-5′TTCTTTTTCTTCTCTTT3′

10 d-5′CTCAAAGAGAAGAAAAAGAACT3′
11 r-5′CUCAAAGAGAAGAAAAAGAACU3′

12 d-5′CTTCCAAGAGAAGAAAAAGAAGAGAAACTAGA3′
13 d-3′GAAGGTTCTCTTCTTTTTCTTCTCTTTGATCT5′

14 d-5′CTCA*C*AGAGAAGAAAAAGAACT3′
15 d-5′CTCA*G*AGAGAAGAAAAAGAACT3′

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Table 1. Melting Temperatures for Duplexes and Triplexes

	duplexes[a]				triplexes[b]	
	target **10** (DNA)		target **11** (RNA)		target **12** + **13**	
ODNs	T_m	ΔT_m/mod	T_m	ΔT_m/mod	T_m	ΔT_m/mod
1	41.5	+2.5	−	−	26	+2
2	44.5	+2.75	53.5	+3	28	+2
3	42.3	+3.3	−	−	27	+3
4	45.5	+3.25	55.5	+4	28.5	+2.25
5	41	+2	−	−	25.3	+1.3
6	42.5	+1.75	51.5	+2	27	+1.5
7	43	+4	−	−	26.5	+2.5
8	47	+4	54.5	+3.5	28.5	+2.25
9	39	−	47.5	-	24	−

[a] 1 μM concentrations in ODNs (each strand) in a 10 mM sodium cacodylate buffer, pH 7, containing 100 mM NaCl. [b] 1 μM concentrations in duplex target and 1.5 μM in the third strand in a 10 mM phosphate buffer, pH 7, containing 140 mM KCl and 5 mM $MgCl_2$. ΔT_m/mod is ±1 °C for modified ODNs **1**, **3**, **5**, and **7** and ±0.5 °C for modified ODNs **2**, **4**, **6**, and **8**.

Table 2. Thermodynamic Parameters for Duplex Formation[a]

duplexes	ΔH (kcal·mol^{-1})	ΔS (cal·mol^{-1}·K^{-1})	$\Delta G^{37°C}$ (kcal·mol^{-1})
2 + **10**	−111.5	−322.1	−11.6
4 + **10**	−110.2	−316.8	−12
9 + **10**	−105.4	−308.1	−9.8
2 + **11**	−142.6	−406.9	−16.4
4 + **11**	−166.3	−477	−18.4
9 + **11**	−126.9	−366.7	−13.2

[a] Parameters were derived from T_m^{-1} vs log [C_m] plots. Duplex concentrations were [C] = 10^{-6} M, 2.5 × 10^{-6} M, 5 × 10^{-6}M, and 10^{-5} M in a 10 mM sodium cacodylate buffer, pH 7, containing 100 mM NaCl. Errors in ΔH and ΔS are estimated as ±10%.

A slight increase in stability was observed with ODN **2** containing two amino functions per nucleoside, and the strongest stabilization was obtained with ODNs **8** and **4** containing, respectively, one or two guanidinium groups per modified nucleoside. These results indicate that the presence of positive charges in the major groove of the duplexes has a strong stabilizing effect on the duplex structures in opposition to a literature report concerning similar "zwitterionic" DNA.[10] The thermodynamic parameters of the duplex formation for the modified ODNs **2**, **4**, and the natural ODN **9** with both the DNA (ODN **10**) and the RNA (ODN **11**) targets were determined by a plot of $1/T_m$ versus log[C_m]. Comparison of the data (Table 2) clearly shows an enthalpic advantage and an entropic disadvantage for duplex formation with the modified ODNs over the unmodified ODN with both the DNA and the RNA targets. The changes of ΔH and ΔS are compensatory, and the calculated $\Delta G^{37°C}$ of the duplex formation is in favor of a higher stability of the modified duplex as expected from the T_m values. The influence of the NaCl (0.1−0.75 M) concentration on duplex stability was studied for the modified ODN **4** and reference **9** hybridized with the DNA target **10**. For modified ODN **4**, as expected due to the presence of positively charged substituents, the increase in stability, when ionic strength increases, is less than that obtained with the unmodified ODN **9** (ΔT_m = 12 °C in the case of the modified duplex and ΔT_m = 15 °C in the case of the unmodified duplex).

Melting studies of the triplexes were performed in salt concentration and pH that approach physiologic. Of the two transitions observed during the melting of each triplex (data not shown) the one with higher T_m value (around 63 °C), the same for all complexes, corresponded to the melting of the target duplex **12** + **13**, and the one with lower T_m's, to the dissociation of the third strand. In all cases, the presence of modified nucleosides in the third strand of the triple helices had a stabilizing effect. However, the stabilization is less important than in the case of duplex formation. As observed for the duplexes, the presence of two amino groups per modified nucleoside was also more efficient than the presence of only one in stabilizing triplex structures. However, the presence of the guanidinium groups did not seem to be more efficient than the presence of the amino groups. This is probably due to a problem of steric hindrance. Our results confirm that the reduction of the global negative charge number on one strand is an important parameter in stability of duplex and triplex structures. Future work will be aimed at the preparation of other ODN sequences to verify the properties of these new modified nucleosides and at the characterization of the interactions involved in such modified duplex and triplex structures.

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Supporting Information Available: Synthesis and characterization of new monomer synthon dU^{BA}, mass spectrometry data for modified ODNs **1**−**8**, and data concerning the binding studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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