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Modulation of *i*-motif thermodynamic stability by the introduction of UNA (unlocked nucleic acid) monomers

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ABSTRACT

The influence of acyclic RNA derivatives, UNA (unlocked nucleic acid) monomers, on *i*-DNA thermodynamic stability has been investigated. The 22 nt human telomeric fragment was chosen as the model sequence for stability studies. UNA monomers modulate *i*-motif stability in a position-depending manner. The largest destabilization is observed for position C14, while UNA placed in position A12 causes significant increase of *i*-DNA thermodynamic stability. CD curves of UNA-modified variants imply no structural changes relative to the native *i*-motif.

C-rich oligonucleotides may adopt *i*-motif structures (*i*-DNA) with a core based on intercalation of C–C$^+$ base pairs.[1] One of the cytosine moieties of each base pair must be protonated to form H-bonds (Fig. 1a), which requires acidic pH of the solution. *i*-DNA structures are characterized by different intercalation and folding topologies depending on the sequence. In general the core is formed by two parallel, right-handed DNA duplexes containing C–C$^+$ hemiprotonated base pairs intercalating with each other by head-to-tail interactions.[1,2] C-rich fragments were found in natural sequences of telomeric ends, centromers, introns, and satellite DNA suggesting them to be important for regulation of biological processes.[3,4]

Modified *i*-motif structures are promising tools applicable in nanotechnology, diagnostics and therapeutics.[2,5–7] Knowledge about *i*-motif structural changes induced by various chemical modifications is therefore important towards design of new therapeutics or nanodevices with desired properties, and studies of the influence of different chemical modifications on an *i*-motif structure are warranted.

One of the best known natural *i*-motif sequence is the 22 nt fragment of human telomeric DNA (HT DNA) with the sequence 5′-d[(CCCTAA)$_3$CCCT]. It was found that this *i*-DNA fragment constitutes the 5′-tail at the telomere end of chromosomes in the S phase of replication and directly correlates with the proliferative rate of human cell cultures.[8–10] According to NMR investigations the C-rich HT DNA fragment folds intramolecularly.[11] The core of this *i*-motif consists of six hemiprotonated C–C$^+$ base pairs with the outer 2′-deoxycytidine situated at the 5′-end of each stretch resulting in 5′E (5′ extremity) intercalation topology (Fig. 1b). Moreover, the core is connected by an internal TAA loop placed in the narrow groove and two external TAA loops bridging the wide grooves.

We determined the thermodynamic stability of 22 nt UNA-modified C-rich fragments of HT DNA. UNA (unlocked nucleic acid) (Fig. 2) is an acyclic RNA analogue lacking the C2′–C3′ bond within the ribose moiety. The flexible UNA monomers are useful tools to modulate the stability of duplexes. In general, UNA monomers significantly decrease duplex stability, but are able to either increase or decrease base-pairing specificity depending on their positioning

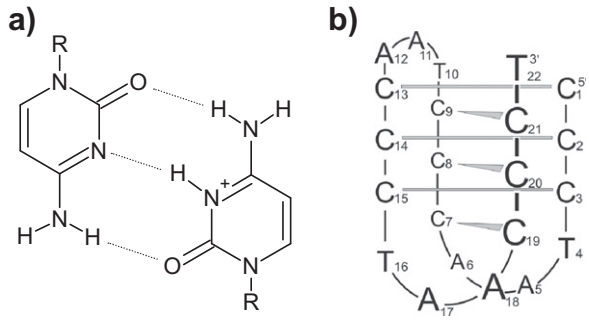

Figure 1. Characteristics of the HT DNA *i*-motif: (a) hemiprotonated C–C$^+$ base pair, (b) structure of intramolecular human telomeric DNA *i*-motif with 5′E intercalation topology.

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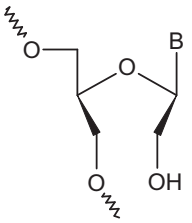

Figure 2. Structure of an unlocked nucleic acid (UNA) monomer.

within a duplex.[12–14] It has furthermore been reported that UNA-modified oligonucleotides are compatible with RNase H activity[15,16] and that strategic UNA modifications improve gene silencing specificity of siRNA constructs.[17–21] UNA monomers were found as efficient modulators of quadruplex thermodynamic stability being able to change the binding affinity and biological properties of a quadruplex-based thrombin binding aptamer (TBA) and improve its potency to inhibit fibrin-clot formation.[22]

The 22 nt fragment of human telomeric DNA was modified by single UNA substitution in various positions: two positions of the core (terminal C1 and internal C14), all positions of loops, and the terminal position resulting in twelve different UNA modified variants (Table 1). Names of modified HT DNA variants are marked by underlined italic font to differentiate from UNA monomer position within an *i*-motif variant. The thermodynamic stability of these sequences was studied by the UV melting method. Measurements were performed in two buffers with different pH values (4.8 and 5.6) to evaluate pH-dependence of the stability which is characteristic for an *i*-motif structure.[1] Moreover the melting curves were recorded at two different wavelengths (295 and 265 nm). Denaturation of the samples at 295 nm resulted in hypochromic changes while at 265 nm in hyperchromic changes, which are in accordance with typical *i*-motif behavior.[23] The longer wavelength was included in this study in accordance with previous experiments which indicated measurements at 295 nm as optimal to study the process of *i*-motif thermal unfolding.[23–25] The thermodynamic parameters obtained from melting curves recorded at the two different wavelengths at pH 5.6 were identical within the limits of error whereas the ones derived from denaturation profiles measured at pH 4.8 showed higher variability (Supplementary data). Nevertheless, the data obtained at pH 4.8 and 5.6 reveal the same major trends ($\Delta\Delta G^{\circ}_{37}$). However, the lower accuracy of measurements performed at pH 4.8 suggests that the conditions are suboptimal for studies of thermodynamic stability of UNA-modified HT DNA, and we therefore decided to limit the discussion to the results obtained at pH 5.6.

As expected, UNA monomers placed in position C1 or C14 cause significant loss of thermodynamic stability ($\Delta\Delta G^{\circ}_{37}$ = 1.92 and 3.30 kcal/mol, respectively; Table 1). Additionally, positive values of Gibbs' free energies of those two variants indicate that at pH 5.6 and at physiological temperature the predominant species is the unfolded form. The destabilization is most probably due to disruption of interactions within an *i*-motif core which are essential for structure formation which is confirmed by more significant destabilization of *i*-DNA modified by UNA-C at the internal position (C14) of the core than at the terminal position (C1). Moreover, this behavior is in accordance with previously reported data showing that in general the influence of various modifications on thermodynamic stability of duplexes and quadruplexes decreases when shifting a modification towards oligonucleotide ends.[26–28] Analysis of enthalpy-entropy compensation for *C1* and *C14* variants also imply distortion of *i*-motif core. In both cases unfavorable enthalpy changes dominate over favorable entropy effects suggesting disruption of hydrogen bonding and/or *i*-motif hydration.

In contrast, any UNA modification of the internal loop is stabilizing. Thermodynamic stability of UNA-modified *i*-DNA increases in the order *U10* < *A11* < *A12*. The lower stability of the *U10* and *A11* variants relative to *A12* is presumably due to structural differences. It was reported that T10 in the unmodified HT DNA stacks on the *i*-motif core, while A11 is situated above and also involved in stacking interactions.[11] On the contrary, A12 is positioned outward relative to the core. Moreover, it was suggested that hydrogen-bonding is present between T10 and T22. H-bonds across water molecules are furthermore observed between A11 and T22. The increased flexibility introduced by UNA in position U10 or A11 therefore likely causes perturbation in stacking interaction and/or hydrogen bond formation resulting in the overall gain of thermodynamic stability being lower than for A12. Analysis of enthalpy and entropy terms confirms the above hypothesis since *U10* and *A11* are characterized by less favorable ΔH° and more favorable ΔS° relative to *A12*. The increased stability of *U10*, *A11*, and *A12* variants relative to unmodified HT DNA is presumably due to improved stacking and hydrogen bonding since the modified variants are characterized by more favorable enthalpy and unfavorable entropy values. The pronounced flexibility of the acyclic UNA monomers may be ideal for obtaining stabilized conformations and in consequence makes UNA a new member of extremely narrow group of modifications stabilizing i-motif structures such as PNA,[29–31] LNA,[2] 3′-*S*-phosphorothiolates[32,33] or 2′-deoxy-2′-fluorocytidine.[34]

Table 1
Thermodynamic parameters of i-motif formation of the 22 nt fragment of human telomeric DNA with single UNA (**X**) substitutions[a]

Name	Sequence	Average of curve fits—295 nm					
		$-\Delta H^{\circ}$ (kcal/mol)	$-\Delta S^{\circ}$ (eu)	ΔG°_{37} (kcal/mol)	T_M (°C)	$\Delta\Delta G^{\circ}_{37}$ (kcal/mol)	ΔT_M (°C)
HT DNA	d(CCCTAACCCTAACCCTAACCCT)	68.9 ± 1.1	216.7 ± 3.5	−1.73 ± 0.02	45.0	0	0
C1	d(**C**CCTAACCCTAACCCTAACCCT)	61.7 ± 1.9	199.5 ± 6.2	+0.19 ± 0.07	36.1	1.92	−8.9
C14	d(CCCTAACCCTAAC**C**CTAACCCT)	58.2 ± 3.1	192.8 ± 10.3	+1.57 ± 0.08	28.9	3.30	−16.1
U4	d(CCC**U**AACCCTAACCCTAACCCT)	72.1 ± 1.3	226.4 ± 4.2	−1.92 ± 0.09	45.5	−0.19	0.5
A5	d(CCCT**A**ACCCTAACCCTAACCCT)	69.5 ± 1.0	219.8 ± 3.0	−1.36 ± 0.04	43.2	0.37	−1.8
A6	d(CCCTA**A**CCCTAACCCTAACCCT)	70.4 ± 0.7	222.4 ± 2.1	−1.43 ± 0.02	43.4	0.30	−1.6
U10	d(CCCTAACCC**U**AACCCTAACCCT)	75.3 ± 1.0	235.6 ± 3.1	−2.25 ± 0.04	46.5	−0.52	1.5
A11	d(CCCTAACCCTA**A**CCCTAACCCT)	80.8±0.7	251.8 ± 2.2	−2.67 ± 0.04	47.6	−0.94	2.6
A12	d(CCCTAACCCTAA**A**CCCTAACCCT)	84.0 ± 0.7	259.7 ± 2.0	−3.40 ± 0.03	50.1	−1.67	5.1
U16	d(CCCTAACCCTAACCC**U**AACCCT)	74.4 ± 1.0	233.3 ± 3.3	−2.03 ± 0.04	45.7	−0.30	0.7
A17	d(CCCTAACCCTAACCCT**A**ACCCT)	73.9 ± 1.1	232.6 ± 3.4	−1.73 ± 0.03	44.4	± 0.00	−0.6
A18	d(CCCTAACCCTAACCCTA**A**CCCT)	72.1 ± 1.5	227.4 ± 4.8	−1.54 ± 0.02	43.8	0.19	−1.2
U22	d(CCCTAACCCTAACCCTAACCC**U**)	75.9 ± 0.8	238.8 ± 2.4	−1.78 ± 0.02	44.4	−0.05	−0.6

[a] Solution: 100 mM KCl, 10 mM sodium cacodylate, pH 5.6.