Remarkably Stable Parallel-Stranded Oligonucleotides Containing 5-Methylisocytosine and Isoguanine

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The polymorphic nature of double-stranded DNA is well established.[1] A common feature of three major families of A-, B-, and Z-DNA duplexes is the antiparallel orientation of the constituent strands. Parallel-stranded DNA (ps-DNA) is a new family of DNA conformations experimentally confirmed in 1988, in which complementary strands have the same 5′-3′ orientation and form reverse Watson−Crick A-T base pairs.[2] Numerous thermodynamic studies revealed that the parallel-stranded duplexes melt with considerably lower transition temperatures than the corresponding antiparallel duplexes.[2] Thus, 3′-p-3′- or 5′-p-5′-linked hairpins or relatively long oligomers such as 25-mers have been employed to investigate the thermodynamic behavior and reactivities of ps-DNA.[3] We reasoned that incorporation of 2′-deoxy-5-methylisocytosine (iC) or 2′-deoxyisoguanine (iG) as a new artificial base would stabilize parallel-stranded duplexes by forming three hydrogen bonds with guanine and cytosine, respectively.[4] We now report herein that iC- and iG-containing oligomers can form a parallel-stranded duplex of a comparable stability with a normal antiparallel duplex even at a decamer level.

PM3 semiempirical calculations in the gas phase suggested that both iC-G (−12.0 kcal) and iG-C (−13.7 kcal) base pairs are more stable than a reverse Watson−Crick A-T base pair (−4.6 kcal) and should possess stability comparable with a Watson−Crick G-C base pair (−11.9 kcal) (Figure 1).[5] Thus, iG- and iC-containing deoxydecanucleotides (ODN I−III) were synthesized according to the standard β-cyanoethyl phosphoramidite chemistry on a DNA synthesizer (Table 1).[6] The β-cyanoethyl phosphoramidites of protected iC and iG were prepared according to the reported procedure.[7] After standard deprotection with concentrated ammonia, the oligomers were purified by reverse phase HPLC. Concentration and composition of oligomers were determined by complete digestion of oligomers with snake venom phosphodiesterase and bacterial alkaline phosphatase to mononucleosides.

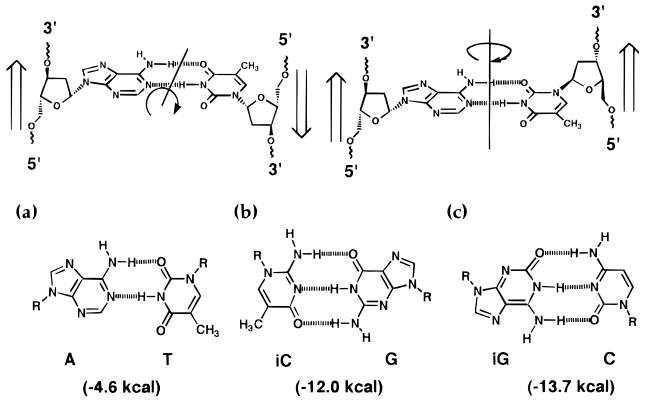

Figure 1. Schematic representation of antiparallel orientation of Watson−Crick (W-C) A-T base pair (upper left) and parallel-oriented reverse W-C A-T base pair (upper right). The 2-fold symmetry axes of sugar−phosphate backbone are indicated. Reverse W-C A-T base pair (a) and the putative iC-G (b) and iG-C base pair (c). Numbers in parenthesis are energies of base pair formation using PM3 semiempirical calculation. The energies of base pair formation are calculated by subtracting the energies of the geometry-optimized *N*-methylated base pair from the energies of the individual geometry-optimized *N*-methylated bases as reported procedure by Kollman *et al*.[5]

In order to investigate duplex formation, UV spectra of the mixture of ODN I and 5′-d(ACGTGCCTGA)-3′, which can form full-matched base pairs when aligned in a parallel orientation, were examined at various temperatures. As shown in Figure 2a, the mixture exhibited a thermally induced hyperchromicity. The 1:1 stoichiometry for complex formation of each strand was confirmed by UV mixing curve experiments.[8] Figure 2b shows a thermal denaturation profile of ODN I and 5′-d(ACGTGCCTGA)-3′ (p-1) together with p-2 and p-3, showing a cooperative UV melting behavior from base-stacked ps duplex to a single strand. A further indication for the ps-DNA duplex was obtained from CD spectrum of 1:1 mixture of ODN I and 5′-d(ACGTGCCTGA)-3′ as shown in Figure 2c. In accord with the previous observation,[2,3] the CD of p-1 showed a typical negative maximum dichroism near 260 nm, very similar to that observed for antiparallel duplexex.

In order to investigate the recognition ability of iC- and iG-containing oligomers toward single-stranded DNA or RNA oligomers, hybridization properties of various oligomers were next examined. The oligomer sequences and the melting temperatures for duplex formation are listed in Table 1. The thermal denaturation data demonstrated that iC- and iG-containing ps-DNAs (p-1, p-2, and p-3) have comparable stabilities or a slightly weaker stability compared with the corresponding parent antiparallel (ap) duplexes (ap-1, ap-2, and ap-3), respectively. Natural oligomers that would have to form a parallel-stranded complex did not show any sign for parallel strand formation even under high salt conditions (c-1, c-2, and c-3).

The data also show a high specificity for iG-C and iC-G base pairs. A remarkable T_m decrease was observed for a single mismatch opposite iC or iG ranged from 24 to 8 °C either at low-salt or high-salt conditions (pm-1−pm-6). It was also found that ODN I−III form ps-DNA-RNA hybrids (ph-1, ph-2, and ph-3) of comparable stabilities with those of normal antiparallel DNA-RNA hybrids (aph-1, aph-2, and aph-3).

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Table 1. Melting Temperatures of Parallel- and Antiparallel Duplexes[a]

		T_m (°C)				T_m (°C)	
duplex	sequence	at low salt[b]	at 1.0 M NaCl	duplex	sequence	at low salt[b]	at 1.0 M NaCl
p-1	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT G C CT GA)-3′	25	46	p-2	5′-d(TAiCAiCiGiGAiCT)-3′ (ODN II) 5′-d(AT GT G C CT GA)-3′	14	45
ap-1	5′-d(ACGTGCCTGA)-3′ 3′-d(TGCACGGACT)-5′	25	56, 55[d]	ap-2	5′-d(ATGTGCCTGA)-3′ 3′-d(TACACGGACT)-5′	25	53, 44[d]
c-1	5′-d(ACGTGCCTGA)-3′ 5′-d(TGCACGGACT)-3′	nd[c]	36[e]	c-2	5′-d(ATGTGCCTGA)-3′ 5′-d(TACACGGACT)-3′	nd[c]	nd[c]
ph-1	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-r(A C GU G C CU GA)-3′	24	50	ph-2	5′-d(TAiCAiCiGiGAiCT)-3′ (ODN II) 5′-r(AU GU G C C U GA)-3′	14	48
aph-1	5′-r(ACGUGCCUGA)-3′ 3′-d(TGCACGGACT)-5′	23	53, 52[d]	aph-2	5′-r(AUGUGCCUGA)-3′ 3′-d(TACACGGACT)-5′	22	49, 47[d]
pm-1	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT G A CT GA)-3′	nd[c]	37 (−9)[f]	p-3	5′-d(TiGAAiCiGiGAiCT)-3′ (ODN III) 5′-d(ACTT G C C T GA)-3′	18	50
pm-2	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT G G CT GA)-3′	nd[c]	38 (−8)[f]	ap-3	5′-d(ACTTGCCTGA)-3′ 3′-d(TGAACGGACT)-5′	20	52, 49[d]
pm-3	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT G T CT GA)-3′	nd[c]	33 (−13)[f]	c-3	5′-d(ACTTGCCTGA)-3′ 5′-d(TGAACGGACT)-3′	nd[c]	nd[c]
pm-4	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT A C CT GA)-3′	nd[c]	26 (−20)[f]	ph-3	5′-d(TiGAAiCiGiGAiCT)-3′ (ODN III) 5′-r(ACUUG C CUGA)-3′	14	50
pm-5	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT C C CT GA)-3′	nd[c]	22 (−24)[f]	aph-3	5′-r(ACUUGCCUGA)-3′ 3′-d(TGAACGGACT)-5′	17	46, 45[d]
pm-6	5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I) 5′-d(A C GT T C CT GA)-3′	nd[c]	32 (−14)[f]				

[a] Thermal denaturation profiles were obtained under the conditions described in Figure 2. Melting temperatures (T_m) were calculated as the maximum in a plot of $\Delta Abs_{260}/\Delta T$ *vs* temperature. The mismatch base pairs are shown in an open font. The abbreviations are as follows: p, parallel DNA-DNA duplex; ap; antiparallel DNA−DNA duplex; ph, parallel DNA−RNA hybrid; aph, antiparallel DNA−RNA hybrid; pm, parallel-mismatched DNA−DNA duplex; c, control natural decamers. [b] Measurement at low-salt conditions was conducted in 10 mM sodium phosphate, 1 mM EDTA (pH 7.0). [c] Cooperative melting behavior was not detected. [d] The numbers are the predicted T_m values calculated by using published parameters.[9] [e] The melting behavior is presumably due to the formation of antiparallel partial duplex formation with complementary five base pairs. [f] The numbers in parentheses were the T_m decrease relative to p-1.

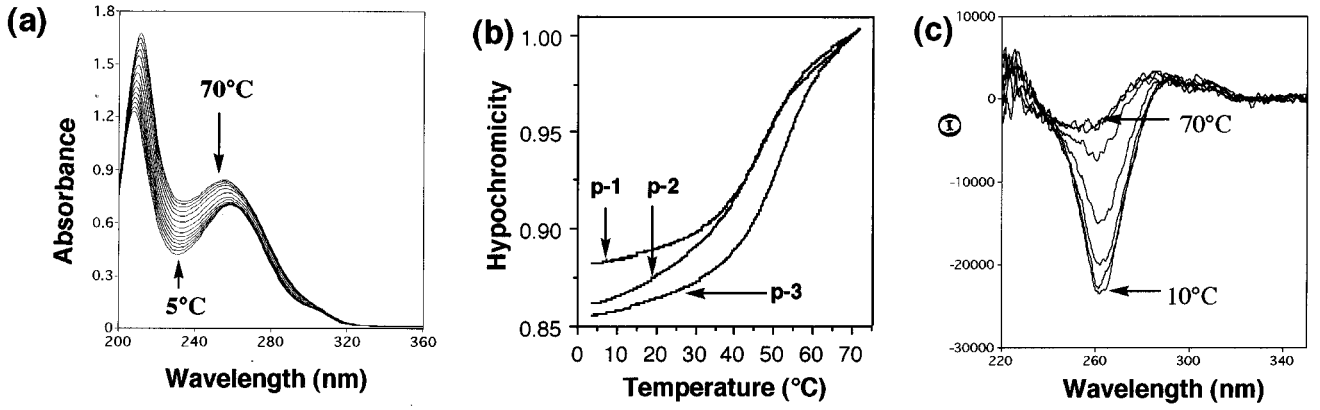

Figure 2. Thermal denaturation profiles of iC- and iG-containing oligomers (0.05 mM base concentration) in the presence of complementary strand (0.05 mM base concentration). Measurements were conducted in 10 mM sodium phosphate buffer containing 1 mM EDTA (pH 7.0) and 1 M NaCl. (a) UV absorbance spectra of 5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I)/5′-d(ACGTGCCTGA)-3′ (p-1) at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70 °C. (b) UV melting profiles of p-1, p-2, and p-3. The absorbance of the oligomers was monitored at 260 nm from 2 to 80 °C with a heating rate of 1 °C per minute. The hypochromicity of the oligomer was obtained from the observed absorbance devided by the absorbance at 70 °C. (c) CD spectra of 5′-d(TiGiCAiCiGiGAiCT)-3′ (ODN I)/5′-d(ACG TGCCTGA)-3′ (p-1) at 10, 20, 30, 40, 50, 60, and 70 °C.

The present studies demonstrated that iC- and iG-containing oligomers can form a remarkably stable parallel-stranded duplex with DNA or RNA oligomers and that both iC and iG bases can effectively discriminate mismatches. The present results also indicate that single-stranded DNA or RNA oligomers can be recognized by iC- and iG-containing oligomers in a parallel orientation. Therefore, parallel strand formation is now added to the repertoire of designed sequence-specific agents in antisense therapeutics. A detailed analysis of the structure of iC- and iG-containing ps duplex by ^{1}H NMR NOE-restrained refinement as well as molecular dynamics calculations is in progress.

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