Modified Triplexes from Oligonucleotides Bridged by Two Cholane-3,24-diol (3α,5β) Units†

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Since the first discovery of triple helix (triplex),[1] it has been suggested that a biologically important three-stranded complex could be constructed from single-stranded RNA and double-stranded DNA. In general, a triplex is formed when a third strand binds in the major groove of a B-form double helix (duplex). The duplex must be composed of a homopurine-homopyrimidine sequence, which represents up to 1% of eukaryotic genomes.[2] Although direct evidence of the participation of triplexes in biological processes has yet to be obtained, a growing body of data suggests that triplexes are involved in the regulation of DNA replication, transcription ("antigene therapy"), recombination, and development.[3] In addition, triplexes have been demonstrated to be stable under physiological conditions and to inhibit various enzymes, including RNA polymerase,[4] DNase, and RNase.[5]

Recently, we synthesized a novel phosphoramidite reagent, 24-*O*-(4,4'-dimethoxytrityl)cholane-3,24-diol-3-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite (3α,5β) (**L**), and used it to develop hairpin oligonucleotides.[6] We became interested in extending this research to the development of stable triple-stranded nucleic acids incorporating two hairpin moieties (Figure 1);[7] such stable triplexes may be useful tools for studying the biological roles and genetic applications of naturally occurring triplexes.

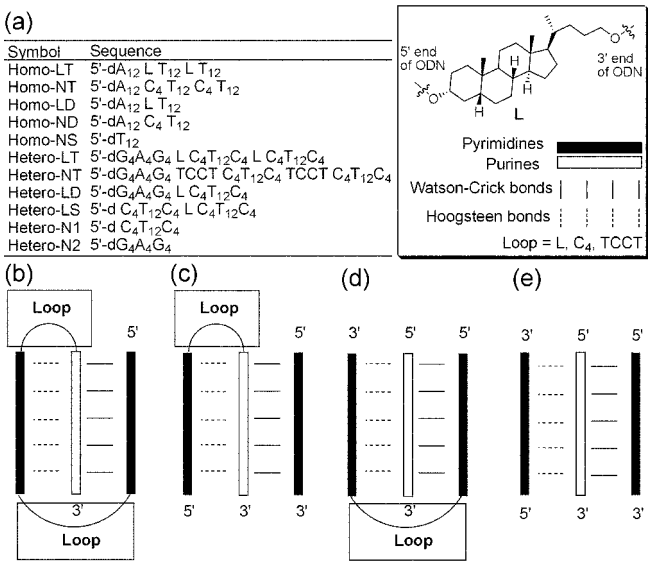

Figure 1. Possible triplex structures of the designed ODNs: (a) Sequences of the synthetic ODNs; (b) **Homo-LT**, **Homo-NT**, **Hetero-LT**; (c) **Homo-LD or Homo-ND/Homo NS**, **Hetero-LD**/**Hetero N1**; (d) **Hetero-LS**/**Hetero-N2**; (e) 2 (**Hetero-N1**)/**Hetero-N2**.

We synthesized the requisite modified oligodeoxynucleotides (ODNs) with high coupling efficiencies using an automated DNA synthesizer (Figure 1a) and confirmed their successful syntheses through MALDI-TOF mass spectral analysis. The ODNs **Homo-LT**, **Homo-NT** and **Hetero-LT** are palindromers containing two hairpin moieties; we expected that these modified ODNs might form triplexes that feature intramolecular hydrogen bonding patterns. Table 1 summarizes the melting temperature data of the ODN systems. In general, the intrastrand triplexes were more stable than the interstrand triplexes and replacement of C_4 hairpin residues by the **L** group provided extra stability to the triplexes. **Homo-LT** and **Homo-NT** exhibit single melting temperatures (entries 1 and 2) that reflect the fact that the melting of the Hoogsteen strands occurs at the same temperature as does that of the Watson-Crick strands (*i.e.*, the strands melt simultaneously). In addition, we note that

Table 1. Melting Temperatures of Synthetic ODNs[a]

Entry	Name	T_m (°C) at pH 5.0	T_m (°C) at pH 6.0	T_m (°C) at pH 7.2
1	**Homo-LT**	–	–	76 (71[b])
2	**Homo-NT**	–	–	69 (62[b])
3	**Homo-LD / Homo N1**	–	–	17, 75 (17[b])
4	**Homo-LS/Homo-N2**	–	–	19, 70 (19[b])
5	**Hetero-LT**[c]	65, 82	73	72
6	**Hetero-LD**[c]	52	70	71
7	**Hetero-LD/Hetero-N1**[c]	46	44, 69	16, 71
8	**Hetero-LS/Hetero-N2**[c]	77	44, 66	34, 53
9	**2(Hetero N1)/Hetero N2**	56 (46[c])	36, 63 (31[c])	63 (35[c])

[a]Values of T_m were determined by measuring changes in absorbance at 260 nm (cuvette, 1-cm path length) as a function of temperature in 10 mM buffer solution [either sodium acetate (pH 5.0), Tris-acetate (pH 6.0), or Tris·HCl (pH 7.2) buffer solution] containing 10 mM of NaCl and 20 mM of $MgCl_2$. The total ODN concentration was 3 μM. The temperature was raised at a rate of 1.0 °C/min. [b]The data were obtained by monitoring at 284 nm. [c]The buffer solution was prepared without NaCl or $MgCl_2$ and the solution was monitored at 260 nm.

†Dedicated to Professor Yong Hae Kim for his distinguished achievements in organic chemistry.

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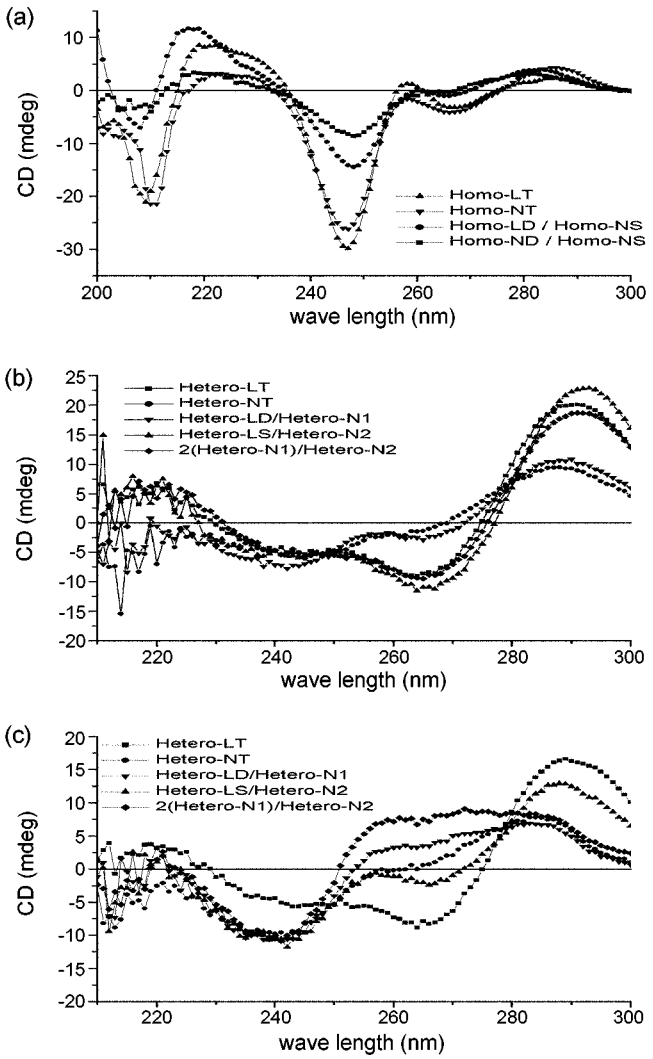

Figure 2. CD spectra of ODNs recorded at 260 nm in buffer solutions at (a) pH 7.2 (in 10 mM NaCl and 20 mM $MgCl_2$), (b) pH 5 [same buffer as (a)], and (c) pH 7.2 (in the absence of NaCl and $MgCl_2$). The conditions are described in Table 1. All spectra were recorded at 10 °C.

Homo-LT has higher values of T_m (measured at both 260 and 284 nm) than does **Homo-NT**. In the cases of the hetero-sequences (entries 5 to 9), we observe that the thermal stability of the third strand in the triplex increases as the solution becomes more acidic, consistent with the fact that protonated C more effectively binds to major groove of C-G base paring to form triplex.[7b] The strands of **Hetero-LT** melt simultaneously at both pH 6 and 7, but in a stepwise manner at pH 5 (entry 5). By comparing this phenomenon with the results presented in entry 6, we believe that the first melting curve of **Hetero-LT** reflects the melting of the duplex and the second reflects melting of the third strand.[8]

Figure 2 presents the superimposed CD spectra of the triplexes. The appearance of the strong negative CD band (210-220 nm) in Figure 2a indicates the triplex formation. The CD spectra of the ODNs having hetero-sequences (Figures 2b-c) are consistent with those obtained in preceding studies.[9] From the electronic effect provided by protonated C, the typical spectra of a nucleic acid duplex in the B conformation shifted slightly toward longer wave-length. In addition, **Hetero-LT** formed its triplex under neutral as well as acidic pH conditions.

In conclusion, we have designed and synthesized intra-strand triplexes using a novel phosphoramidite monomer prepared from lithocholic acid as a structural scaffold upon which we attached the hairpin residues. We have confirmed the nature of the secondary structures of these ODNs by determining their values of T_m and by performing semi-empirical analyses of CD spectroscopic data. These analyses reveal that the modified ODNs form highly stable triplexes through intramolecular hydrogen bonding between their complementary sequences of ODNs. The **L** moiety provides greater stability to the hairpin structure than exists in natural DNA hairpin structures.

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