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Stabilization of the *i*-motif structure by the intra-strand cross-link formation

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ABSTRACT

The *i*-motif structures are formed by oligonucleotides containing cytosine tracts under acidic conditions. The folding of the *i*-motif under physiological conditions is of great interest because of its biological role. In this study, we investigated the effect of the intra-strand cross-link on the stability of the *i*-motif structure. The 4-vinyl-substituted analog of thymidine (T-vinyl) was incorporated into the 5′-end of the human telomere complementary strand, which formed the intra-strand cross-link with the internal adenine. The intra-strand cross-linked *i*-motif displayed CD spectra similar to that of the natural *i*-motif at acidic pH, which was transformed into a random coil with the increasing pH. The pH midpoint for the transition from the *i*-motif to random coil increased from pH 6.1 for the natural one to pH 6.8 for the cross-linked one. The thermodynamic parameters were obtained by measuring the thermal melting behaviors by CD and UV, and it was determined that the intra-strand cross-linked *i*-motif is stabilized due to a favorable entropy effect. Thus, this study has clearly indicated the validity of the intra-strand cross-linking for stabilization of the *i*-motif structure.

Guanine-rich single stranded DNA can form a stable four-stranded DNA second structure, that is, the G-quadruplex (G4-DNA).[1] DNA sequences with the potential to form G4-DNA are frequently found in a genome,[2] such as a telomere[3] and the promoter regions,[4,5] and thought to form functionally relevant G4 DNA structures.[6] A number of ligands have been developed to detect or stabilize the G4 DNA structures.[1] C-rich DNAs are associated with G-rich DNAs as the complementary strands and also form four-stranded DNA helices.[7,8] In this four-stranded C-rich DNA, two parallel duplexes are complexed in an antiparallel fashion by alternatively intercalating hemiprotonated cytosine–cytosine base pairs. This unique intercalated structure, called the *i*-motif, requires acidic pH for its folding due to protonation of one of the cytosines of the base pairs. The open and four-stranded form of the *i*-motif sequences are interchangeable depending on the pH,[9–13] and applied in nanotechnology.[14] In the meantime, the binding ligands and chemical modification that stabilize the *i*-motifs have been investigated to reveal its intracellular existence and biological roles.[15] Nevertheless, only a few studies reported the *i*-motif folding at neutral pH.[16–18] In this study, we

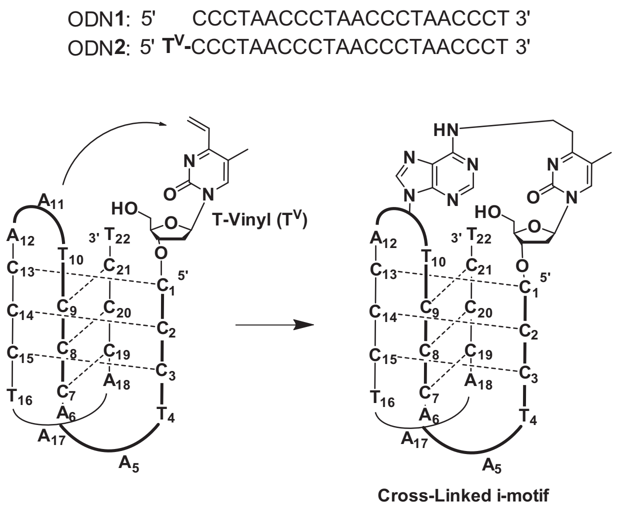

Figure 1. The sequences for the folding of the *i*-motif used in this study and expected intra-strand cross-linking.

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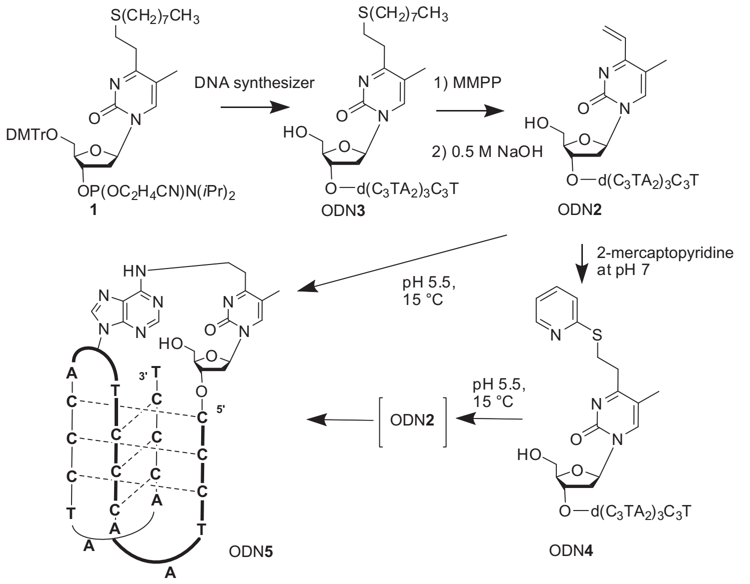

Scheme 1. Synthesis of ODN**2** with T-vinyl, ODN**4** with its 2-thiopyridinyl protection and the formation of the cross-linked ODN**5**.

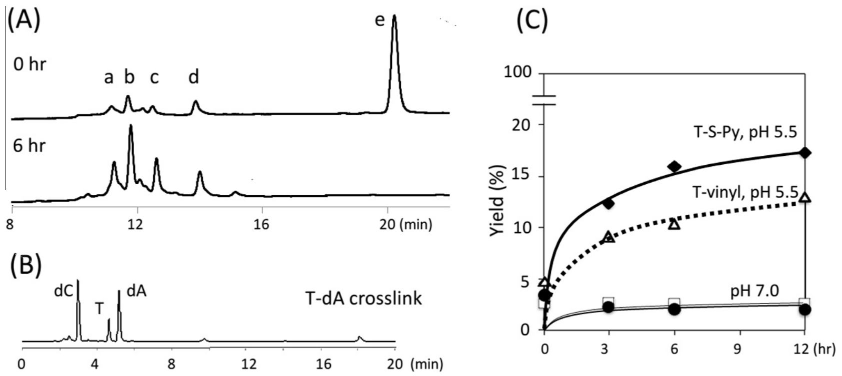

Figure 2. (A) HPLC Charts of the cross-link formation using ODN**4**. (a) non ODN-derived materials, (b) H_2O adduct of ODN**2**, (c) ODN**5**, (d) ODN**2**, (e) ODN**4**. (B) HPLC Chart of hydrolysates of ODN**5**. (C) The time-course of the cross-link reactions. The cross-linking reaction was performed using 10 μM ODN in the buffer containing 10 mM Na cacodylate and 100 mM KCl at pH 5.5 and 15 °C.

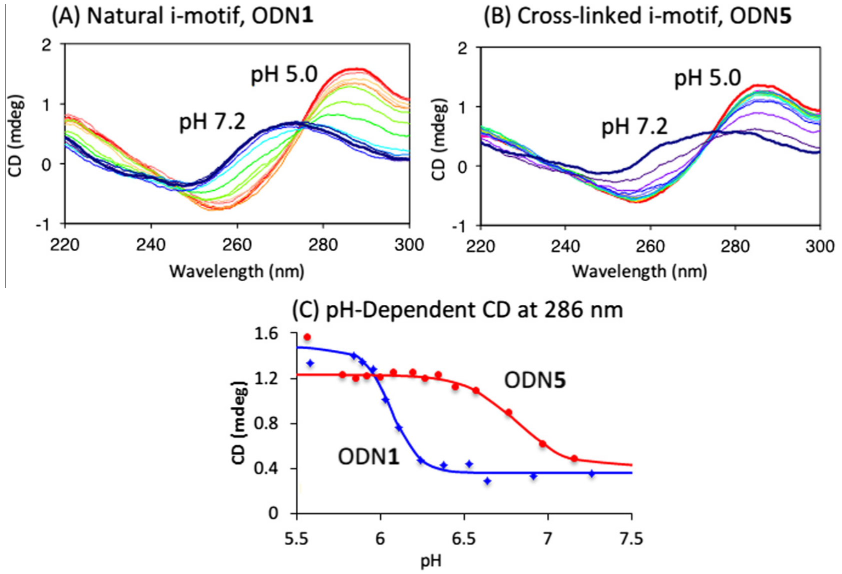

Figure 3. (A) pH-dependent CD of ODN**1**. (B) pH-dependent CD of ODN**5**. (C) pH-dependent CD intensity at 286 nm. CD spectra were measured using 2 μM ODN in 10 mM sodium cacodylate buffer containing 100 mM KCl at 20 °C.