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Formation and Stability of a Janus-Wedge Type of DNA Triplex

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We describe here the formation and stability of a Janus-Wedge (J-W) type of triple helix: a complex formed from two DNA target strands and a third probe strand capable of base pairing with the Watson−Crick faces of the two target sequences.

Conventional DNA triplexes are formed by a third strand binding to the functional groups on the Hoogsteen face of purine residues.[1,2] These binding sites are present in the major groove of duplex DNA and can be used to target specific polypurine sequences using a polypyrimidine[1,2] (forming T-A-T and C^+-G-C base triplets) or polypurine[3] (forming A-A-T and G-G-C base triplets) probe sequence. The disadvantage of the conventional approach is that sequences of polypurines are the sole targets. The challenge to generalized targeting is the intervening pyrimidines within a polypurine sequence; pyrimidine targeting approaches include derivatives that employ only a single hydrogen bond,[4] recognition of each base pair as a unit,[5] or being able to bind a purines in either strand.[6] However, a variety of targeting approaches[7−9] have resulted in only moderate successes.

A Janus-Wedge (J-W) triple helix, a recognition motif first suggested by Lehn[10] in his work with heterocycles, involves the ability of the incoming third strand to hydrogen bond with the Watson−Crick (W−C) faces of the two target strands (see Figure 1a). Two central questions can be posed about this recognition motif: (i) What is the stability of a triplex based upon the triplet structure illustrated in Figure 1a? (ii) Can a J-W triplex be formed by strand invasion of a native W−C duplex? Here we answer the first of those questions.

To examine the formation and stability of a J-W triplex we prepared an 8-mer T + C target site bracketed with 11 Watson−Crick (W−C) base pairs on either side (Figure 1b). The two pyrimidine bases should permit binding by the Janus-Wedge base (W) to both W−C faces forming a series of base triplets such as that illustrated in Figure 1a. A corresponding triplet involving a purine−pyrimidine pair would exhibit a slightly different geometry. In this initial study, formation of the J-W structure does not need to compete with W−C base pairing. For the W residue we used 6-amino-pseudocytidine attached to a peptide nucleic acid (PNA) backbone. The peptide backbone was chosen for two reasons: (i) It is neutral and limits charge−charge repulsion effects. (ii) PNA sequences can bind DNA duplexes by strand invasion,[11] a process that should be advantageous for the targeting of native W−C duplexes.

To prepare the J-W strand, an acetoxy linker was attached to the 5-position of the readily available 2,6-diaminopyrimidin-4-one. The para-exocyclic amino group was protected as the CBZ derivative; the corresponding ortho-amino group proved to be largely unreactive. The 8-mer synthesis started with an L-lysine (K) residue to assist the aqueous solubility of the PNA oligomer as well as to provide some complementary charge−charge interactions between the PNA and target sequence. The product W_8K oligomer was purified by HPLC and identified by MALDI-TOF mass spectrometry.

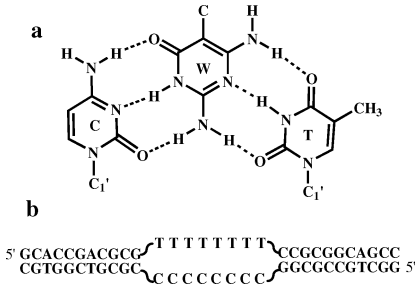

Figure 1. (a) A Janus-Wedge base triplet: the third-strand residue W binds to the W−C faces of both target residues, (b) the $11dC_811-11T_811$ target sequence.

To probe for complex formation, the W_8K oligomer was added to each of the single-stranded target strands as well as to the duplex itself, and the mixtures were analyzed by nondenaturing PAGE (Figure 2). The two DNA single strands are found in Lanes 5 and 6, while in Lanes 1 and 2 one equivalent of the W_8K strand has been added to each. The T_8-target strand is more effectively shifted to a lower mobility species (Lane 2) than is the dC_8-target strand (Lane 1) suggesting more effective binding to the former. Mixing the $11T_811$ and $11dC_811$ strands resulted in a lower-mobility species (Lane 4) interpreted to be the duplex of Figure 1b.

Addition of one equivalent of the W_8K strand to the duplex target resulted in complete shift of the duplex band to a lower-mobility species that we interpret as being the J-W complex (Lane 3).

The gel shift experiment indicates that complex formation occurs, but it cannot clarify the nature of binding. It appears that the W_8K strand binds more effectively to the T_8 strand than the dC_8 strand (compare Lanes 1 and 2 of Figure 2), and in the triplex it could, in principle, bind only to the T_8 strand. We performed a chemical probing experiment with bromide and monoperoxysulfite known[12] to differentiate between single-stranded and double-stranded dC

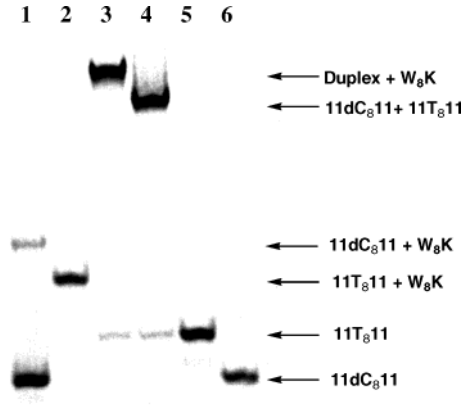

Figure 2. Gel shift assay for W_8K binding to the ss and ds 30-mers of Figure 1b. (Lane 1) $11dC_811 + W_8K$. (Lane 2) $11T_811 + W_8K$. (Lane 3) duplex + W_8K. (Lane 4) $11dC_811 + 11T_811$ duplex. (Lane 5) $11T_811$. (Lane 6) $11dC_811$.

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residues. Treatment of the duplex target with the bromide reagent resulted in cleavage at essentially all of the dC residues of the dC_8 target, but not those present in the duplex regions. After addition of the W_8K strand to the duplex, a second chemical probing experiment indicated that none of the dC residues were susceptible to cleavage (Supporting Information). This experiment indicates that the W_8K strand interacts with the dC_8-target sequence as well as with the T_8-target sequence.

The orientation of the N- and C-termini of the W_8K strand in the three-stranded complex was determined by tethering a phenanthroline residue to the N-terminus of the W_8K strand. After addition of $CuSO_4$ and a thiol this reagent results in cleavage of DNA[13] in the vicinity of the Cu-phenanthroline. The results of this experiment (Supporting Information) indicated that binding occurs with the N-terminus oriented toward the 5′-terminus of the $11dC_811$ strand (and toward the 3′-terminus of the $11T_811$ strand).

The proposed J-W complex results from the W_8K strand entering the double-stranded complex through the major groove of the target base pairs (see Figure 1a). An alternative binding mode would have it enter through the minor groove. Since this initial study uses a homopolymer and the acceptor/donor hydrogen bonding pattern for the W−T interaction is symmetrical, the base pairing orientation must be discerned on the basis of the interactions with the dC strand. We prepared shorter (14-mer) target strands containing either a central dC_8 or $d(C_2iso^mC_4C_2)$ sequence. 5-Methyl-isocytidine (iso^mC) should form three stabilizing hydrogen bonds with the W residue if it enters from the minor groove, but should be destabilized if oriented to enter from the major groove (Figure 3). We could not prepare the fully substituted iso^mC_8 due to acid depyrimidination during assembly, although earlier work[14] reported on the synthesis of iso^mC_{10} sequences. While a mixture of the 8-mer dC_8 the W_8K strand resulted in clearly definable A_{260} vs temperature transition, and a T_m value of 23.3 °C,[15] the $d(C_2iso^mC_4C_2)$ sequence gave no discernible transition, suggesting destabilizing interactions between the W and iso^mC residues. This experiment suggests that the type of triplex formed is similar to that illustrated in Figure 1a with the J-W strand entering from the major groove.

Thermal denaturation of the J-W triplex formed from the duplex target of Figure 1b and the W_8K strand resulted in two transitions (Supporting Information). The midpoint of the first transition occurred near 43 °C, while the midpoint of the second occurred near 70 °C. The latter transition was present in the absence of the W_8K strand and is interpreted and reflecting the helix-to-coil transition for the duplex target. The early transition then reflects the J-W triplex to DNA duplex + W_8K transition. Since these transitions were clearly separated, it was possible to perform a van't Hoff analysis on a series of transitions obtained at varying total concentrations and obtain thermodynamic parameters characterizing the J-W triplex (Table 1).

We examined three different transitions involving the two 8-mer duplexes dC_8−W_8K, T_8−W_8K, and the $11dC_811$−$11T_811$−W_8K triplex. For the three complexes, ΔG values of −7.35, −10.5, and −15.2 kcal/mol, respectively, were obtained. The $\Delta G°_{25}$ values for the two duplexes and the corresponding calculated K_D values are

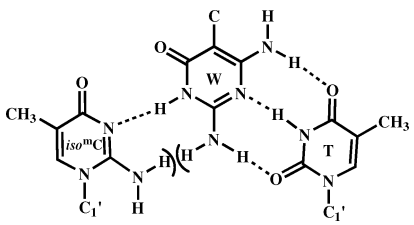

Figure 3. Destabilizing/missing H-bond interactions between W and iso^mC.

Table 1. Thermodynamic Parameters for Complex Formation[a]

	dC_8–W_8K	T_8–W_8K	W_8K triplex
$\Delta H°$ (kcal/mol)	−83.8 ± 0.5	−4.5 ± 0.4	−95.7 ± 1.9
$\Delta S°$ (cal/mol °K)	−256 ± 2	−214 ± 2	−270 ± 6
$\Delta G°_{25}$ (kcal/mol)	−7.35 ± 0.07	−10.5 ± 0.1	−15.2 ± 0.3
K_D[19]	4.2×10^{-6} M	2.0×10^{-8} M	7.4×10^{-12} M

[a] Standard deviations reflect the reproducibilities of the van't Hoff analyses.

consistent with the gel shift data of Figure 2, which indicates that formation of the dC_8−W_8K duplex is less favored than is the formation of the T_8−W_8K duplex. The thermodynamic data indicate differences in stabilities ($\Delta\Delta G \sim 3$ kcal/mol). This difference may reflect strand polarity. To form three interresidue H-bonds in the dC_8−W_8K duplex, the dC_8 strand must adopt the polarity that appears to be less preferred (with the amino terminus of the W_8K strand oriented toward the 5′ terminus of the dC_8 strand). The formation of the triplex occurs with a $\Delta G°_{25}$ of −15.2 kcal/mol, a value that is very similar to the sum of the $\Delta G°_{25}$ values for both duplexes (−17.85 kcal/mol). This thermodynamic analysis confirms that triplex formation by W_8K occurs with binding to both of the DNA target strands. Finally, the magnitude of the stabilization that accompanies J-W triplex formation (absent competing W-C pairing) is substantially greater than conventional DNA triplexes,[16,17] or hairpin triplexes[18] of the same or in some cases longer-sequence lengths.

Experiments related to the second question regarding the ability of related sequences to undergo strand invasion are presently under study. The disruption of a Watson−Crick dA_8·dT_8 target will require an energetic cost of ∼7.1 kcal/mol[20] with a remaining ∼8 kcal/mol of stabilization energy. Effective strand invasion will likely require sequences longer than 8-mers.

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Supporting Information Available: Synthetic schemes and procedures, and a variety of gel and thermodynamic analyses (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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