DNA Triple Helices with C-Nucleosides (Deoxypseudouridine) in the Second Strand

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Formation of three-stranded helices, or triplexes, by nucleic acids is a well-known phenomenon which involves a third strand interacting through the formation of specific H-bonds with one of the two antiparallel strands in a Watson–Crick (W·C) duplex. Because of the stability afforded by making at least two H-bonds in a base-pairing interaction, and because only purines in the W·C base pairs T(U)·A and C·G possess two adjacent H-bonding sites in the major groove of a double helix, formation of triplexes has been limited almost exclusively to sequences wherein all of the purine bases occur on one strand (i.e., homopyrimidine·homopurine duplex + third strand).[1] The most well-characterized triplex is one in which third-strand pyrimidines bind to second-strand purines through the A=T base-pairing geometry first shown by Hoogsteen,[2a] and proposed[2b] and later verified[2c,d] for triplexes having U·A=U and T·dA=T base triads. In stability studies for oligomers of equal length, the third strand in such triplexes typically dissociates from the purine strand before duplex dissociation; i.e., second=third-strand interactions are weaker than W·C-strand interactions. Here we report that homopyrimidine third strands can bind to a W·C 13-mer duplex having four deoxypseudouridine residues in the second strand. Triplex formation is not observed, however, when the second strand contains deoxyuridine at the same residue positions in substitution for this pyrimidine C-nucleoside. In addition, one of the triplexes formed in this system has a thermal stability which is substantially greater than that of the underlying duplex.

Deoxyribonucleoside 13-mers having nonsymmetrical sequences (Figure 1a) were designed to test the ability of deoxypseudouridine (Ψ) to support third-strand binding when contained in a W·C duplex. The first strand ($1T_{pd}$), or target strand, has a phosphodiester backbone and a sequence of thymidines with four noncontiguous deoxyadenosines (A). Antiparallel second strands have methylphosphonate backbone linkages[3] and sequences in which A pairs with T and either Ψ ($2\Psi_{mp}$) or deoxyuridine ($2U_{mp}$) pairs with A in the first strand. Methylphosphonate ($3H_{mp}$) or phosphodiester ($3H_{pd}$) third strands are parallel to 2 strands such that T·A=T and A·Ψ=C base triads may be formed (Figure 1b). The former triad is the well-characterized pyr·pur=pyr (Watson·Crick=Hoogsteen) geometry in which third-strand thymidine residues provide donors at NH3 and acceptors at O4 for acceptor and donor sites at N7 and NH6, respectively, of deoxyadenosine residues in the second strand. In the other base triad, third-strand deoxycytidine residues provide acceptor–donor pairs at N3–NH4 for donor–acceptor

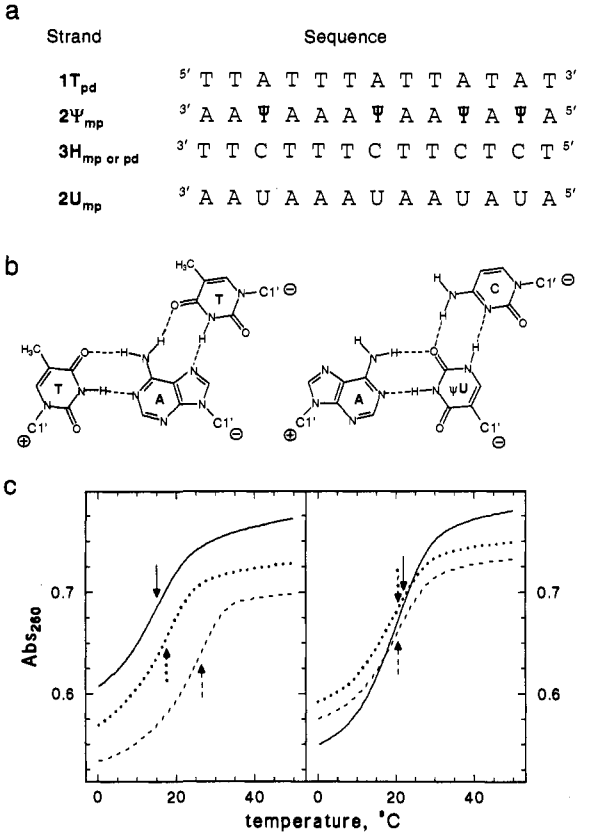

Figure 1. (a) Abbreviations and sequences of the oligomers used in the model system; T (target), Ψ (deoxypseudouridine), U (deoxyuridine), H (Hoogsteen), mp (methylphosphonate) and pd (phosphodiester). (b) H-Bonding schemes for T·A=T and A·Ψ=C base triads; backbone polarities (⊕ = 3′ → 5′, ⊖ = 5′ → 3′) are indicated at the sugar glycosyl carbon, C1′, for each base. (c) UV thermal profiles for mixtures of oligomers at various stoichiometries: (left) $1T_{pd}$:$2\Psi_{mp}$ [3:3] μM (—), $1T_{pd}$:$2\Psi_{mp}$:$3H_{mp}$ [2:2:2] μM (···), $1T_{pd}$:$2\Psi_{mp}$:$3H_{pd}$ [2:2:2] μM (– – –); (right) $1T_{pd}$:$2U_{mp}$ [3:3] μM (—), $1T_{pd}$:$2U_{mp}$:$3H_{mp}$ [2:2:2] μM (···), $1T_{pd}$:$2U_{mp}$:$3H_{pd}$ [2:2:2] μM (– – –). Absorbance at 260 nm *vs* increasing temperature is shown for samples in 0.1 M Na^+ (Cl^-), 0.01 M (Na^+) PO_4^{3-}, 10^{-5} M EDTA, pH 7. Arrows indicate inflection points for each cooperative transition from which T_m's are derived.

sites at NH1–O2 of second-strand deoxypseudouridine residues. Pseudouridine is a minor base which occurs naturally in RNA as a result of highly-conserved, post-transcriptional modification. This C-nucleoside analog differs from standard N-nucleoside uridine residues in that the glycosyl linkage of the pyrimidine base to the sugar occurs at C5 rather than at N1. The H-bonding pattern of Ψ at O4–NH3–O2 (acceptor–donor–acceptor) is identical to that of U at O2–NH3–O4, so that W·C base pairing with adenosine can occur. An extra H-bond donor occurs at NH1 of the Ψ nucleoside in a position isosteric to C5 of U which, by virtue of the pseudodyad symmetry of the T·A and A·Ψ base pairs, will be located in a major groove position of a W·C duplex similar to N7 of A. Therefore, a third strand could potentially bind to A·Ψ base pairs utilizing the donor–acceptor sites at NH1–O2 of Ψ as shown. On the other hand, a double helix containing A and U in the first and second strands will have only one H-bond acceptor at O4 of uridine, in which case a third strand might not be able to associate with the duplex utilizing the single site provided by the standard pyrimidine base.

Thermal profiles of 1:1 $1T_{pd}$:$2\Psi_{mp}$ and $1T_{pd}$:$2U_{mp}$ mixtures (Figure 1c) give helix–coil transition midpoints (T_m's) of 15 and 22 °C, respectively. The $2\Psi_{mp}$ oligomer has a weaker interaction than $2U_{mp}$ with the first strand as evidenced by reduced T_m and base stacking (hypochromicity) in the helical state. The basis of this decrease in stability of the Ψ-containing duplex is

(1) There have been a few reports of triplex formation involving W·C duplexes having one or two pyrimidine insertions within the purine strand; for examples, see: (a) Griffen, L. C.; Dervan, P. B. *Science* **1989**, *245*, 967–971. (b) Griffen, L. C.; Kiessling, L. L.; Beal, P. A.; Gillespie, P.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 7976–7982. (c) Huang, C.-Y.; Cushman, C. D.; Miller, P. S. *J. Org. Chem.* **1993**, *58*, 5048–5049. (d) Huang, C.-Y.; Miller, P. S. *J. Am. Chem. Soc.* **1993**, *115*, 10456–10457.

(2) (a) Hoogsteen, K. *Acta Crystallogr.* **1959**, *12*, 822–823. (b) Felsenfeld, G.; Davies, D. R.; Rich, A. *J. Am. Chem. Soc.* **1957**, *79*, 2023–2024. (c) Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645–650. (d) Rajagopal, P.; Feigon, J. *Biochemistry* **1989**, *28*, 7859–7870.

(3) Methylphosphonate internucleoside linkages, C3′–O3′–PO(CH_3)–O5′–C5′, are nonionic and chiral; for a recent review of the chemistry and applications of these oligonucleotide analogues, see: Ts'o, P. O. P.; Aurelian, L.; Chang, E.; Miller, P. S. *Ann. N.Y. Acad. Sci.* **1992**, *660*, 159–177.

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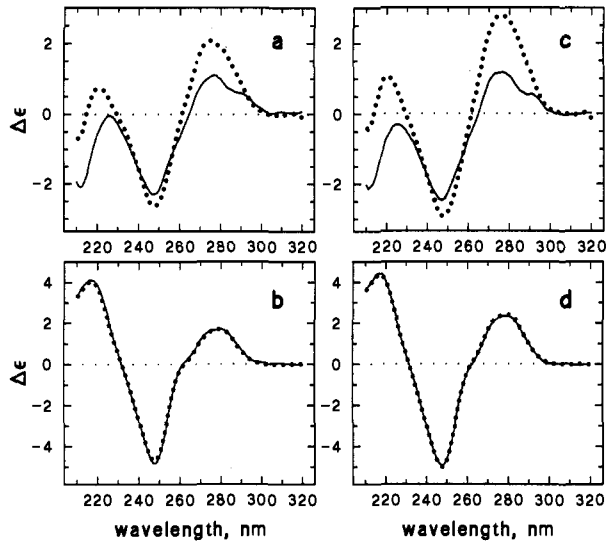

Figure 2. CD spectra at 4 °C for [2:2:2] μM mixtures of oligomers. (a) $1T_{pd}$:$2\Psi_{mp}$:$3H_{mp}$ observed (—); $^2/_3$ ($1T_{pd}$:$2\Psi_{mp}$ [3:3] μM) + $^1/_3$ ($3H_{mp}$ 6 μM) calculated (···). (b) $1T_{pd}$:$2U_{mp}$:$3H_{mp}$ observed (—); $^2/_3$ ($1T_{pd}$:$2U_{mp}$ [3:3] μM) + $^1/_3$ ($3H_{mp}$ 6 μM) calculated (···). (c) $1T_{pd}$:$2\Psi_{mp}$:$3H_{pd}$ observed (—); $^2/_3$ ($1T_{pd}$:$2\Psi_{mp}$ [3:3] μM) + $^1/_3$ ($3H_{pd}$ 6 μM) calculated (···). (d) $1T_{pd}$:$2U_{mp}$:$3H_{pd}$ observed (—); $^2/_3$ ($1T_{pd}$:$2U_{mp}$ [3:3] μM) + $^2/_3$ ($3H_{pd}$ 6 μM) calculated (···). Spectra were obtained for the same samples used in the UV thermal profiles.

unclear. Previous reports have shown that two contiguous pseudouridine residues in an RNA duplex can stabilize *vs* A·U base pairs,[4a] whereas substitution of 1-methyldeoxypseudouridine for thymidine significantly destabilizes DNA duplex formation.[4b] Sequence effects and backbone composition most certainly play roles in these observations; however, it is clear that both $2\Psi_{mp}$ and $2U_{mp}$ may interact with $1T_{pd}$ to form a double helix, presumably *via* W·C base pairing interactions. Thermal profiles of 1:1:1 mixtures of 1:2:3 strands show strikingly different results for $2\Psi_{mp}$ and $2U_{mp}$ strands. Mixtures of 1 equiv of either $3H_{mp}$ or $3H_{pd}$ strands with 1 equiv of the $1T_{pd}$·$2\Psi_{mp}$ duplex have hypochromicities which are approximately equivalent to the $1T_{pd}$·$2\Psi_{mp}$ duplex. The T_m of the $1T_{pd}$:$2\Psi_{mp}$:$3H_{mp}$ mixture (17.5 °C) is slightly greater than that of the $1T_{pd}$·$2\Psi_{mp}$ duplex; however, the T_m for the $1T_{pd}$:$2\Psi_{mp}$:$3H_{pd}$ mixture has increased substantially to 26.5 °C.[5] Mixtures of the deoxyuridine-containing duplex and third strands, on the other hand, show slightly reduced T_m's and hypochromicities which are about two-thirds of those observed for the duplex alone. As the pyrimidine third strands have no hypochromicity on their own under these conditions, thermal profiles indicate that they interact substantially with the $1T_{pd}$·$2\Psi_{mp}$ duplex and not with the $1T_{pd}$·$2U_{mp}$ duplex.

These results are further confirmed by circular dichroism (CD) measurements and calculations. CD spectra of samples from the UV thermal profiles were observed at 4 °C, a temperature at which any strand association which might occur can be monitored. The observed spectrum of a 1:1:1 mixture of $1T_{pd}$:$2\Psi_{mp}$:$3H_{mp}$ is quite different from the weight-averaged sum of spectra observed for the $1T_{pd}$·$2\Psi_{mp}$ duplex and for $3H_{mp}$ by itself (Figure 2a). In contrast, the spectrum observed for a 1:1:1 mixture of $1T_{pd}$:$2U_{mp}$:$3H_{mp}$ is almost exactly reproduced by summing spectra independently observed for the $1T_{pd}$·$2U_{mp}$ duplex and $3H_{mp}$ (Figure 2b). These results are strong evidence that the intended third strand, $3H_{mp}$, can interact with the deoxypseudouridine-containing duplex, $1T_{pd}$·$2\Psi_{mp}$, to form a new helical complex which involves base-stacking interactions, whereas it does not interact with the deoxyuridine-containing duplex, $1T_{pd}$·$2U_{mp}$, under these conditions. Similar results are found for 1:1:1 mixtures of $1T_{pd}$:$2\Psi_{mp}$:$3H_{pd}$ and $1T_{pd}$:$2U_{mp}$:$3H_{pd}$ (Figure 2c,d).

To our knowledge, this is the first report of a base-pairing interaction between deoxypseudouridine and deoxycytidine bases, as well as the first demonstration of a triplex containing the A·Ψ=C base triad. In addition, the increased thermal stability of the $1T_{pd}$·$2\Psi_{mp}$=$3H_{pd}$ triplex as compared to the $1T_{pd}$·2Ψ or U_{mp} duplexes is remarkable. The data presented here show that a homopyrimidine third strand can bind to a W·C duplex of mixed pyrimidine–purine content when the pyr residues in the second strand contain an additional site for H-bonding to the third strand. This additional site is provided by using a pyrimidine analog, deoxypseudouridine, in the A·Ψ base pair. A similar W·C pairing for guanosine residues in the first strand may be proposed for the cytidine analog, deoxypseudoisocytidine (ψiC), in a G·ψiC base pair.[6a] By using these two pyrimidine C-nucleoside analogues, along with adenosine and guanosine, in a second strand with W·C complementarity, any naturally occurring sequence in a target nucleic acid may serve as the first strand in a triplex.[6b] Efforts are underway in this laboratory to utilize both of these C-nucleosides in a second strand to enable triplex formation at single-stranded sites of unrestricted sequence. In addition, the effects of backbone composition on triplexes containing C-nucleosides are being examined, e.g., targeting of RNA sequences by the addition of two oligonucleotides (having natural or analog backbones) as second and third strands.

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Supplementary Material Available: Experimental procedures regarding preparation of deoxypseudouridine methylphosphonamidite, oligomer synthesis and characterization, sample preparation, and UV and CD spectrophotometry and spectroscopic data for single-strand and double-strand samples (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(5) We have found that a T_{pd}·dA_{mp}=T_{pd} triplex (T_m = 47 °C) is also more stable than a T_{pd}·dA_{mp}=T_{mp} triplex ($T_m \leq 30$ °C) in a 16-mer oligopyrimidine: oligopurine model system. Trapane, T. L. Ph.D. Thesis, Biochemistry Dept., The Johns Hopkins University, 1993.

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