An Inverted Motif for Oligonucleotide Triplexes: Adenosine–Pseudouridine–Adenosine (A–Ψ–A)

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Received November 8, 1994

Since the discovery of the poly(U)–poly(A)–poly(U) nucleic acid triple helix,[1] a variety of base-triple motifs other than U–A–U have been characterized.[2] Nonetheless, nearly all of these motifs utilize a purine rather than a pyrimidine base of a Watson–Crick duplex for binding the third strand. This is in part a result of purines having two hydrogen bond donor/acceptor groups in the major groove of a double helix available for interaction with a third strand, whereas only one such group is present in the common pyrimidine nucleotides. Alternative base triples with pyrimidines as the point for third strand attachment can be envisioned where standard pyrimidine nucleotides are replaced by nonstandard ones bearing two major groove accessible donor/acceptor atoms. One of the simplest motifs of this type inverts the original U–A–U triple and utilizes the natural hypermodified ribonucleoside pseudouridine as the nonstandard pyrimidine component to give A–Ψ–A (Figure 1). We report herein the characterization of this inverted base-triple. Independent of our own efforts, Ts'o and coworkers have recently reported a different triplex motif based on pseudouridine that employs its additional hydrogen-bonding capabilities.[3]

A tridecanucleotide bearing a central pseudouridine embedded in the midst of twelve deoxyadenosines, **Ψ-1** (Table 1), was synthesized by automated methods, and purified by HPLC. The composition of **Ψ-1** was chosen because any triplex structure formed between it and complementary oligomers was anticipated to have a unique signature at 284 nm in a UV versus temperature profile, based on our experience with a similar standard parent sequence, d-T_{12} paired with d-A_{12} in other work.[4] Pseudouridine was used in place of deoxypseudouridine due to instability of this nucleoside when the 2'-hydroxyl group is absent.[5] Pseudouridine phosphoramidite was synthesized essentially as described by McLaughlin.[6] Incorporation of pseudouridine into **Ψ-1** was verified by its digestion to nucleoside monomers using calf spleen phosphodiesterase along with bacterial alkaline phosphatase and HPLC analysis. No α-pseudouridine was detected in the digest.[7] Complete calf spleen nuclease

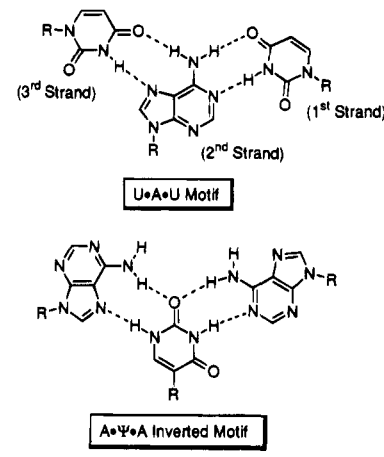

Figure 1.

digestion indicates pseudouridine is incorporated as its 3'-phosphodiester since this enzyme is known not to cleave 2'-phosphodiester linkages.[8] The integrity of **Ψ-1** was further verified by MALDI-TOF mass spectrometry, reversed-phase HPLC, and end-labeling followed by PAGE analysis. **Ψ-1** was judged to be greater than 95% pure using the latter two methods.

UV versus temperature profiles for **Ψ-1**, its standard base counterpart bearing uridine (U-**2**) and dA_{12} (A-**3**), in the presence of complementary oligomers are shown in Figure 2. The results from these experiments are summarized in Table 1. All UV versus temperature profiles were obtained in both the forward and reverse directions with essentially no hysteresis and less than 1 °C difference in T_m.

When **Ψ-1** is combined with 2 equiv of its complement, the stoichiometric amount for triple helix formation, two clear transitions are observed in the resulting UV versus temperature profile at 260 nm (Figure 2A, panel 1, upper curve), whereas at 284 nm a single transition is observed that coincides with the lower melting of the two transitions at 260 nm (Figure 2A, panel 2, upper curve). We interpret the lower melting transition to be denaturation of a triplex to a duplex and the higher melting transition as denaturation of a duplex to single strands. These data also suggest that the A–Ψ–A inverted motif is viable in the context of a triplex based on the T–A–T motif.

To gain additional evidence in support of the triplex and duplex transition assignments, **Ψ-1** was combined with 1 equiv of its complement, the stoichiometric amount for double helix formation, and the UV versus temperature profiles recorded. A single transition is observed at 260 nm, and no transition is seen at 284 nm (Figure 2A, panels 1 and 2, lower curves). Here, the T_m of the single transition observed at 260 nm coincides with that of the higher melting transition in the triplex stoichiometry case and supports its assignment as duplex denaturation in the latter.

To ascertain to what extent the A–Ψ–A inverted motif stabilizes or destabilizes triplex structure, the triple helix was examined between U-**2** and two equivalents of the same d-TTTTTTATTTTTT complementary sequence used in experiments with **Ψ-1**. In this situation the dA in the third strand should form a mismatch with the second strand rU. The UV versus temperature profile for this case yields two transitions at 260 nm and a single

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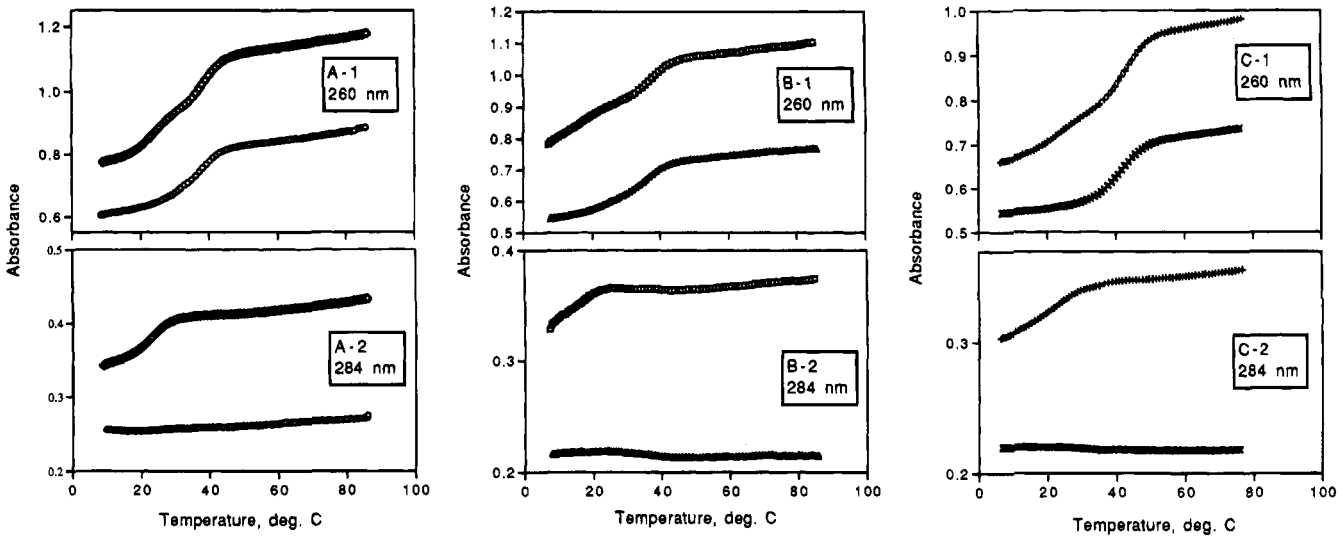

Figure 2. (A) UV absorbance profiles versus temperature for dAAAAAA-rΨ-dAAAAAA (Ψ-**1**) in the presence of 2 equiv (upper curves in panels A-1 and A-2) and 1 equiv (lower curves in panels A-1 and A-2) of d-TTTTTTATTTTTT at the wavelengths indicated. (B) UV absorbance profiles versus temperature for dAAAAAA-rU-dAAAAAA (U−**1**) in the presence of 2 equiv (upper curves in panels B-1 and B-2) and 1 equiv (lower curves in panels B-1 and B-2) of d-TTTTTTATTTTTT. (C) UV absorbance profiles versus temperature for dA_{12} (A−**3**) in the presence of 2 equiv (upper curves in panels C-1 and C-2) and 1 equiv (lower curves in panels C-1 and C-2) of d-T_{12}. All profiles were obtained in 1 M NaCl, 10 mM sodium phosphate, 0.1 mM EDTA at pH 7.

Table 1

oligonucleotide	equiv of complement	T_m (°C) 260 nm	T_m (°C) 284 nm
dAAAAAA-rΨ-dAAAAAA (Ψ-**1**)	2[b]	24.6, 38.2	23.6
	1[b]	37.2	
dAAAAAA-rU-dAAAAAA (U-**2**)	2[b]	16.8, 37.6	16
	1[b]	35.3	
dA_{12} (A-**3**)	2[c]	21.8, 42.3	20.8
	1[c]	41.3	

[a] Experimental conditions in all cases were as noted in the legend of Figure 2. [b] Complementary oligonucleotide: d-TTTTTTATTTTTT. [c] Complementary oligonucleotide: d-T_{12}.

transition at 284 nm (Figure 2B, panels 1 and 2, upper curves). The T_m for the triplex to duplex transition is found to be 8 °C lower than the corresponding transition for Ψ-**1**, consistent with favorable A−Ψ third and second strand pairing. Additional support for A−Ψ third and second strand pairing derives from comparison of third strand T_m's between the Ψ-**1** and A-**3** triplexes (Table 1). This comparison shows that the A−Ψ−A inverted motif leads to increased stability of the Ψ-**1** triplex over the A-**3** triplex which has an equivalent number of T−A−T triples.

An issue that our data do not address directly concerns which faces of the third-strand adenine and the second-strand pseudouridine pair with one another in the inverted motif. This ambiguity derives from the fact that there are identical pairing patterns with respect to the formation of two hydrogen bonds for the alternative faces present in both cases. The inverted motif as represented in Figure 1 invokes bonding between the Hoogsteen face of a syn-oriented adenine in the third strand and the analogous face in an anti-oriented pseudouridine. An anti conformation of pseudouridine would be consistent with its known structure in tRNAs[9] and an RNA duplex;[6] bonding to the Hoogsteen face of adenine would relate the A−Ψ third and second strand pair to the corresponding T−A one by pseudodyad symmetry.

Inverted base-triples may allow for complete generalization of the triple helix by permitting recognition of any element of a suitably pyrimidine-modified double helix. Such helices should be accessible by in vitro amplification methods using nonstandard nucleoside triphosphates.[10] At least three second- and third-strand pairing motifs in addition to the one discussed here are possible by permutation of hydrogen-bond donor and acceptor patterns. This fact raises the possibility of increasing the informational content of nucleic acid triplexes by mating these additional patterns with pyrimidines involved in nonstandard Watson−Crick pairs.[11] Finally, double helices capable of forming continuous triplex structures on a given strand have open to them a new pathway for replication.

Acknowledgment. Support for this work by the NIH (GM-47375) and NASA is gratefully acknowledged. The Midwest Center for Mass Spectrometry is thanked for obtaining mass spectra of oligonucleotides.

Supplementary Material Available: General experimental procedures and characterization data for all compounds (6 pages).

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