FTIR Study of a Nonclassical dT_{10}*dA_{10}-dT_{10} Intramolecular Triple Helix

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ABSTRACT: Many early investigations on triple helices have been devoted to the study of the triplex formed by dT*dA-dT base triplets in which the third strand is oriented parallel to the dA strand. We now describe an intramolecular triple helix with dT*dA-dT base triplets in which the pyrimidine third strand is oriented antiparallel, formed by folding back twice the tridecamer dT_{10}-linker-dA_{10}-linker-dT_{10} (linker = pO(CH2CH2O)3p). Third-strand base pairing to the target strand, sugar conformation, and thermal denaturation of the triplex have been studied by Fourier transform infrared spectroscopy. Our results confirm that when the third-strand orientation is reversed from parallel to antiparallel with respect to the target strand, the third-strand hydrogen-bonding scheme is changed from Hoogsteen to reverse Hoogsteen. The sugar conformation in this triple helix is of the S type (C2′endo/anti, B family form) for all strands. Our results are discussed with respect to models for triplexes proposed as intermediates in homologous recombination [Zhurkin, V. B., Raghunathan, G., Ulyanov, N. B., Camerini-Otero, R. D., & Jernigan, R. L. (1994) *J. Mol. Biol. 239*, 181−200].

Watson−Crick duplexes are known since the late 1950s to be able to interact with single-stranded nucleic acids to form triple helices (Felsenfeld et al., 1957). Since then, numerous studies have been reported concerning inter- and more recently intramolecular triplexes using DNA and/or RNA oligomers [for review see, Hélène and Toulmé (1990), Maher (1992), and Cheng and Pettitt (1992)]. All of this work shows that a polypurine sequence of duplex DNA can be recognized in the major groove by a third DNA or RNA strand, either all pyrimidine or all purine. In the first case, (pyr*pur-pyr),[1] the third strand is oriented parallel with respect to the purine target strand and the third-strand binding scheme is of the Hoogsteen type (T*A-T and C^+*G-C base triplets; Arnott & Selsing, 1974; Arnott et al., 1976; Moser & Dervan, 1987; Le Doan et al., 1987; De los Santos et al., 1989; Rajagopal & Feigon, 1989). In the second case, (pur*pur-pyr), the observed base triplets are A*A-T and G*G-C (Fresco, 1963; Broitman et al., 1987; Letai et al., 1988; Beal & Dervan, 1991; Pilch et al., 1991; Durland et al., 1991; Radhakrishnan et al., 1993). Very recently another class of triple helices has been proposed, in particular as intermediates in homologous recombination (Shchyolkina et al., 1994; Zhurkin et al., 1994). The third strand is oriented parallel to the homologous strand of the Watson−Crick duplex and can contain all four bases.

In the present work we have prepared and studied by FTIR spectroscopy such a triple helix containing T*A-T base triplets, in which both dT strands are parallel and thus antiparallel to the dA strand, (apdT*dA-dT). FTIR spectroscopy has proved to be well suited for the study of double- and triple-helical nucleic acid structures (Taillandier & Liquier, 1992). The results are compared with previous data obtained for the classical parallel triple helix containing the T*A-T base triplets (pdT*dA-dT) (Liquier et al., 1991; Howard et al., 1992). We find that when the third-strand orientation is reversed from parallel to antiparallel with respect to the dA strand, the third-strand H-bonding scheme is changed from Hoogsteen to reverse Hoogsteen. Such a modification has also been observed in the case of the parallel (pαdT*dA-dT) triple helix formed with a third strand containing α anomeric thymidines instead of the usual β anomeric thymidines. In the (pαdT*dA-dT) triple helix, the αdT third strand is oriented parallel to the dA Watson−Crick strand and bound following a reverse Hoogsteen scheme (Liquier et al., 1993).

Two models have been proposed for recombinant triple helical T*A-T motifs obtained by energy minimization computations (Zhurkin et al., 1994). One of them, the so-called collapsed form, has its sugar rings in the C2′endo domain. FTIR spectroscopy can be used to determine sugar geometries, and we observe that in the antiparallel (apdT*dA-dT) triplex the D-2′-deoxyribose of all strands is in a S-type geometry (C2′endo/anti, B family form).

MATERIALS AND METHODS

The tridecamer dT_{10}-linker-dA_{10}-linker-dT_{10}, where linker = pO(CH2CH2O)3p, was purchased from Genset. Purity was controlled by reverse phase HPLC (acetonitrile gradient in triethylammonium acetate buffer), and salt content was adjusted by elution on a G10 column. The stock solution was annealed at 80 °C for 10 min and then cooled down to 4 °C during 24 h.

FTIR spectra were recorded using a Perkin-Elmer 1760 spectrophotometer coupled to a PE7700 minicomputer. The sample solution was deposited between ZnSe windows at a 3 mM strand concentration in 100 mM NaCl. The temperature was monitored using a Specac temperature controller in the 5−60° range. Fifteen scans were accumulated.

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[1] In the triple-helix notation an "*" represents third-strand binding, either Hoogsteen or reverse Hoogsteen, while a "-" represents the Watson−Crick base pairing of the target duplex. The prefix p or ap means that the third strand is oriented parallel or antiparallel with respect to the dA strand of the target duplex.

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Table 1: Proposed Assignments for In-Plane Double-Bond Base Vibrations[a,b]

	dA_{12} ss	dT_{12} ss	dA_{12}-dT_{12} duplex	pdT*dA-dT triplex[c]	apdT*dA-dT triplex		
temp, °C	25	25	25	25	10	35	60
assignt, cm^{-1}							
C2=O2 (T; RH)					1712		
C2=O2 (T; WC or H)			1696	1699	1698	1696	
C2=O2 (T; F)		1692				1692	1692
C4=O4 (T)		1663	1662	1659	1659	1661	1662
ring (T; WC)			1641	1644		1641	
ring (T; H, RH, or F)		1632		1633	1631	1631	
ND2 (A; F)	1626						1627
ND2 (A; WC)			1622			1622	

[a] Spectra recorded in D_2O solutions. [b] T, thymine; A, adenine; ss, single strand; apdT*dA−dT, antiparallel triple helix; pdT*dA−dT, parallel triple helix; WC, base involved in Watson−Crick base pairing; H, base involved in Hoogsteen base pairing; RH, base involved in reverse Hoogsteen base pairing; F, free base. [c] Liquier et al., 1991.

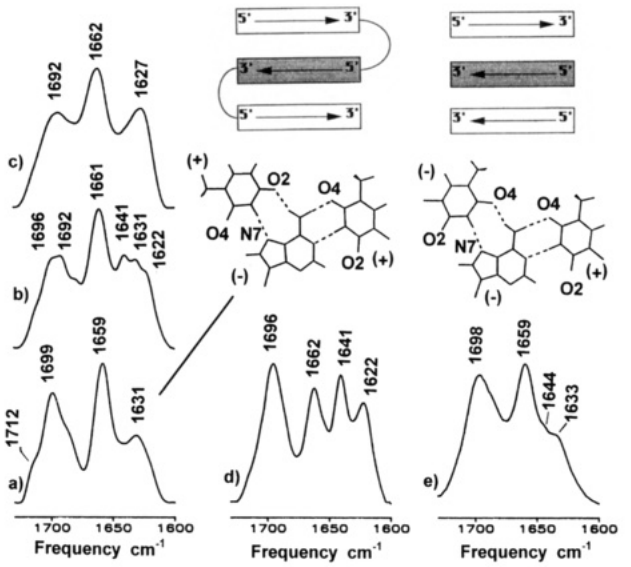

FIGURE 1: FTIR absorption spectra recorded in D_2O solutions (spectral region containing the base in-plane double-bond stretching vibration bands) of the dT_{10}-linker-dA_{10}-linker-dT_{10}, (a) at 10 °C (antiparallel apdT*dA-dT triple helix), (b) at 35 °C, and (c) at 60 °C, the dA_{12}-dT_{12} duplex (d) at 25 °C, and the parallel pdT*dA-dT triple helix (e) at 25 °C. Models for Hoogsteen and reverse Hoogsteen third-strand binding and third-strand orientation are shown: + and − indicate the strand orientations, the shaded region indicates the polypurine dA strand, and the nonshaded region indicates the polypyrimidine dT strands.

Deuteration experiments were performed by drying the sample H_2O solution under nitrogen and redissolving in the same volume of D_2O (>99.8% purity, purchased from Euriso-top CEA) at pH 7.

For UV experiments the oligomer was dissolved in NaCl solution (50−200 mM), pH 7, at a concentration of 1.2 μM strand determined spectrophometrically using ϵ_{262} = 9490 L/mol·cm. Absorption spectra and absorbance versus temperature profiles were recorded with a Kontron Uvikon 941 spectrophotometer. Temperature was varied (0.1 °C per minute) by circulating liquid using a Huber water bath controlled by a Huber PD415 temperature programmer.

RESULTS

Formation of the Antiparallel Triple Helix. Evidence of the formation of the intramolecular dT_{10}-linker-dA_{10}-linker-dT_{10} triple helix with the third pyrimidine strand oriented antiparallel (apdT*dA-dT triplex) is presented in Figure 1a−e in the form of FTIR spectra recorded at different temperatures (spectra recorded in D_2O solution). The spectral region shown contains absorption bands assigned to in-plane double-bond stretching vibrations of the bases. These bands are known to be particularly sensitive to base-pairing interactions. For comparison, the spectra of the dA_{12}-dT_{12} Watson−Crick duplex and of the classical poly dT*poly dA-poly dT triple helix with the third poly dT strand oriented parallel with respect to the poly dA duplex strand (pdT*dA-dT triplex) (Liquier & al., 1991) are given in Figure 1d,e.

Four absorption bands in the spectrum of the duplex have been respectively assigned to vibrational modes of thymine (C2=O2 stretch, 1696 cm^{-1}; C4=O4 stretch, 1662 cm^{-1}; ring C=C and C=N stretch, 1641 cm^{-1}) and adenine (ring C=C and C=N stretch, ND_2 scissoring, 1622 cm^{-1}) (Tsuboi et al., 1969). When the parallel pdT*dA-dT triple helix is formed, the relative intensity of the 1622 cm^{-1} adenine band is drastically reduced, showing that this base is involved in new hydrogen-bonding interactions with the third-strand thymine; moreover, two thymine absorptions are detected at 1644 and 1633 cm^{-1}, corresponding respectively to bases involved in Watson−Crick base pairing and to third-strand Hoogsteen-bound thymines (Figure 1e, Table 1; Liquier et al., 1991). Similarly in the spectrum of dT_{10}-linker-dA_{10}-linker-dT_{10} at 10 °C (Figure 1a), the relative intensity of the adenine band at 1622 cm^{-1} is drastically decreased, showing that the adenine base is involved in an interaction with the third-strand thymine. In this case, however, the triple helix formed, due to its sequence, is necessarily the antiparallel apdT_{10}*dA_{10}-dT_{10} triplex.

Base-Pairing Scheme in the Antiparallel Triplex. The FTIR spectrum of the antiparallel apdT*dA-dT triplex (Figure 1a) contains a high-wavenumber C2=O2 carbonyl stretching vibration band at 1712 cm^{-1} that is not present in the pdT*dA-dT triplex (Figure 1e). This reflects a different binding of the third strand. In the Hoogsteen third-strand base-pairing model found for the pdT*dA-dT triplex, the C2=O2 carbonyl groups of both thymines in the base triplet are free and a single absorption band is observed in the spectrum at 1698 cm^{-1}. In contrast in the reverse Hoogsteen third-strand base-pairing model, two different C2=O2 carbonyl groups exist, and thus two different absorptions are expected, corresponding to free and hydrogen-bonded carbonyls. The presence of two carbonyl vibration absorptions at 1712 and 1699 cm^{-1} in the antiparallel triple-helix spectrum can be correlated with the existence of two different C2=O2 carbonyl groups in the structure and with a reverse Hoogsteen binding mode for third-strand thymines. This

signature of a reverse Hoogsteen thymine binding has been previously reported in the case of αdT*dA-dT triple helices containing alpha (α) anomeric third-strand thymidines instead of beta (β) anomeric third-strand thymidines (Liquier et al., 1993). In that case, the αdT third strand of the αdT*dA-dT triple helix is oriented parallel with respect to the dA strand and bound to the dA strand through reverse Hoogsteen hydrogen bonds (LeDoan et al., 1987; Praseuth et al., 1988). A high-wavenumber carbonyl absorption was observed in the αdT*dA−dT triple-helix FTIR spectrum at 1712 cm^{-1}. The dT*dA-dT base motif with reverse Hoogsteen third-strand binding can be observed in a parallel as well as in an antiparallel triple helix. The conversion from a Hoogsteen to a reverse Hoogsteen third dT strand can be obtained either by inverting the third dT strand orientation from parallel to antiparallel or by keeping the third-strand parallel orientation but replacing the third-strand βdT anomers by αdT anomers.

Thermal Denaturation. The antiparallel apdT*dA-dT triple helix has been observed in the 7−15 °C temperature range. Above 15 °C biphasic melting of the triplex occurs, as observed by FTIR. The spectrum recorded at 35 °C (Figure 1b) no longer presents the high-wavenumber C2=O2 band at 1712 cm^{-1} characteristic of the reverse Hoogsteen third-strand binding. There are now two absorptions at 1696 and 1692 cm^{-1} corresponding, respectively, to a C2=O2 vibration of thymine involved in Watson−Crick base pairing (in the dA_{12}-dT_{12} duplex) and of free thymine (single-stranded dT_{12}) (Table 1). Simultaneously, there is the adenine band located at 1622 cm^{-1}, which is normal for an adenine base involved in Watson−Crick base pairing. Thus, the spectrum at 35 °C reflects the coexistence of a dA_{10}-linker-dT_{10} duplex with a non-base-paired dT_{10} dangling end. If the temperature is increased to 60 ° (Figure 1c), then the spectrum is again modified, and due to the melting of the dA_{10}-linker-dT_{10} loop absorption bands are now located at positions corresponding to single-stranded dT_{12} (1692 cm^{-1} instead of 1699 cm^{-1}) and dA_{12} (1627 cm^{-1} instead of 1622 cm^{-1}) (Table 1).

The melting in dT*dA-dT triple helices can be followed by modifications in the interactions at the level of the ND_2 group of adenines estimated by measurement of the relative intensity of the adenine infrared absorption located around 1625 cm^{-1}. Figure 2a gives a plot of the variation of the relative intensity of this band with temperature in the case of the antiparallel apdT*dA-dT triple helix. Biphasic melting is readily apparent, with the first transition reflecting dissociation of the third strand, followed by a second transition reflecting the melting of the dA_{10}-linker-dT_{10} duplex.

The UV melting profile (Figure 2b) is similarly biphasic, with the first transition located between 23 and 27 °C in the 50−200 mM NaCl range. As expected for unimolecular structures, denaturation is not oligomer concentration dependent (3 mM oligomer for the FTIR profile and 1.2 μM for the UV profile).

Sugar Conformations. FTIR spectroscopy allows direct characterization of sugar geometries in nucleic acid structures using well-established marker bands sensitive to sugar pucker in the 900−800 cm^{-1} region. An absorption band ~840 cm^{-1} reflects S-type sugars (C2′endo/anti, B family form) whereas a band at ~865 cm^{-1} is characteristic of N-type sugars (C3′endo/anti, A family form) (Taillandier & Liquier, 1992). Figure 3 shows FTIR absorption spectra in H_2O

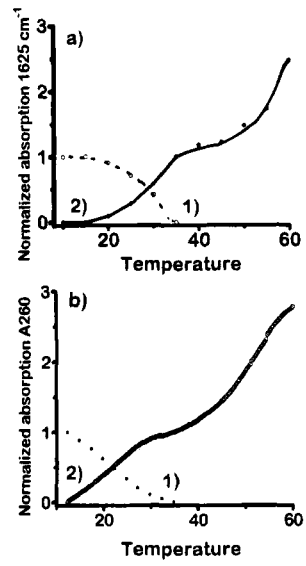

FIGURE 2: Thermal denaturation of the antiparallel triplex: (a) plot of the relative intensity of the adenine absorption located around 1625 cm^{-1} as a function of molar triplex fraction (curve 1) and temperature (curve 2) and (b) plot of the UV A_{260nm} absorption as a function of molar triplex fraction (curve 1) and temperature (curve 2).

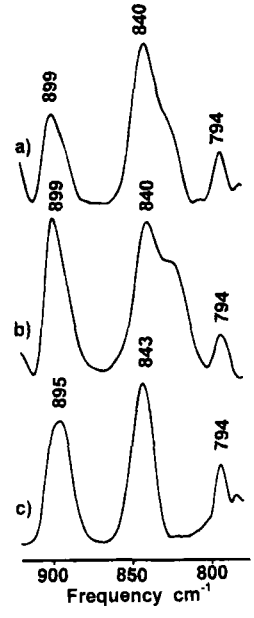

FIGURE 3: S-type sugar conformations. FTIR absorption spectra recorded in H_2O solutions of (a) the antiparallel apdT*dA-dT triple helix, (b) the parallel pdT*dA-dT triple helix, and (c) the dA_{12}-dT_{12} duplex.

solution of the apdT*dA-dT triplex (Figure 3a) and, for comparison, the spectra of the pdT*dA-dT triplex (Figure 3b) (Liquier et al., 1991) and of the dA_{12}-dT_{12} duplex (Figure 3c). In this region the spectrum of the dA_{12}-dT_{12} duplex contains a band at 843 cm^{-1}, indicative of S-type sugar geometry. Similarly the spectra of the apdT*dA-dT triplex and of the pdT*dA-dT triplex show a strong band at 840 cm^{-1}, also indicative of S-type sugars. However, there is a major shoulder at 822 cm^{-1} which could reflect the presence of two slightly different sugar geometries, both belonging to the B-form genus. There is absolutely no contribution detectable near 865 cm^{-1}, showing that all sugars in the apdT*dA-dT triplex adopt S-type geometry similarly to what

was previously characterized for the pdT*dA-dT triple helix (Liquier et al., 1991; Howard et al., 1992).

DISCUSSION

Intramolecular triple helices formed by oligomers able to fold back twice allows one to unambiguously fix the third-strand orientation. In the present work we have characterized the geometry of the triple helix containing dT*dA-dT base triplets with an antiparallel orientation of the third pyrimidine strand with respect to the purine strand. The importance of such triple helices comes from their possible existence as intermediates in homologous recombination in the presence of recA protein (Camerini-Otero & Hsieh, 1993; Rao et al., 1993). The dT*dA-dT base triplet is one of the four possible triplets expected in such antiparallel triple helices (the dA*dA-dT motif is currently under study in the laboratory). Our FTIR results clearly show that the base-pairing scheme in the antiparallel dT*dA-dT triple helix is, as stereochemistry demands, different from the classical parallel dT*dA-dT triple helix, such as observed for poly dT*poly dA-poly dT. When the third-strand orientation is inverted (from parallel to antiparallel), its binding scheme changes from Hoogsteen to reverse Hoogsteen. This result agrees with the stereochemistry proposed for the third-strand thymine of the T*(A-T) triplet in the recombination triplex (Hsieh et al., 1990). Such a geometry is more favorable than the recently proposed position of the third-strand thymine which stabilizes the T*(A-T) triplet in the recombinant triplex by interactions with A and T bases of both Watson–Crick duplex strands (Zhurkin et al., 1994). These authors propose two different forms for the triplex: a collapsed form and an extended form. Their computations show that the collapsed form should have sugar rings in the C2′endo domain, while in the extended form the sugars would be in C3′endo conformation. Our spectroscopic measurements clearly indicate that in the antiparallel apdT*dA-dT triple helix all sugars have a S-type (C2′endo) geometry. This is in favor of a "collapsed-type" geometry model, but with third strand-thymine binding following a reverse Hoogsteen scheme and not interacting with both Watson–Crick bases simultaneously. The study of the induction of a transition from this collapsed form to a supposed extended form of antiparallel triplexes is in progress.

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