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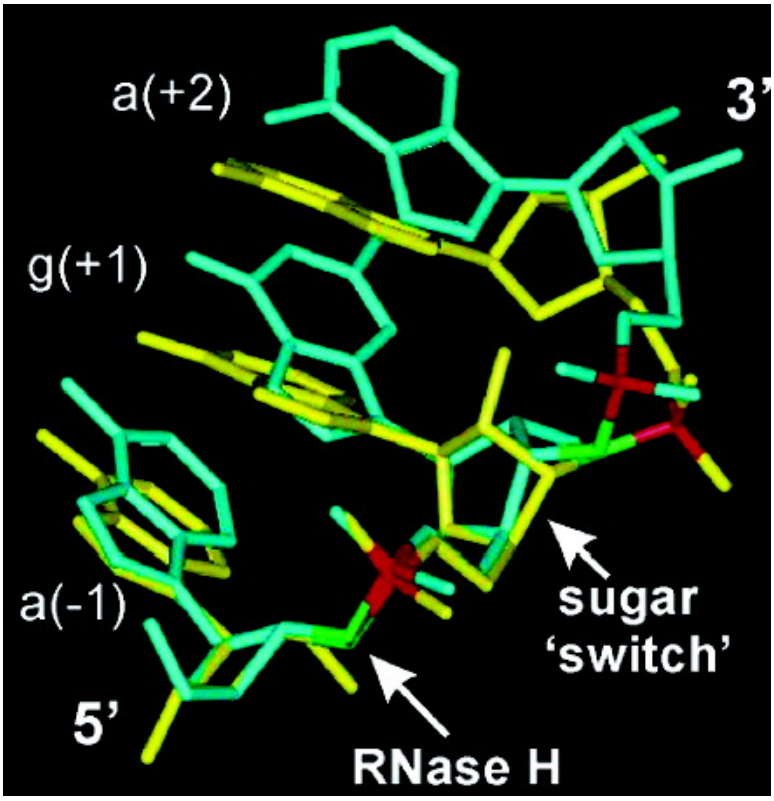

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A Ribose Sugar Conformational Switch in the LTR-Retrotransposon Ty3 Polypurine Tract-Containing RNA/DNA Hybrid

Hye Young Yi-Brunozzi,[†] Danielle M. Brabazon,[‡] Daniela Lener,[†] Stuart F. J. Le Grice,*[,†] and John P. Marino*[,§]

HIV Drug Resistance Program, NCI-Frederick, Frederick, Maryland 21702, Department of Chemistry, Loyola College in Maryland, Baltimore, Maryland 21210, and Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, and National Institute of Standards and Technology, Rockville, Maryland 20850

Received May 25, 2005; E-mail: marino@umbi.umd.edu; slegrice@ncifcrf.gov

Reverse transcriptase (RT)-associated ribonuclease H (RNase H) precisely removes the tRNA and polypurine tract (PPT) primers of (−)-strand and (+)-strand DNA synthesis, respectively, to generate the 3′ and 5′ terminal sequences of double-stranded viral DNA essential for subsequent integration.[1] The fidelity of these processing events is critical to a number of subsequent steps in the retroviral replication cycle. Previous studies have shown that the PPT-containing RNA/DNA hybrids are specifically selected by their cognate RT's, suggesting that unique structural features are recognized by RT to orient the RNase H domain for specific cleavage at the PPT/U3 junction.[2−5] However, the molecular mechanism by which an RT processes its cognate PPT remains largely unknown despite numerous investigations of both nucleic acid and RT sequence requirements. To probe structural features that mediate its recognition and processing, a 20 bp RNA/DNA hybrid duplex (Figure 1A), which includes the full PPT sequence of the *Saccharomyces cerevisiae* LTR-retrotransposon Ty3, has been investigated using solution NMR spectroscopy. Here, we report an unexpected sugar pucker switch from C3′- to C2′-endo pucker for the ribose at position +1, relative to the RNase H cleavage site, in the RNA strand of the Ty3 RNA/DNA PPT hybrid that has been detected through analysis of double-quantum filtered correlation spectroscopy (DQF-COSY) experiments.[6] Modeling of the structural distortions in the RNA/DNA hybrid that may result from this intrinsic feature of the Ty3 PPT suggests how the sugar pucker switch may contribute to the specificity of RNase H cleavage at the PPT/U3 junction.

Formation of the 20 bp RNA/DNA PPT hybrid was verified using native gel electrophoresis, and a T_M for the hybrid of 69 °C was determined from a UV-detected thermal melt (Figure S1). Assignment of the ^{1}H and ^{13}C resonances of the Ty3 RNA/DNA PPT hybrid has been made using standard analysis of NOESY, DQF-COSY, and natural abundance ^{13}C HMQC experiments[7,8] (see Supporting Information for details). Despite the significant chemical shift overlap generally observed in spectra of RNA/DNA hybrids, almost complete assignment has been obtained for the imino, H1′, H2′/H2″, and aromatic/H5 resonances. As expected based on the native gel and melting analysis, the NMR data indicate that the RNA/DNA strands adopt a stable hybrid duplex, as defined by a single set of resonances for almost all residues, that is characterized by an overall A-form-like helical geometry (Figure 1 and Figures S2 and S3).

Figure 2A shows an expansion of the ribose H1′ to H2′ correlated region of a DQF-COSY spectrum acquired for the 20 bp RNA/DNA PPT hybrid duplex. Surprisingly, a relatively strong H1′,H2′ cross-peak is observed in this region of the spectrum, which is

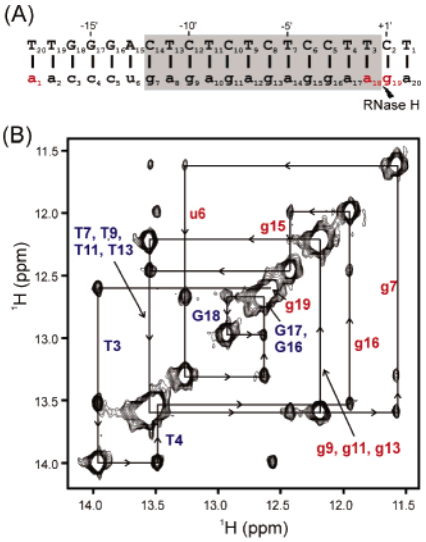

Figure 1. (A) Ty3 polypurine tract-containing RNA/DNA duplex sequence. The PPT sequence is shaded with the (+)-RNA strand in lower case type and the DNA strand in upper case. The RNase H cleavage site is indicated, and RNA nucleotides which display heterogeneous sugar conformations are highlighted in red. The numbering of bases is indicated 5′ to 3′ for each strand, and corresponding positions relative to the RNase H cleavage site are indicated above the DNA strand. (B) An expansion of the imino to imino correlated region of a 2D water flip-back watergate NOESY (τ_{NOE} = 150 ms) experiment applied to the Ty3 RNA/DNA hybrid in 90% H_2O/10% D_2O at 283 K. The data were acquired using Bruker DMX600 spectrometer and TXI HCN probe. The proton carrier frequency was set to 4.75 ppm. The data were acquired with sweep widths of 12 000 Hz in both dimensions, 4K complex points in t_2, 512 complex points in t_1, and 64 scans per increment. The "NOE walk" is shown by lines with arrows, and assignments (RNA, red; DNA, blue) are indicated. Note that imino resonances are not observed for T1, T19, and T20.

assigned to the ribose sugar of residue g19. Significantly weaker COSY cross-peaks are also observed and have been assigned to a1, as would be expected for a terminal residue, and a18. In the region of the spectrum shown in Figure 2A, if a ribose sugar adopts a C3′-endo (or North) pucker, as is the case in canonical A-form backbone geometry (Figure 2B), correlations between ribose H1′ and H2′ protons are not typically observed since ^3J(H1′,H2′) vicinal couplings are small (<2 Hz). In contrast, if a ribose sugar adopts a C2′-endo (or South) pucker, as is found in B-form backbone geometry, ^3J(H1′,H2′) couplings can assume relatively large (6−8 Hz) values, and correlations between H1′ and H2′ protons are observed in a COSY spectrum. While terminal residues in RNA helices often exhibit conformational flexibility, allowing them to exchange between sugar conformations and thus exhibit sizable, averaged ^3J(H1′,H2′) couplings, internal residues within duplex structures do not normally exhibit such dynamic behavior. In the

[†] NCI-Frederick.
[‡] Loyola College in Maryland.
[§] University of Maryland.

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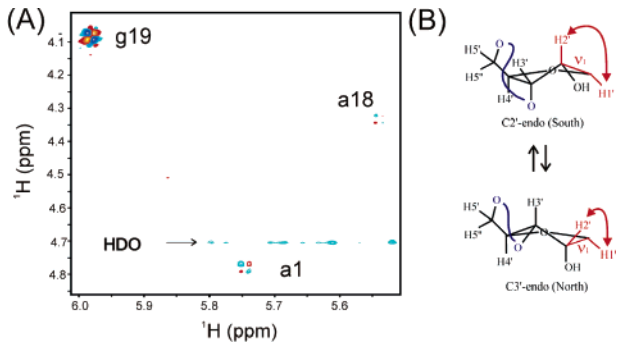

Figure 2. (A) Ribose H1′−H2′ region of a DQF-COSY spectrum applied to a 1.2 mM sample of the 20 bp RNA/DNA Ty3 duplex in 99.96% D_2O at 30 °C. Positive and negative signals of the antiphase multiplets are in blue and red, respectively, and cross-peak assignments are indicated. Residual HDO signal is indicated. Note that no DNA correlations are found in this region of the spectrum. The data were acquired using Bruker DMX500 spectrometer and TXI HCN probe. The proton carrier frequency was set to 4.704 ppm, residual solvent suppressed using presaturation, and acquisition parameters with sweep widths of 5000 Hz in both dimensions, 2K complex points in t_2, 480 complex points in t_1, and 48 scans per increment. (B) Schematic of the C2′- and C3′-endo ribose sugar puckers. The H1′,H2′ vicinal coupled protons (ν_1) are shown in red. The difference in the relative positions of the backbone 5′ and 3′ oxygen atoms in the two states are highlighted in blue.

case of the Ty3 PPT hybrid, the cross-peak observed for residue g19(+1) suggests that the ribose of this residue is in a conformational equilibrium between C2′- and C3′-endo sugar puckers. The ^3J(H1′,H2′) coupling measured for g19, using the procedure of Titman−Keeler,[9] was ∼4 Hz and is consistent with a sugar conformation that is in fast exchange between C2′- and C3′-endo sugar puckers. The weak H1′,H2′ cross-peak observed for the ribose of a18(−1) indicates that this residue proximal to the cleavage site may also be in conformational exchange with a small population of C2′-endo sugar pucker. Structural heterogeneity, which may result in part from these sugar pucker switches, is also evidenced by the observation of the exchange broadening of certain cross-peaks and the duplicity of others around the RNase H cleavage site in the NOESY experiments (Figure S3). While residue g19-(+1) is only one base step in from the end of the hybrid, there is no evidence to indicate that the observed sugar pucker switch is a consequence of end fraying. For example, imino protons are observed for all but the terminal base pair, and NOE patterns indicate a well-stacked, base-paired geometry at this end of the duplex. In addition, while a weak H1′,H2′ cross-peak is observed for a1, no such cross-peak is observed for residue a20. This difference in behavior of the 5′ and 3′ terminal bases of the RNA strand is again consistent with imino proton spectra (Figure 1), which suggest that fraying is more pronounced at the a1 end of the duplex. In general, the observation of a specific ribose sugar pucker switch is in contrast to previous solution NMR studies of rR:dY hybrids, where the conformation of the sugars in the RNA strand has been found to uniformly adopt C3′-endo conformations, while the deoxyribose sugars in the DNA strand were found to exhibit averaged vicinal couplings.[10−13]

An A- to B-transition in the g(+1) sugar pucker at the Ty3 PPT/U3 junction would significantly alter the backbone conformation of the RNA/DNA hybrid relative to a uniform A-form backbone, creating both a local distortion as well as a potentially more long-range kinking of the helix. This can be highlighted by overlaying the a(−1), g(+1), a(+2) step at the Ty3 PPT/U3 junction modeled with uniform A-form backbone geometry with the same step where g(+1) ribose is in a C2′-endo conformation (Figure S4). It is noteworthy that the sugar pucker switch induces a structural alteration that propagates to the 3′ side of the RNA strand. In particular, the conformational change induced by the A- to B-type sugar pucker transition in the g(+1) ribose results in a significant change in the phosphodiester backbone trajectory of the RNA strand, which alters the presentation of backbone phosphates and 2′-hydroxyl groups 3′ of this residue. In contrast, the conformation of the phosphodiester backbone on the 5′ side of this residue and immediately 3′ of the RNase H cleavage site is not perturbed by the sugar pucker switch. This observation implies that part of the mechanism governing RNase H fidelity may be through distortion of the RNA/DNA helix one base ahead of the scissile bond.

A similar ribose sugar pucker switch has also been observed in the crystal structure of a rR:dY model duplex, derived from the 5′ end of the HIV-1 PPT sequence.[14] In addition, an NMR structure of the junction formed at the HIV-1 minus strand initiation site has revealed a deoxyribose sugar switch one base step away from the tRNA−DNA junction.[13] The observed sugar pucker switch in the Ty3 PPT is also consistent with previous chemical foot-printing studies implicating noncanonical behavior of base pairs at position +1 in the HIV-1 PPT sequence.[5] These observations, together with the results described here, suggest that sugar pucker switches may represent a general mechanism for guiding the alignment of RNA/DNA PPT substrates for RNase H cleavage. Taken together with the observations of distortions and mispairing of bases upstream of the cleavage site in the co-crystal of HIV-1 RT with its cognate PPT,[15] the sugar pucker switch at position +1 in the Ty3 PPT may contribute an additional "distortable" feature that ensures proper alignment of the RNase H active site for cleavage at the PPT/U3 junction.

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Supporting Information Available: Native gel and UV melt of the 20 bp Ty3 PPT hybrid (1); Expanded plot of DQF-COSY (2a), natural abundance ^{13}C HMQC (2b), and D_2O NOESY (3) spectra acquired for the 20 bp Ty3 PPT hybrid. Model of the sugar switch induced structural change at the U3/PPT junction (4). This material is available free of charge via the Internet at http://pubs.acs.org.

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