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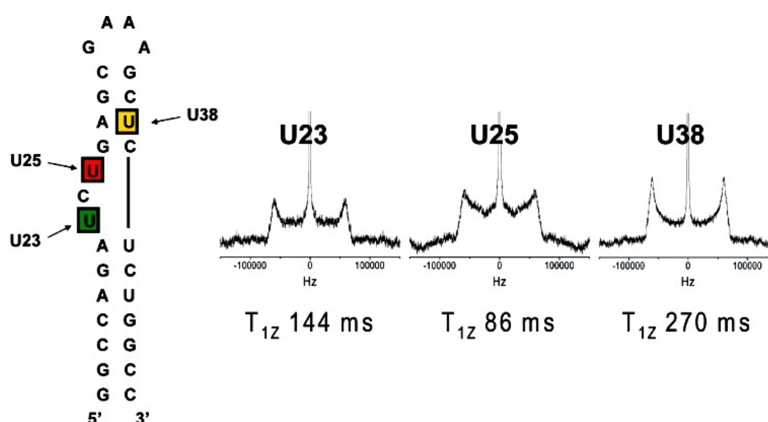


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# Solid-State Deuterium NMR Studies Reveal μs-ns Motions in the HIV-1 Transactivation Response RNA Recognition Site

Greg L. Olsen, Dorothy C. Echodu, Zahra Shajani, Michael F. Bardaro, Jr., Gabriele Varani, and Gary P. Drobny*

*Departments of Chemistry and Biochemistry, University of Washington, Seattle, Washington 98195*

Received October 14, 2007; E-mail: Drobny@chem.washington.edu

The HIV-1 transactivation response (TAR) RNA provides a classic example of adaptive protein-RNA recognition[1,2] and is of key importance for viral replication. Transcription of the HIV genome is dependent upon binding of the viral regulatory protein Tat at a three-nucleotide bulge linking two short helices comprising the TAR hairpin[3] (Figure 1). The inherent flexibility of this bulge allows it to undergo a conformational transition upon binding of Tat protein or even of a single arginine, generating the structure required to form a specific protein−RNA complex.[1,2] The structures of the free RNA and of the Tat- or arginine-bound RNA are known; how the RNA traverses the intervening conformational landscape is not. In order to understand how TAR functions, it is necessary to characterize the full range of motions accessible to this RNA. Such knowledge would provide mechanistic insight into how the conformational changes occur and how the dynamics information required for Tat binding is encoded in the TAR sequence.

Solution NMR studies have begun to uncover complex dynamics in many RNAs, revealing rich local and collective dynamics in the ns−ps and ms−μs time scales.[5−12] However, solution NMR methods fail to capture motions that occur in the μs-ns time scale. In contrast to solution relaxation methods, deuterium solid-state NMR line shape measurements are particularly sensitive to motions in the μs-ns range. We recently observed extensive motions within this dynamic window in the DNA target of a methyltransferase using solid-state NMR[13] and wished to investigate whether TAR RNA was dynamically rich within the μs-ns time scale as well as in the faster and slower regimes.

Previous $^2$H solid-state NMR investigations of RNA dynamics have studied uniformly C8-labeled duplex samples, precluding delineation of the motional properties of individual residues.[14−16] Here we report the first site-specific $^2$H solid-state NMR study of RNA. The solid-state experiments were used to probe three sites with different structural characteristics.[3,6] U23 and U25 are both single-stranded, but while U23 is stacked on a neighboring base, U25 is neither base-paired nor stacked. U38 is part of a base-paired helix; U23 and U38 are critical for TAR function (Figure 1a). Quadrupole echo line shape and $T_{1Z}$ relaxation data were collected for 5,6-$^2$H uridine nucleobase labels introduced at these three positions in separate 29-nucleotide TAR constructs. Each RNA sample was hydrated to 16 waters per nucleotide, to reproduce conditions under which nucleic acid motions have been shown to be similar to those observed in solution.[16−18] Under these conditions, local hydration of the nucleic acid backbone and bases is substantially complete so that local dynamics are essentially solutionlike; introduction of additional waters only adds to bulk hydration, facilitating global tumbling and negating the advantages of the solid-state investigation.

Motions occurring at rates in the $10^6−10^9$ Hz range modulate the quadrupolar line shape.[20] The deuterium line shape is thus especially useful in revealing dynamics too slow to be detected by solution relaxation measurements (ns-ps) or too fast to be detected by rotating frame relaxation measurements (ms-μs). Motions occurring at rates greater than the Larmor frequency "preaverage" the electric field gradient tensor and effectively scale the width of the deuterium spectrum. The effects of these motions can be expressed in terms of order parameters. For rates slower than the Larmor frequency ($\upsilon_0 \sim 10^8$ Hz) but faster than the quadrupolar coupling constant ($\upsilon_Q = e^2qQ/h$; $\sim 1.7 \times 10^5$ Hz for the present work), the modulation of the powder pattern is more complex.[21] However, deviation from a static Pake powder pattern—often visible as trajectory-dependent spectral averaging between the 'horns' of the powder pattern—is diagnostic for motions on this time scale (Figure 2).

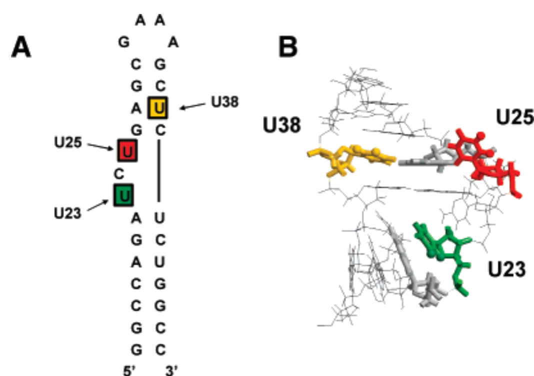


***Figure 1.*** a) Secondary structure of HIV-1 TAR RNA, showing the three sites where 5,6-$^2$H deuterium labels were introduced for solid-state NMR studies. b) Close-up view of the structure of unbound TAR,[4] showing the different structural contexts of the three nucleotides studied here. Colored spheres denote 5,6-$^2$H nucleobase label positions. U23 (green) is single-stranded but stacked on the adjacent base (A22, in gray); U25 (red) is largely disordered; and U38 (yellow) is base paired with A27 (in gray) and part of a canonical Watson−Crick paired helix. U23 and U38 play critical roles in the binding of Tat protein; U25 functions as a spacer.[1,2]

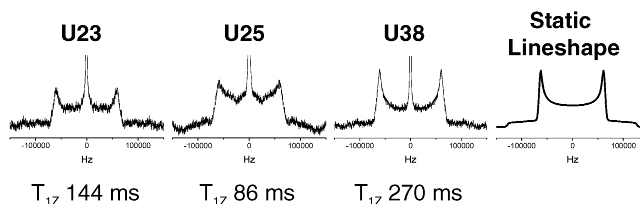


***Figure 2.*** Deuterium solid-state NMR spectra and corresponding Zeeman spin−lattice ($T_{1Z}$) relaxation times for 5,6-$^2$H labels at positions U23, U25, and U38 in TAR RNA, with a static reference line shape simulated using MXET1.[19] Spectra were acquired at room temperature; all samples were hydrated to 16 waters per nucleotide to ensure conditions similar to those observed in solution[16−18] (see the Supporting Information). The narrow central isotropic component in the experimental spectra is due to residual deuterium in the water of hydration.

The $^2$H solid-state NMR line shapes from TAR reveal the presence of distinct dynamics at each of the three sites (Figure 2).

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***Table 1.*** $^{13}$C Solution NMR Relaxation Data and Corresponding ModelFree Order Parameters for C6 and C1′ Sites in TAR Residues U38 and U23[a]

| | U38 – C6 | U23 – C6 | U38 – C1′ | U23 – C1′ |
|---|---|---|---|---|
| $T_1$ | 354 | 328 | 497 | 330 |
| $T_{1\rho}$ | 26 | 29 | 38 | 49 |
| NOE | 1.14 | 1.32 | 1.25 | 1.43 |
| $S^2$ | 0.97 | 0.82 | 0.95 | 0.67 |

[a] All relaxation times are in milliseconds. The average C6 relaxation rates for double-helical residues were 355 ms ($T_1$); 25 ms ($T_{1\rho}$); 1.16 (NOE); and 0.96 ($S^2$). The average double-helical C1′ values were 534 ms ($T_1$); 37 ms ($T_{1\rho}$); 1.23 (NOE); and 0.93 ($S^2$).

U38 retains the features of a Pake doublet, similar to line shapes observed previously for bases in double-stranded DNA devoid of significant internal motions.[22,23] In contrast, both U23 and U25 clearly show line shape modulations indicative of motion on the intermediate spectral time scale. Consistent with the presence of distinct motions at these three sites, the Zeeman spin−lattice relaxation time ($T_{1Z}$) was 270 ms for U38 but was considerably shorter for U23 and U25 (144 and 86 ms, respectively).

In order to compare the dynamics observed by solid-state NMR with what is observed in solution, we also measured $^{13}$C solution NMR relaxation rates for C6/C8 and C1′ sites throughout TAR, including the three sites probed in the solid-state experiments described above. While U25 was not sufficiently resolved from other resonances in the spectrum of TAR to enable measurement of its relaxation properties, results were obtained for U23 and U38. Data were analyzed using the model-free approximation, to determine in particular whether slower motions or conformational exchange ($R_{ex}$) were present and to establish the degree of spatial restriction ($S^2$) at these sites. The base (C6) data for U38 in these experiments (Table 1) are indicative of restricted motions consistent with a rigid helical residue (lower heteronuclear NOE and high $S^2$ order parameter) and are thus in good agreement with the solid-state data and previous $^2$H relaxation measurements in solution.[24] The shorter C6 $T_1$ seen for U23, in combination with the relatively large $T_{1\rho}$ and NOE values observed here, suggests the presence of ns−ps base motion at this position. Intriguingly, the C1′ solution NMR data suggest that the sugar−phosphate backbone at U23 is less restricted than the base itself in the free RNA, as was also observed by deuterium solution NMR.[24] Notably, $R_{ex}$ values for C6 and C1′ as determined from the model-free analysis at both positions were negligibly small, suggesting the absence of slower (ms-$\mu$s) base and furanose motions at these sites, in agreement with previous work.[6,25]

The solid-state results demonstrate that TAR RNA dynamics involving residues essential to Tat binding include not only the faster time scale motions probed by solution relaxation measurements but also a significant component in the $\mu$s-ns time scale. Motion in the $\mu$s-ns regime is clearly apparent in the strongly modulated $^2$H powder patterns for both U23 and U25; the trend in relaxation times, $T_{1Z}$(U38) > $T_{1Z}$(U23) > $T_{1Z}$(U25), indicates progressively greater dynamics near the $^2$H Larmor frequency, from the rigid helical environment of U38 to the partially stacked environment of U23 to the relatively unconstrained environment of U25. These motions were not observed in solution relaxation measurements, as they were not fast enough to affect relaxation parameters nor slow enough to modulate solution line shapes. However, they may have been responsible for the reported discrepancy between order parameters calculated from solution relaxation or from residual dipolar couplings (RDCs).[26] Smaller order parameters for RDCs probably reflected additional motions that averaged their values yet did not affect the relaxation rates, as we report here. The slower motions we detect may also be connected to the interhelical motions observed by domain elongation.[11]

To our knowledge, this work represents the first site-specific $^2$H solid-state NMR study of RNA dynamics. Given the importance of understanding the functional roles of dynamics in RNA, and the demonstrated presence of motions in ranges accessible either to solution or to solid-state measurements—but not to both—the parallel application of solid-state and solution NMR methods will be necessary to construct a complete picture of the motions present in TAR and other RNAs. Our results in both DNA and RNA also suggest that this generally overlooked dynamic window ($\mu$s-ns) may be equally rich in proteins as it clearly is in nucleic acids.

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**Supporting Information Available:** Materials and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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