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Contrasting Views of the Internal Dynamics of the *Hha*I Methyltransferase Target DNA Reported by Solution and Solid-State NMR Spectroscopy

Paul A. Miller,[†] Zahra Shajani,[†] Gary A. Meints,[§] Dorothy Caplow,[†] Gil Goobes,[†] Gabriele Varani,[†,‡] and Gary P. Drobny*,[†]

Departments of Chemistry and Biochemistry, University of Washington, Seattle, Washington 98195, and Department of Chemistry, Missouri State University, Springfield, Missouri 65897

Received August 31, 2006; E-mail: drobny@chem.washington.edu

The enzymatic methylation of deoxyribonucleic acid (DNA) is essential for many biological processes[1−3] and has therefore been studied in considerable detail.[4−6] The structure of the ternary complex of the *Hha*I methyltransferase with its DNA target [5′-(dGATAGC̲GCTATC)-3′]$_2$ and the methyl-donating cofactor *S*-adenosyl L-methionine (AdoMet) demonstrated that the substrate cytosine is extruded from its normally base-paired position.[4] Through this conformational change, the carbon at the C5 position on the base of the underlined cytosine becomes accessible in the enzyme's catalytic pocket. The interactions observed between the protein and the extruded base explain the stabilization of this highly distorted structure[4] but do not provide a mechanism or pathway to flip the base outside of the double helix. What are the energetically favorable pathways that allow base extrusion? Are they sequence dependent?

Intriguingly, preliminary deuterium solid-state NMR studies identified increased motion and flexibility at *Cyt-Gua* steps[7] and at methyltransferase binding sites.[8] These studies demonstrated marked quenching in dynamics upon methylation,[8] even as the crystal structures displayed only small perturbations from unmethylated DNA.[9,10] On the basis of these results, we hypothesize that local DNA dynamics promotes methylation by lowering energetic barriers for the conformational changes requisite for *Hha*I binding.

Here, we used solid state and solution NMR conjointly to probe internal motions at six positions near the *Hha*I enzyme recognition site for the [5′-(dGATAGC̲GCTATC)-3′]$_2$ DNA dodecamer.[4] Solid-state ^{2}H line shape (Figure 1a) and inversion recovery (Figure 1e) data were collected for six DNA samples containing deuterons at the H2″ positions of residues Ade4−Ade10. The DNA was hydrated to 11−13 waters per nucleotide, to reach conditions where motions in the solid are very close to those observed in solution[11,12] and to establish that the line shapes do not differ simply as a result of differential hydration. Consistent with our hypothesis that methylation sites would have a characteristic dynamic signature, we observed remarkable variations in line shape and longitudinal relaxation times (T_{1Z}) for residues framing the methylation site (Cyt6) (Figure 1a). Cyt6 and the nearby Cyt8 exhibit T_{1Z} values (30 and 22 ms, respectively) that are nearly half the value of their neighbors (Figure 1e). These same residues also have a noticeable modulation of the line shape, suggesting considerable motional averaging at the Larmor frequency (∼76.76 MHz). Nearby residues are not nearly as flexible, as demonstrated by the Pake doubletlike line shapes and much larger T_{1Z} (59−82 ms). By modeling similar line shapes as described elsewhere,[13] we established that the H2″ nuclei experience small amplitude librations (∼10°) of the CD bond

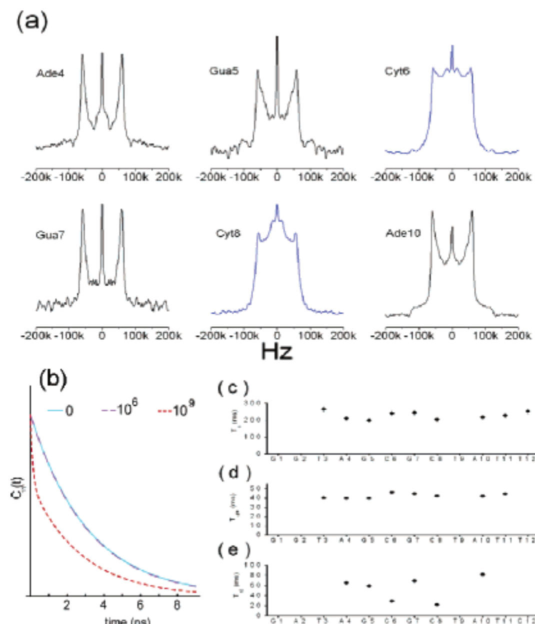

Figure 1. (a) Deuterium quadrupolar echo pulse sequence line shapes (2.6 μs 90° pulse with 40 μs echo delay; changes in signal-to-noise are due to different concentrations in each sample); (b) Simulated decay of the C_{11} component of the correlation function describing the motions of CD/CH bonds embedded in a rigid-rodlike DNA molecule tumbling at 3.9 ns and subject to internal motion at the indicated frequencies. In the absence of internal diffusion (blue), the decay is monoexponential; for fast internal motions, the decay is strongly multiexponential (red), reflecting the modulation of the correlation functions by multiple motional mechanisms. Slower internal motions become invisible in the correlation functions (purple). (c−e) Relaxation parameters for C-H2″: (c) solution T_1, (d) solution $T_{1\rho}$, (e) solid-state T_{1Z}. Experimental procedures are given in the Supporting Information.

at frequencies faster than the quadrupolar interaction (∼174 kHz), whereas H2″ nuclei on Cyt6 and Cyt8 experience large amplitude motions (∼36°) at similar frequencies. These effects are specific for the *Cyt-Gua* target DNA sequence as other DNAs revealed no significant variation in T_{1Z} or line shapes between purine or pyrimidine residues (Meints, G. A.; Hatcher, M. Ph.D. Thesis, University of Washington).

We next examined whether the *Hha*I target DNA has a sequence-dependent motional signature in solution as well, by measuring NMR relaxation properties in the liquid state. By preparing a uniformly ^{13}C and ^{15}N-labeled sample (for synthetic reasons, we used a nonpalindromic dsDNA dodecamer containing the core methyltransferase recognition sequence, as shown in the Supporting Information) we were able to measure dynamics at many more sites simultaneously than is practical with solid-state deuterium NMR. Relaxation times (T_1, $T_{1\rho}$) were collected at 125 MHz for carbon

[†] Department of Chemistry, University of Washington.
[‡] Department of Biochemistry, University of Washington.
[§] Missouri State University.

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nuclei on the bases (C6, *C*2, and C8), the furanose ring (C1′, *C*2′, C3′, C4′), and the phosphodiester backbone (C5′). In marked contrast to the solid-state results, solution relaxation times along the DNA sequence are remarkably similar for all residues, with the exception of the terminal base-pairs where fraying at the end of the helix leads to increased local mobility (Figure 1c,d). Thus, if we had only conducted solution state NMR relaxation studies, we would have concluded that the *Hha*I methylation target site has a rigid structure. Yet the solid-state data demonstrate that the cytidine that is methylated by *Hha*I experiences considerably increased local conformational fluctuations compared to other residues.

It is very unlikely that the differences between the two techniques are attributable to different local internal dynamics. DNA local motions in the solid state become essentially solution-like as the molecule becomes hydrated to levels used in the present investigation.[11,12] Rather, the two NMR methods differ because motions in the ns−μs time scale responsible for modulation of the solid-state line shapes are not observable in solution relaxation measurements. We calculated rotational correlation functions for the diffusive motion of CD/CH bonds experiencing both local fluctuations at different rates and global rigid-rodlike rotational motion with a correlation time of 3.9 ns, as observed for this DNA (Figure 1b). The correlation function, ($C_{11}(t)$), representative of the anisotropic component of the total correlation function is plotted for various internal diffusion rates. The results underscore the fact that internal motions occurring on time scales longer than the macromolecule tumbling time may not be discerned in the overall correlation function that governs magnetization relaxation in solution and therefore are not easily determined by standard solution relaxation measurements. The motions observed in the solid-state experiments are also too fast to modulate the chemical shift and therefore would also be unobservable in standard relaxation dispersion experiments. However, novel solution NMR experiments designed for slower motions (μs scale) in proteins[15] may extend the time range detectable in these experiments.

In summary, solid-state NMR studies demonstrate that the *Hha*I DNA methylation target site possesses an intrinsically dynamic structure, with sequence specific local motions on a time scale of ∼10 to 100 ns. Measurements of dynamics for several nucleotides in the vicinity of the methylation site identify two residues that experience significant mobility in the absence of the methylase. The ability to observe ns−μs motions of oligonucleotides and proteins renders solid-state NMR an important but underexploited avenue to study biological motion. The quantitative analysis of the line shape results is underway to understand the physical nature of the motions observed in the methyltransferase site and their relationship to enzymatic activity.

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Supporting Information Available: Materials and method and relaxation data for C1′ and base resonances. This material is available free of charge via the Internet at http://pubs.acs.org.

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