The effects of sequence context on base dynamics at TpA steps in DNA studied by NMR

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ABSTRACT

Base dynamics, heretofore observed only at TpA steps in DNA, were investigated as a function of sequence context by NMR spectroscopy. The large amplitude conformational dynamics have been previously observed in TₙAₙ segments where n ≥ 2. In order to determine whether the dynamic characteristics occur in more general sequence contexts, we examined four self-complementary DNA sequences, [d(CTTTANATNTAAAG)₂] (where N=A, C, T, G and N=complement of N). The anomalous broadening of the TpA adenine H₂ resonance which is indicative of large amplitude base motion was observed in all nine unique four nucleotide contexts. Furthermore, all the adenine H₂ resonances experienced a linewidth maximum as a function of temperature, which is a characteristic of the dynamic process. Interestingly, the temperature of the linewidth maximum varied with sequence indicating that the thermodynamics of TpA base dynamics are also sequence dependent. In one example, neither a T preceding nor an A trailing the TpA step was required for base dynamics. These results show that base dynamics, heretofore observed in only a few isolated sequences, occurs at all TpA steps which are either preceded or followed by a thymine or adenine, respectively, and may be characteristic of all TpA steps in DNA notwithstanding sequence context.

INTRODUCTION

Studies of the structure and dynamics in TₙAₙ segments of DNA have followed an initial interest in unusual electrophoretic behaviour of A:T rich sequences of DNA. When AₙTₙ segments are ligated inphase, they experience retarded electrophoretic migration, which has been widely interpreted as a resulting from macroscopic DNA curvature (1,2). Studies involving solution-state NMR led to a better understanding of the molecular dynamics in these sequences although the mechanism for DNA curvature is still not fully understood (3–6). Goodsell and Dickerson have recently developed a program that calculates DNA bending and curvature using three different categories of DNA bending models (7). In an intriguing observation, it was shown that simply reversing the order from AₙTₙ to TₙAₙ abolished the unusually retarded electrophoretic patterns (8) raising questions concerning the structural differences between AₙTₙ and TₙAₙ DNA segments. There are clear differences in secondary structure when AₙTₙ and TₙAₙ DNA segments are compared. In AₙTₙ sequences, the DNA assumes a unique structure referred to as B DNA (9) characterized by a gradual and increasingly compressed minor groove which reaches a minimum at the ApT step. The ApT step is distinguished by slow base pair opening kinetics (10) and by high resistance to hydroxy radical cleavage (11). NMR structural studies of TₙAₙ segments in DNA have been reported for the trp promoter (12) and for several sequences containing restriction sites where cleavage occurs precisely at the TpA step (13,14). From those studies, it was found that the TpA adenine base experiences large amplitude (20–50°) and slow motion (10 ms–1 µs) (14) which is correlated with transitions between several distinct conformational states (15,16). These conformational transitions have also been reported for the mutant trp promoter (17,18). In contrast to the notable chemical properties observed at ApT steps, in TₙAₙ segments, where two segments of B are abutted at a junction defined by the TpA step, the TpA site is a highly reactive center for hydroxy radical cleavage (11), its base pair lifetime is not unusually long (10), and the minor groove reaches a local maximum at the site (14). We (14) and others (13) have observed a number of unusual structural characteristics at the TpA step in TₙAₙ segments of DNA, e.g. a high negative propeller twist, large rise, negative buckle, and large opening (Lingbeck et al., submitted to Biochemistry). These structural features may explain the increased flexibility of TₙAₙ segments in DNA compared with AₙTₙ in studies using Monte Carlo calculations reported by Zhurkin et al. (19). Recently we have investigated the effect of methylation on TpA base dynamics (Lingbeck et al., submitted to Biochemistry) and structural origin of the base dynamics by comparing the structure of [d(CGAGGTITAAACCTCG)]₂ with a derivative methylated at the TpA step. Both the poor base stacking which is associated with the TpA base dynamics and the motional broadening of the adenine-H₂ resonance were abolished upon N6-methylation of the TpA adenine.

The conformational dynamics at TpA steps have been primarily observed in TₙAₙ segments where n ≥ 2 (12,14). Here, in order to determine if the dynamic characteristics occur in more general sequence contexts, we examined four self-complementary DNA sequences, [d(CTTTANATNTAAAG)₂] (where N=A, C, T, G

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and N= complement of N). Our results indicate that TpA base dynamics, heretofore noticed in only a few isolated cases (12,14), occur in all eight sequence contexts where the TpA step is either preceded or followed by a T or A, respectively. In one example, where the TpA step is neither preceded nor followed by an A, base dynamics remain apparent. This indicates that base dynamics may be characteristic of all TpA steps in DNA notwithstanding sequence context. The large amplitude, slow conformational dynamics and distinct structural features observed at TpA steps, which might be considered unusual in random DNA sequences, appear to be 'characteristic' of TpA steps rather than 'unusual' or 'exceptional'. Therefore, it is important to contemplate the broader implications of TpA steps when considering the chemistry and molecular interactions that take place at TpA steps in DNA. Such considerations may be especially important for understanding protein recognition of DNA sequence, e.g. the TATA-box binding protein (TBP) which causes a 'kink' at the first TpA step upon binding to DNA (20). Furthermore, a number of restriction endonucleases recognize DNA segments that contain TpA sites and which cleave the DNA precisely at these steps, e.g. Scal cleaves at the TpA in AGTACT, Rsal cuts at the TpA in GTAC and AblIII cleaves at the TpA in TTATAA. DNA damage occurs with sequence specificity under certain circumstances, e.g. the observation of sequence-dependent levels of UV damage in A:T rich regions of DNA (21). Finally, site-specific drug binding has been observed selectively at TpA steps and not ApT sites in certain DNA sequences (22).

MATERIALS AND METHODS

Sample preparation

The DNA oligomers, [d(CTTAAATTTAAAG)2], [d(CTTTACATGTAAAG)2], [d(CTTTAGATCTAAAG)2] and [d(CTTTATATATAAAG)2] were synthesized on a 10 µmole scale using cyanoethyl phosphoramidites on an Applied Biosystems DNA/RNA synthesizer. The samples were purified by reverse-phase HPLC on a Waters LC4000 and exchanged into 100 mM NaCl, 20 mM phosphate buffer and 0.25 mM EDTA by flow-dialysis. The samples were then lyophilized and redissolved in 600 µl D2O for NMR analysis.

NMR spectroscopy

All temperature studies were performed at 750 MHz using a Varian UNITYplus 750 MHz NMR spectrometer. One-dimensional spectra were collected using 16384 complex points with an acquisition time of 1.024 s and a spectral width of 8000 Hz. The digital resolution was 0.488 Hz/point and the linewidths were measured using the dres command in the VNMR (Varian Associates, Inc.) software package. The dres command uses a width at half height algorithm to calculate linewidths. An inversion recovery pulse sequence was used to acquire partially recovered spectra in order to selectively observe adenine H2 resonances. This method is effective since the T1 values for adenine H2 protons are much longer than other protons in the sample. From these data it was possible to resolve most of the resonances that were overlapped in the fully relaxed spectra. After each temperature adjustment the sample was allowed equilibrate at the new temperature for 10 min before data collection. Linewidths measured by this method were found to be reproducible within an uncertainty of ±1 Hz. The field homogeneity was adjusted until the water resonance was no more than 3–4 Hz. Adenine H2 resonances were assigned from two dimensional NOESY data. 2D NOESY spectra were also acquired at 750 MHz using a spectral width of 7930 Hz, 50–200 ms mixing time and 200–400 τ increments. Data were processed on an SGI Onyx using Felix 2.30 and apodized with a 90° shifted sine bell function matched to the data size in both dimensions.

RESULTS AND DISCUSSION

Large amplitude base dynamics at TpA steps in DNA are most easily detected by the broadening of the intrinsically narrow non-exchangeable adenine H2 resonances at these sites. The linewidth of the adenine H2 resonances is intrinsically narrow relative to all other types of protons in DNA because the H2 proton is relatively isolated from other protons in DNA helix. Consequently, line broadening due to the effects of dipolar or J coupling is minimal. This makes the broadening due to base dynamics easily detectable compared with signals of other protons which have naturally broader resonance linewidths. For example, the adenine H8 proton, whose linewidth is dominated by dipolar broadening and the N3H imino protons, whose linewidth is dominated by chemical exchange, have also been shown to be broadened by TpA base dynamics (14), (Lingbeck et al., submitted to Biochemistry). However, the natural linewidths of the H8 and N3H protons are greater and the change in linewidth due to conformational dynamics is not as large. Alternatively, the base dynamics can be detected by non-mono- tonic changes of the H8 resonance frequency with increasing temperature (15). The dynamic process which gives rise to resonance broadening involves a large amplitude motion of the nucleotide base about the glycosidic bond. The resonance broadening results from ring current fluctuations due to base motion on an intermediate timescale with respect to the chemical shift difference between different conformational states (14). Figure 1 shows how sequence context affects both the broadening
Figure 2. Temperature dependence of adenine H2 linewidths measured at 750 MHz in the following DNA oligomers: (A) A5- and A7-H2 and (B) A11-, A12- and A13-H2 resonances in [d(CTTTAAATTTAAAG)2]; (C) A5- and A7-H2 and (D) A11-, A12- and A13-H2 resonances in [d(CTTTACATGAAAG)2]; (E) A5- and A7-H2 and (F) A11-, A12- and A13-H2 resonances in [d(CTTTAGATCTAAAG)2]; (G) A5-H2 and (H) A11-, A12- and A13-H2 resonances in [d(CTTTATATATAAAG)2].

and chemical shift of TpA adenine H2 resonances in four 14 base pair sequences. The spectra are shown at temperatures corresponding to the TpA adenine H2 linewidth maxima (see below). In the control sequence, [d(CTTAAATTTAAAG)2], which has TpA sites at T4:A5 and at T10:A11, the A5- and A11-H2s were noticeably broadened by motion as expected in comparison to all the other H2s in the sequence (Fig. 1A). For example, notice the difference in linewidths between the A11-H2 and A13-H2 resonances. Further inspection of Figure 1 shows that both the TpA adenine-H2 and other adenine-H2 linewidths vary dramatically depending on the sequence context. This indicates either a significant range in the amplitude and rate of base motion or a variation in the nearest neighbor ring current contributions at the TpA steps in these sequences. Consistent with large ring current contributions to H2 chemical shifts, which are in part responsible for resonance broadening by the mechanism described above, a large variation of H2 chemical shifts is observed with varying sequence context. Two mechanisms are responsible for the observed changes in chemical shifts. First, it is known that proton resonances in duplex DNA change monotonically with tempera-
ture for normal unwinding of the DNA helix (15). This accounts for the observed difference in the observed chemical shift of the A13-H2 resonance in Figure 1A (T = 35°C) compared with that observed in Figure 1B–D (T = 24°C). Secondly, changes in ring current shifts with sequence context cause changes in resonance positions at one temperature, e.g. notice how the A11-H2 resonance changes in Figure 1B–D. Additionally, residual nearest neighbor effects of broadening are observed for some bases which are probably not experiencing local nucleotide dynamics (14), e.g. note that the linewidth of A12-H2 is intermediate between that of A11-H2 and A13-H2.

The sequences, which all have TpA steps at T4:A5 and T10:A11, were designed such that half of the TTTAAA segment in the control sequence is preserved while either the nucleotide preceding or following the TpA step is varied. This allows for the direct investigation of whether the thymine preceding or the adenine trailing the TpA step is required for TpA base dynamics. At the first TpA step which has the sequence context TTATAA, the poly:T region preceding the TpA step is preserved while only the nucleotide following the TpA step is varied. Inspection of Figure 1 shows that the pattern of linewidths varies significantly depending on the identity of the nucleotide following the TpA step. For example, in the control sequence the A5:A6:A7 linewidths vary from 15 Hz at A5 to 4 Hz at A7. This pattern is nearly identical to that observed in the A11:A12:A13 segment (Fig. 1A). However, when A6 is replaced by C6, the A5 is slightly sharper and the A7 resonance is significantly broader (Fig. 1B). The minor sharpening of the A5-H2 could result from a change in the rate or amplitude of base dynamics at the TpA site or from a reduced magnitude of ring current modulation upon substitution of a cytosine for an adenine (23) but the significant broadening of the A7 resonance indicates the onset of motional broadening not seen in the control sequence. It appears that base dynamics similar to those found at TpA steps occur at the CpA step in this sequence context. When A6 is replaced by G6, A7 remains sharp (Fig. 1C) signifying broadening of A7 is specific to the CpA step. When the A6 is replaced by T6, the sequence contains four TpA steps and all four TpA adenine H2 resonances are motionally broadened. It was not possible to resolve the A7- and A9-H2 resonances due to severe overlap but both resonances are clearly broad compared with A12 and A13 (Fig. 1D). At the second TpA site, the context pattern is TNTAAA so that the poly:A run following the TpA step is preserved and only the nucleotide preceding the TpA step is varied. In this sequence context, the A11:A12:A13 linewidths vary from 19 Hz at A11 to 3 Hz at A13. The pattern of line broadening from A11 to A13 is quite constant regardless of the identity of the base preceding the TpA step (Fig. 1B–D).

Resonances broadened due to conformational dynamics such as those found at TpA steps have temperature-dependent linewidths and experience a maximum linewidth at some temperature. Figure 2 shows how the H2 resonance linewidths vary with sequence as a function of temperature. For clarity, the data are segregated into panels showing the behavior of A5-, A6- and A7-H2 linewidths in Figure 2A, C, E and G, and the A11-, A12- and A13-H2 linewidths are shown in Figure 2B, D, F and H. In all sequence contexts, the A5-H2 linewidth undergoes a maximum as a function of temperature, albeit the temperature of the linewidth maximum varies from 24 to 35°C with sequence context. Furthermore, the linewidth at maximum is reduced from ~15 Hz in the control sequence to ~10 Hz when the TpA step is preceded by either C or G whereas the when A6 is replaced by T6, the linewidth maximum remains near 18 Hz. In the control sequence, the linewidth of the A6- and A7-H2 resonances are insensitive to temperature. In contrast, when the TpA is preceded by a cytosine, the A7-H2 linewidth appears temperature dependent compared with the behavior of A7 in either the control sequence or when A7 is preceded by G6. Figure 2G shows that the A5-H2 resonance in the sequence [d(CCTTTATATAAAG)] also experiences a maximum as a function of temperature. In the same sequence, the H2 resonances of the A7 and A9 residues, which also occur at TpA steps, and which are clearly broadened by dynamics (Fig. 1B), were not resolvable, hence only one resonance is shown in Figure 2G.

When the temperature-dependence of linewidth of the H2 resonances of A11, A12 and A13 is compared (Fig. 2B, D, F and H), as expected the linewidth of the A11-H2 resonance experiences a maximum as a function of temperature in all sequences. The temperature of the linewidth maximum varied however from 20 to 35°C depending on sequence context and the magnitude of the linewidth maximum varied from ~14 to ~19 Hz. Interestingly, in all other sequence contexts, the temperature range over which the linewidth was noticeably affected was quite constant at nearly 30°C in contrast with the relatively narrow range of ~10°C for the A11-H2 in the CTTTTATATATAAG sequence. This may indicate the presence of concerted dynamics occurring at TATA(14) sites. In all the sequences, the A12-H2 linewidth is much narrower than that of A11 but it shows a similar, but smaller in magnitude, temperature dependence. This is due to a ‘nearest-neighbor’ effect due to residual ring current fluctuations at the TpA (14).

In summary, resonance broadening characteristic of base dynamics was found at the TpA steps in all nine possible nucleotide contexts studied. This demonstrates that large amplitude base dynamics can be expected at all TpA steps in DNA when a thymine precedes, or when an adenine follows, a TpA step. In CTTTTATATATAAG which contains four TpA steps, two of which have an ATAT sequence context, all H2 resonances were broadened and temperature dependent indicating that a preceding T or a trailing A is not always required for TpA base dynamics. Elsewhere in a report of the structure of a DNA octamer, (24) [d(GTAATG)]-[d(CATTATAC)] H2 resonance broadening was reported at both the T4:A5 and T12:A13 steps, illustrating that dynamics occurs at the TpA in both ATAT and TTAT sequence contexts. These combined observations imply that conformational dynamics may occur at all TpA steps in DNA. We are currently investigating the immediate sequence context dependence of TpA base dynamics in sequences of the form N1TAN2 where both of the base preceding and following the TpA step are independently varied. We will report on these studies elsewhere. Collectively, these results indicate that conformational dynamics at TpA steps are more widespread in DNA sequences than considered in the past. While the evidence of dynamics at TpA steps is clear in all sequence contexts studied, subtle variations in TpA adenine H2 linewidths, temperatures of linewidth maxima, widths of the dynamic transitions and the magnitude of nearest neighbor effects are also evident. This is especially true when the nucleotide following the TpA step is varied, e.g. the TpA adenine H2 resonance sharpens noticeably when C or G follows the TpA step. In contrast, when the residue preceding a TAA triplet is varied the base dynamics are quite insensitive to the identity of the preceding base. This implies that the adenine residue following a TpA step is more critical for the observation of base dynamics than the identity of the residue preceding a TpA step. This is probably due to the large ring current
contribution from base stacking with an adenine (23). These results show that TpA base dynamics can be expected at all the sequence contexts studied here. The variation in the temperature and the magnitude of the linewidth maxima reflect changes in the ring current contributions of neighboring residues and could also indicate that the amplitude, rate and thermodynamics of base motion at TpA steps in DNA are dependent on sequence context. Disentangling the details of the conformational dynamics will require additional investigation.

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