Effect of the Crystallizing Agent 2-Methyl-2,4-pentanediol on the Structure of Adenine Tract DNA in Solution†

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Received July 29, 1996; Revised Manuscript Received September 12, 1996⊗

ABSTRACT: The hydroxyl radical cleavage pattern of bent DNA containing phased adenine tracts has a sinusoidal pattern. Changes in the cleavage pattern of the sequence A_5N_5 in the presence of 2-methyl-2,4-pentanediol (MPD) demonstrate that MPD has an effect on the structure of a bent A-tract region of DNA. The effect of MPD on DNA structure appears to be limited to bent A-tract regions, as we find no significant changes in the cleavage pattern of flanking regions, mixed-sequence DNA, or the unbent A-tract sequence $T_4A_4N_2$.

The structural basis of bending in DNA containing phased adenine tracts is a topic continuously debated. A recent point of contention is the location of the bend in bent DNA. The results of solution-based experiments have been used to infer that the bend is centered in the A-tracts (Hagerman, 1985; Koo et al., 1986; Ulanovsky & Trifonov, 1987). Analysis of crystal structures of A-tract-containing oligonucleotides has led Dickerson and colleagues to the opposite conclusion. They assert that the bend occurs in the G/C-rich regions between A-tracts (Dickerson et al., 1996) and that an adenine tract is in fact straight.

In their report of the crystal structure of an A-tract oligonucleotide, DiGabriele and Steitz (1993) pointed out that the solution and crystal structures of a DNA molecule might indeed be different, because of the presence of the precipitating agent 2-methyl-2,4-pentanediol (MPD)[1] in solutions used to crystallize DNA. This hypothesis was tested by Harvey and co-workers (Sprous et al., 1995), who found that the electrophoretic mobility of DNA containing phased A-tracts is increased in the presence of MPD. As the concentration of MPD was varied over the range 0%−30%, the gel mobility anomaly of A-tract DNA decreased. The idea that MPD affects the curvature of A-tract sequences has gained further support from cyclization kinetics and electron microscopic experiments (Harvey et al., 1995).

The solution-phase experiments performed to date to test the effect of MPD on DNA bending are sensitive to global structural features of the DNA molecule. So while these experiments demonstrate the effect of MPD on the curvature of A-tract-containing DNA, it is difficult to show directly that the structural effect of MPD is localized to the adenine tracts.

Local structural details of bent DNA can be studied using chemical probe methods. We have found previously that the hydroxyl radical produces a highly characteristic cleavage pattern for bent A-tract DNA (Burkhoff & Tullius, 1987). While the frequency of cleavage of mixed-sequence DNA is relatively even, the cleavage pattern of DNA containing a series of phased A-tracts is strikingly modulated. The key feature of the unusual cleavage pattern of bent DNA is a decrease in cleavage from the 5′ to the 3′ end of an A-tract. The frequency of cleavage then increases back to that of mixed-sequence DNA in the flanking base pairs. We have interpreted this cleavage pattern as reflecting the structural consequences of DNA bending through narrowing of the minor groove in the A-tract, and the relative opening of the minor groove in the surrounding mixed-sequence DNA (Burkhoff & Tullius, 1987). A normal cleavage pattern is found for other A-tract-containing DNA molecules that are not bent (Burkhoff & Tullius, 1988; Price & Tullius, 1993).

Here we use the hydroxyl radical to examine the effect of MPD on the structure of A-tract DNA. We obtained hydroxyl radical cleavage patterns for a "classical" bent A-tract (A_5N_5), an A-tract sequence having normal gel mobility ($T_4A_4N_2$), and a mixed DNA sequence, in various concentrations of MPD. We demonstrate that MPD has a specific and dramatic effect on the cleavage pattern of only the bent A-tract sequence. Our results suggest that caution be exercised in extrapolating crystallographic results for A-tract DNA to the structure of bent DNA in solution.

MATERIALS AND METHODS

The construction of pUC18-based plasmids containing inserts of four repeats of either A_5N_5 or $T_4A_4N_2$ has been described previously (Price & Tullius, 1993). A 260 bp *Acc*I-*Pvu*II restriction fragment containing an A-tract insert was 3′-radiolabeled at the *Acc*I site using [α-^{32}P]dCTP and the Klenow fragment of DNA polymerase I. The mixed-sequence DNA molecule we used was the 192 bp *Nde*I-*Eco*0109I restriction fragment from pUC18, 3′-radiolabeled at the *Nde*I site using [α-^{32}P]dTTP.

2-Methyl-2,4-pentanediol was obtained from Aldrich (Milwaukee, WI). Stock solutions of 10%, 20%, 30%, and 40% (volume/volume) aqueous MPD were prepared by serial dilution using water purified with a MilliQ system.

The hydroxyl radical cleavage reaction was carried out on radiolabeled DNA (10 000 dpm) dissolved in 20 μL of a buffer consisting of 50 mM Tris-HCl and 50 mM NaCl (pH 8.0). To this solution was added 50 μL of either a stock

† This research was supported by PHS Grant GM 40894.

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⊗ Abstract published in *Advance ACS Abstracts,* October 15, 1996.

[1] Abbreviations: bp, base pair(s); EDTA, ethylenediaminetetraacetic acid; MPD, 2-methyl-2,4-pentanediol; NMR, nuclear magnetic resonance; Tris, tris(hydroxymethyl)aminomethane.

S0006-2960(96)01880-6 CCC: $12.00 © 1996 American Chemical Society

solution of MPD, or water. The hydroxyl radical-generating reagents were then added to the DNA solution to final concentrations of 50 μM Fe^{2+}, 100 μM EDTA, 0.03% H_2O_2, and 1 mM sodium ascorbate. The volume of the reaction mixture was 100 μL. The reaction was stopped after 2 min by the addition of 100 μL of a solution of 0.0135 M thiourea, 0.0135 M EDTA, and 0.6 M sodium ascorbate. The cleaved DNA was precipitated, subjected to denaturing gel electrophoresis, and analyzed by methods described previously (Price & Tullius, 1993).

RESULTS AND DISCUSSION

We first tested the effect of MPD on the cleavage pattern of A_5N_5, a highly curved DNA sequence. In previous electrophoretic mobility experiments this sequence was found to migrate anomalously slowly, with a relative mobility (R_L) value of 2.07 for a 150 bp fragment (Koo et al., 1986). As seen in Figure 1, MPD dramatically changes the hydroxyl radical cleavage pattern across the A-tract region in the A_5N_5 sequence. The cleavage pattern shows smooth and progressive differences as the MPD concentration is varied from 0% to 20%, in steps of 5%. We note that the minimum concentration of MPD used to crystallize DNA is around 20% (Dickerson et al., 1996).

The sinusoidal hydroxyl radical cleavage pattern in A-tract DNA reflects changes in the width of the minor groove and correlates with bending (Burkhoff & Tullius, 1987, 1988; Price & Tullius, 1993). Elimination of the sinusoidal cleavage pattern as more MPD is added is consistent with a return of the minor groove width to that found in mixed-sequence B-form DNA. As changes in the hydroxyl radical cleavage pattern appear first at the 3′ end of the A-tract, where NMR experiments have shown the minor groove is the narrowest (Kintanar et al., 1987), it is possible that the DNA is straightening as a result of an increase in repulsion between opposite-strand phosphate groups in the presence of MPD. The cleavage pattern outside of the A-tract region remains relatively unchanged in the different concentrations of MPD.

The sequence $T_4A_4N_2$ has been shown to migrate normally on a native polyacrylamide gel (Hagerman, 1986), even though it contains phased adenine tracts. As reported earlier (Burkhoff & Tullius, 1988; Price & Tullius, 1993), the hydroxyl radical cleavage pattern of this sequence is similar to that of mixed-sequence DNA, showing only a slight variation in cleavage (Figure 2). We find that the effect of MPD on the cleavage pattern of this sequence is limited. Therefore while MPD has a substantial effect on the cutting pattern of a bent A-tract sequence, it has only a small effect on a straight A-tract sequence. Likewise, mixed-sequence DNA shows no significant changes in cleavage pattern in the presence of MPD (Figure 3). From these results we conclude that MPD does not dramatically affect the structure of a DNA molecule that does not contain a bent A-tract. Our results for the A_5N_5 sequence and for mixed-sequence DNA are consistent with the observations of others using a variety of techniques sensitive to DNA structure and bending (Sprous et al., 1995; Harvey et al., 1995).

While this manuscript was in preparation, Dlakic et al. (1996) reported the use of several experimental approaches to investigate the effect of MPD on the structure of A-tract DNA. An experiment involving hydroxyl radical cleavage of A-tract DNA was included in their study, and our results are in agreement with theirs. However, the present work includes a more comprehensive series of experiments which clearly demonstrate that the effect of MPD is isolated to bent A-tract regions.

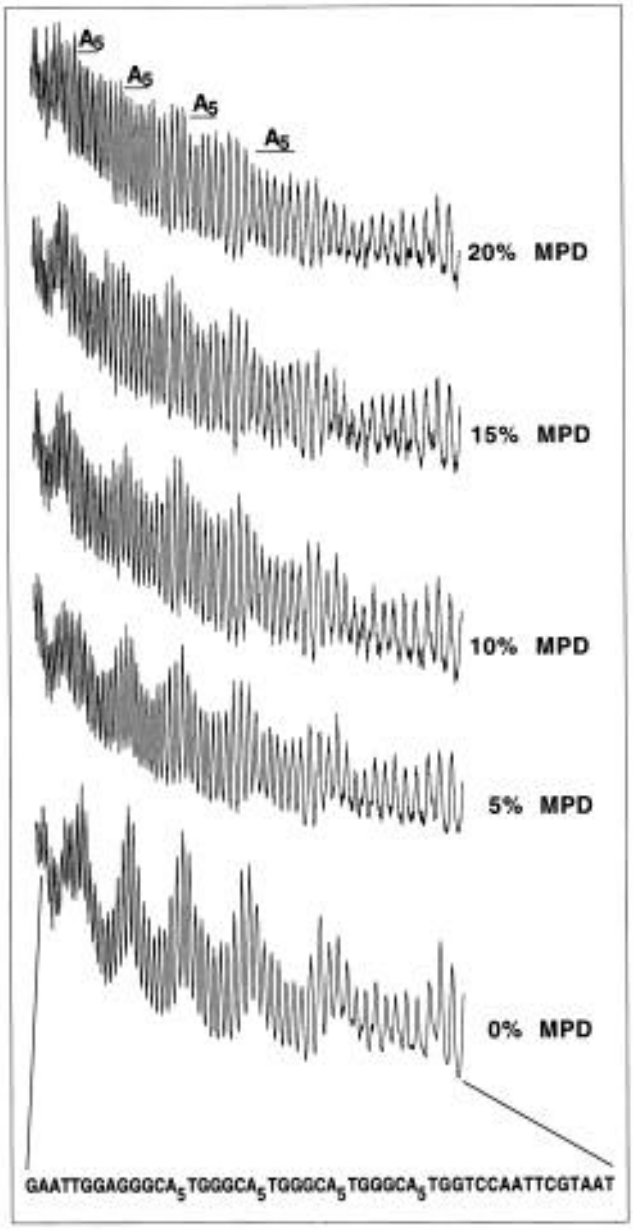

FIGURE 1: Hydroxyl radical cleavage patterns of the bent A_5N_5 DNA sequence. One-dimensional line scans were generated from a phosphorimage of a denaturing gel, using the ImageQuant software package included with the PhosphorImager (Molecular Dynamics, Sunnyvale CA). Labels to the right of the scans indicate the concentration of MPD at which the cleavage pattern was obtained. The locations of the A-tracts are indicated by bars above the top scan. The sequence of this segment of the DNA molecule is shown below the scans. The sequence reads 5′ to 3′, left to right.

MPD is thought to affect the hydration of proteins (Timasheff, 1993), and may affect DNA similarly. It has been suggested that MPD might promote formation of a spine of hydration and thus lead to the straight A-tracts seen in crystal structures (DiGabriele & Steitz, 1993). However, and as DiGabriele and Steitz also pointed out, NMR studies have shown that the residence lifetimes of certain water molecules found in the minor groove of A-tract DNA are quite long (Liepinsh et al., 1992), suggesting that a spine of hydration

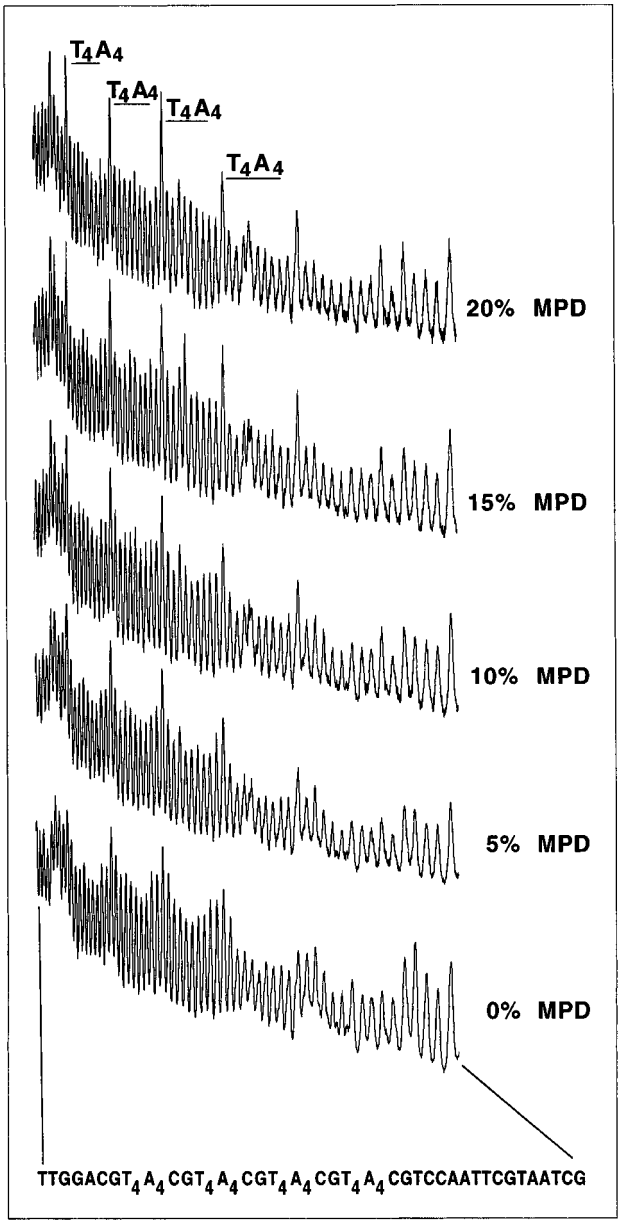

FIGURE 2: Hydroxyl radical cleavage patterns of the straight A-tract sequence $T_4A_4N_2$ in various concentrations of MPD.

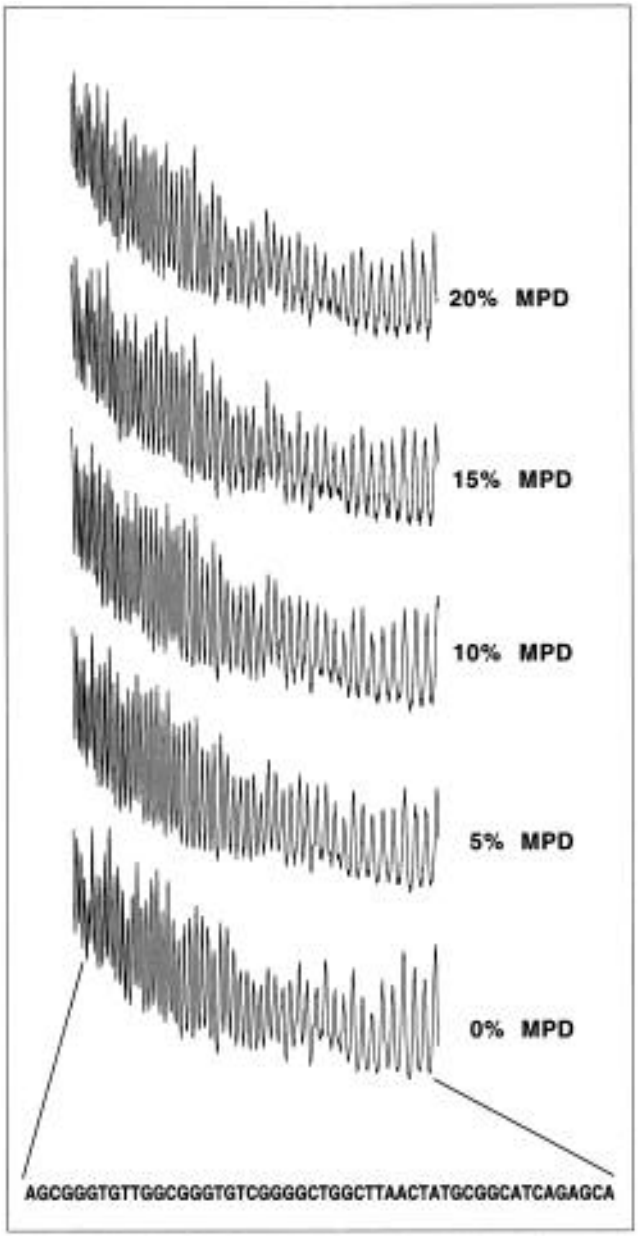

FIGURE 3: Hydroxyl radical cleavage patterns of mixed-sequence DNA in various concentrations of MPD.

also is present in solution in the absence of MPD. Our results indicate that MPD influences the structure of DNA only in regions containing bent A-tracts, where structure may be mediated by the degree of hydration.

CONCLUSIONS

We have shown that in the presence of MPD the hydroxyl radical cleavage pattern of a bent A_5N_5 DNA sequence is dramatically changed, becoming like that of mixed-sequence unbent DNA. Control experiments on a straight A-tract sequence, $T_4A_4N_2$, and on mixed-sequence DNA, show little effect of MPD on the cleavage pattern. Our results therefore indicate that the structure of the adenine tract itself is unusual and is directly related to DNA bending. At concentrations of MPD typically used to crystallize DNA the structure of the adenine tract in solution changes. While it is not possible to show that the major species in solution is in fact the one that crystallizes, our results do counsel caution in using crystal structures to determine the structural basis of DNA bending in solution.

ACKNOWLEDGMENT

We gratefully acknowledge the use of densitometry instrumentation maintained by the Institute for Biophysical Research on Macromolecular Assemblies at Johns Hopkins University, which was supported by an NSF Biological Research Centers Award (DIR-8721059) and by a grant from the W. M. Keck Foundation. We also appreciate helpful conversations with Mensur Dlakic and Rod Harrington.

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BI961880Q