Increased Temperature and 2-Methyl-2,4-pentanediol Change the DNA Structure of both Curved and Uncurved Adenine/Thymine-Rich Sequences†

Niels Erik Møllegaard* and Peter E. Nielsen

Department of Medical Biochemistry and Genetics, The Panum Institute, University of Copenhagen, Blegdamsvej 3c, 2200 Copenhagen N, Denmark

Received February 26, 2003; Revised Manuscript Received May 26, 2003

ABSTRACT: DNA curvature is affected by elevated temperature and dehydrating agents such as 2-methyl-2,4-pentanediol (MPD) (used in crystallization). This effect of MPD has been ascribed to a specific distortion of the structure of adenine tracts (A-tracts), probably through a deformation of the characteristic narrow minor groove. Uranyl photoprobing indicates that a narrowed minor groove is present in all A/T regions containing four or more A/T base pairs. Consequently, this technique may be employed to study conformational changes in other A/T-rich sequences than pure A-tracts. In this study we use uranyl photoprobing to demonstrate that the effect of elevated temperature and MPD is analogous on both "normal" and curve-inducing A/T-rich sequences. The results therefore indicate that under these conditions the minor groove is widened in all A/T sequences and not only in pure A-tracts as previously suggested. Thus, the rather subtle structural difference of AT regions and A-tracts in nonbent DNA versus A-tracts in bent DNA may be quantitative rather than qualitative; i.e., the structure is more persistent and/or rigid in bent DNA.

DNA curvature has been studied for more than a decade but is still not fully understood. It is generally agreed that short runs of adenines (A_n-tracts,[1] $n \geq 4$) positioned at integral turns in the DNA double helix cause macroscopic curvature of the DNA molecule by additive contributions of the helically phased A-tracts. Originally, DNA curvature was detected by anomalously slow migration of DNA fragments containing A-tracts in gel electrophoresis studies (*1*, *2*).

Several models have been put forward to explain the effect of A-tracts on DNA curvature. On the basis of data from solution studies it was proposed that bending is either a result of axial deflections of AA/TT dinucleotide elements in the A-tracts (*3*, *4*) or a result of structural discontinuities at the junction of a straight non-B-form A-tract and a straight normal B-form DNA (*1*). In contrast, the "non-A-tract model" proposes that it is the general mixed sequences between rigid and straight A-tracts that are responsible for DNA curvature, because of phasing of additive roll angles in the general sequence B-DNA. This model has largely been based on crystallographic results (*5*−*8*).

Most studies, however, support the existence of an unusual A-tract structure, containing propeller-twisted base pairs with bifurcated hydrogen bonds and a narrow minor groove (*7*, *8*). The narrow minor groove is believed to be a prerequisite for the special A-tract structure that also results in a characteristic spine of hydration in the minor groove (*9*, *10*).

The dehydrating agent 2-methyl-2,4-pentanediol (MPD), often used in crystal preparation for X-ray crystallography, reduces the anomalous slow gel migration of A-tract DNA fragments (*11*). Different experimental strategies have been employed to address mechanistically this effect of MPD on DNA bending to A-tracts (*12*). Specifically, the effect of MPD on A-tracts has been studied by hydroxyl radical cleavage, showing that MPD dramatically changed the cleavage pattern of a bent A_5N_5 sequence. In contrast, hydroxyl radical cleavage of the straight A-tract sequence $T_4A_4N_2$ and mixed sequences was not affected by MPD. It was concluded that solely A-tract structures of curved DNA are distorted by MPD, thereby providing evidence against the non-A-tract bending model (*12*, *13*).

Gel mobility anomaly of A-tracts is also strongly reduced at elevated temperatures, indicating that DNA curvature diminishes (*14*). By using CD spectroscopy and scanning calorimetry, it has been shown that the non-B-form structure of A-tracts is a distinct low-temperature form, which is disrupted at a premelting transition around physiological temperature, forming a structure similar to general sequence B-DNA (*15*−*17*). This was shown to be the case only for curved DNA including A-tracts, since this premelting event was absent in the uncurved GT_4A_4C sequence and in alternating $(AT)_n$ sequences (*16*−*18*). The characteristic hydroxyl radical cleavage pattern of A-tracts likewise disappears at temperatures higher than 40 °C (*19*).

The effects of elevated temperature and dehydrating agents on DNA curvature have been interpreted as a distortion of the spine of hydration in A-tracts with a concomitant alteration of the DNA structure which eliminates curvature (*12*, *13*, *18*, *20*). Accordingly, it is assumed that a wider

† This work was supported by the Novo Nordisk Foundation, the Lundbeck Foundation, and the Danish Medical Research Council.

* Address correspondence to this author. Phone: +45 35327778. Fax: +45 35396042. E-mail: nem@imbg.ku.dk.

[1] Abbreviations: A-tracts, adenine tracts; MPD, 2-methyl-2,4-pentanediol; NaOAc, sodium acetate.

10.1021/bi0343267 CCC: $25.00
Published on Web 06/26/2003

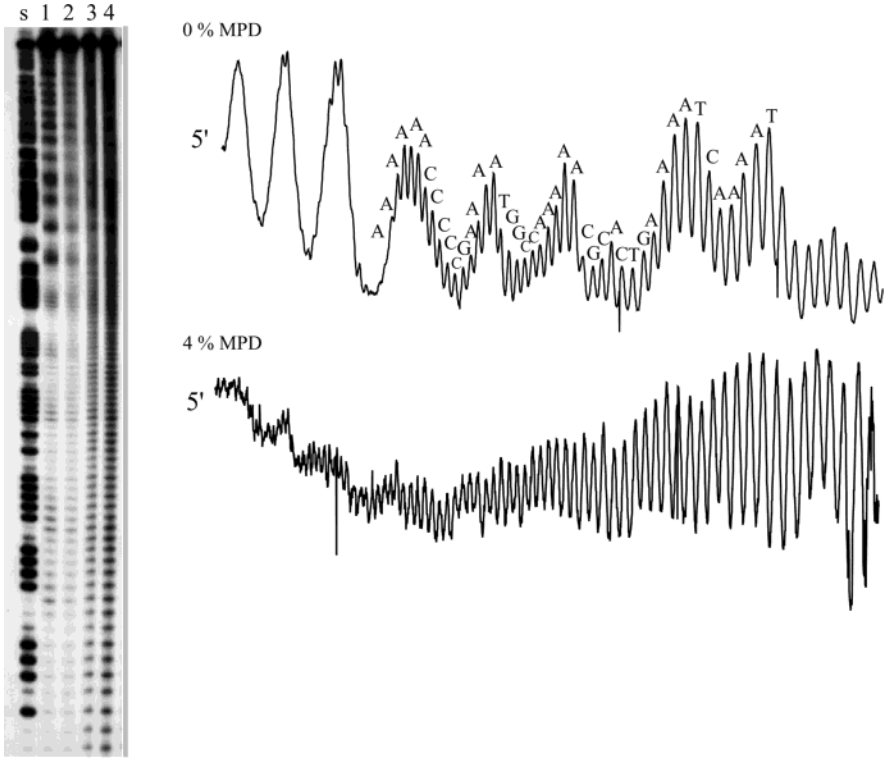

FIGURE 1: MPD effect on uranyl cleavage of kinetoplast DNA. Autoradiograph showing uranyl cleavage at 0%, 2%, 4%, and 8% MPD (lanes 1−4). s is an A/G sequence reaction. Densitometric scan of the autoradiograph showing uranyl reactions in the absence and in the presence of 4% MPD. Uranyl cleavage of DNA is highly dependent on pH in the medium. If the pH is kept below 7, the characteristic pattern presented here appears. However, if the pH is higher than 7 the cleavage is almost equal at all backbone positions. It is therefore important to note that MPD does not affect the pH in the medium. Furthermore, MPD is present at lower concentrations than the one used for crystallization, which is typically 20% (*21*).

minor groove in A-tracts is produced in the presence of MPD and at elevated temperatures, primarily due to a reduction of the base pair propeller twist (*21*).

The structure of A-tract DNA has been intensively studied at the single nucleotide level by hydroxyl radical probing. This method reveals strong cleavage at the 5′ end and weaker cleavage at the 3′ end of A-tracts, which is interpreted to reflect narrowing of the minor groove from the 5′ end toward the 3′ end (*13*). Typically, an unusual hydroxyl radical cleavage pattern is only found in pure A-tracts, whereas a normal cleavage pattern is found in mixed sequences and in A-tracts of noncurved DNA as, for instance, the 5′-TTT-TAAAA sequence. According to this analysis a narrow minor groove is exclusively present in A-tracts of curved DNA (*22*, *23*).

Uranyl photocleavage efficiency has been related to the minor groove width as well, but in contrast to hydroxyl radical probing, hyperreactivity is observed in A/T-rich sequences in general when four or more contiguous adenines/thymines are present. Furthermore, cleavage is progressively increasing toward the 3′ end of A-rich sequences, consistent with a progressive narrowing of the groove toward this end. Interestingly, uranyl hyperreactivity seems to be present in regions containing any combination of four or more adenines/thymines, suggesting that a narrow minor groove is not solely characteristic of pure A-tracts (*24*, *25*). Therefore, uranyl photocleavage can be used to study conformational changes of noncurved A/T regions as well, which is not possible with the hydroxyl radical method.

In the present study we have used uranyl photocleavage as a tool for examination of the effects of temperature and MPD on DNA curvature by comparing uranyl cleavage of curved and noncurved A/T-rich sequences.

MATERIALS AND METHODS

Plasmids. The kinetoplast DNA plasmid pCAT/210 was a gift from Dr. Paul Englund, Baltimore, MD. Construction of pAT_4d is described in ref *25*. The pA_4T_4 and pT_4A_4 were constructed by cloning a multimer sequence of four repeats in the *Bam*HI site of pUC19.

Preparation of Labeled DNA Fragments. pCAT/210 was digested with *Sal*I and radioactively labeled with [α-^{32}P]-dCTP using the Klenow DNA polymerase fragment. Restriction enzyme cutting with *Sst*I results in a fragment of 226 base pairs.

Labeling of the 5′-strand was done by treatment with alkaline phosphatase before labeling with [γ-^{32}P]ATP using polynucleotide kinase. After labeling the linearized plasmid was cleaved with the *Sst*I.

pAT_4d, pA_4T_4, and pT_4A_4 were digested with *Eco*RI, labeled with [α-^{32}P]dATP, and digested with *Pvu*II, resulting in restriction fragments of 294 bp (pAT_4) and 280 bp (pA_4T_4 and pT_4A_4).

The DNA fragments were isolated on 5% native polyacrylamide gels and eluted from the gel slice by diffusion in 90 mM Tris−borate and 1 mM EDTA, pH 7.5.

Uranyl Photocleavage. Purified restriction fragments were subjected to uranyl photocleavage in a volume of 100 μL containing 50 mM NaOAc, pH 6.2, and 1 mM uranyl nitrate. The samples were irradiated in open tubes placed just below a 40W/03 Phillips fluorescent light tube (maximum emission

at 420 nm). After 20 min of irradiation, NaOAc, pH 4.5, was added to a final concentration of 0.2 mM together with 2.5 volumes of ethanol. The tubes were placed on ice for 15 min and thereafter centrifuged for 15 min. The dried pellet was dissolved in 6−10 μL of formamide, 90 mM Tris−borate, and 1 mM EDTA, pH 8.3, containing xylene cyanol and bromophenol blue, and the samples were heated at 90 °C before loading 2 μL onto a 10% denaturing polyacrylamide gel [19:1 acrylamide to methylenebis(acrylamide)]. MPD was added to the indicated concentration before addition of uranyl and subsequent irradiation. Uranyl cleavage at different temperatures was performed by placing samples in a heating block positioned below the light tube. Uranyl was added when the samples had the temperature as indicated in the figure legends. The autoradiograms were obtained by overnight exposure using intensifying screens. A Molecular Dynamics laser densitometry scanner was used to collect data from autoradiographs. Scans were analyzed by using ImageQuant software.

RESULTS

Initially, we examined the effect of MPD and temperature on uranyl photocleavage of a curved kinetoplast DNA fragment that previously has been studied in great detail in terms of DNA curvature (*1*). The results of uranyl photocleavage of the 226 bp *Sal*I−*Sst*I fragment from the kinetoplast sequence containing pPK210/CAT plasmid is shown in Figure 1. The cleavage pattern in the absence of MPD of the A/T-rich regions surrounded by mixed sequence DNA is shown in lane 1 and the upper scan. Inspection of the result reveals that as previously reported (*24*, *25*) uranyl cleavage is significantly enhanced in A/T-rich regions with a maximum around the fourth base from the 5′ end, whereas cleavage probability falls to a minimum within the mixed sequence G/C DNA. This is exactly the pattern expected if minor groove width is the determinant of uranyl cleavage (*26*). Therefore, this cleavage modulation is consistent with the presence of a minor groove narrowing toward the 3′ end in the A/T-rich sequences and a relatively wider minor groove in the mixed sequence DNA. Interestingly, the presence of MPD virtually eliminates the cleavage difference between A/T sequences and general sequences (Figure 1, lanes 2−4 and lower scan). This indicates that a significant structural change in the DNA takes place in the presence of MPD, resulting in an overall evening of the minor groove width.

The effect of temperature on uranyl photocleavage of the kinetoplast fragment is shown in Figure 2. It is clearly demonstrated that uranyl photocleavage pattern is highly temperature dependent. When cleavage is performed at 35 °C, the difference between high and low reactivity along the DNA becomes less pronounced than at 25 °C, and at 45 and 55 °C the cleavage is practically equal at all nucleotide positions. This is in agreement with the results obtained by hydroxyl radical cleavage and supports the conclusion that the sequence-dependent difference in minor groove width is disappearing when the temperature increases (*19*).

The presence of an unusual hydration pattern in other A/T sequences than curved A-tracts has been suggested by NMR studies (*27*, *28*) and X-ray analyses, which may indicate that a narrow minor groove is not solely found in curved A-tracts

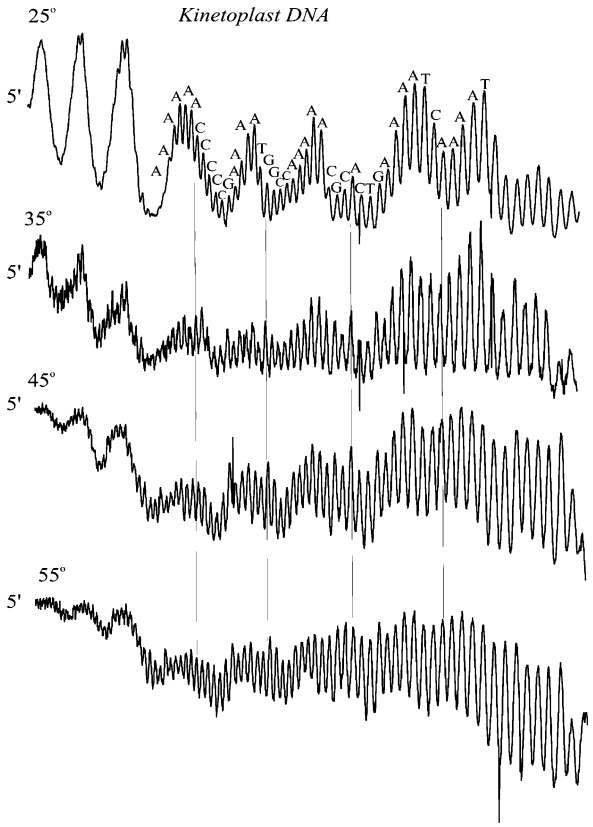

FIGURE 2: Effect of temperature on uranyl cleavage of a kinetoplast DNA fragment. Densitometric scans of an autoradiograph from an experiment analyzing uranyl cleavage at the temperatures indicated. The DNA sequence is annotated on the scan.

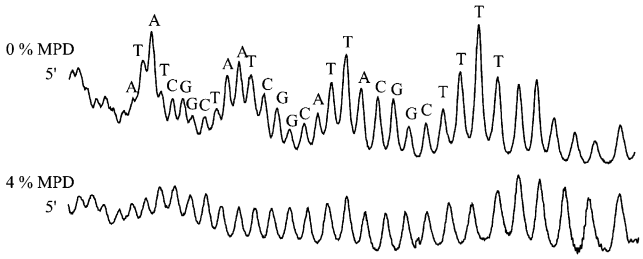

FIGURE 3: MPD effect on uranyl cleavage of various $(A/T)_4$ sites. Densitometric scans of an autoradiograph are shown.

(*29*, *30*). However, spectroscopic and calorimetric studies point out that an unusual hydration pattern is exclusively present in curved A-tract sequences (*15*−*17*).

The effect of MPD and elevated temperature on uranyl cleavage of a noncurved DNA fragment was also examined. This fragment contains several $(A/T)_4$ base pair sequences (including TA steps) with CGGC intervening sequences. We have previously shown that uranyl photocleavage of this DNA exhibits hyperreactivity in the (A/T) tracts and concluded from this observation that a narrowed minor groove is present in all sequences containing four (or more) adenines/thymines (*25*). The effect of MPD on uranyl cleavage of the fragment from pAT_4d is shown in Figure 3. The typical hyperreactivity pattern in the A/T-containing regions completely disappears in the presence of 4% MPD.

In Figure 4 the temperature effect on cleavage of the same fragment is shown. When the cleavage is performed at 25 °C, a clear modulation of the cleavage is present with the

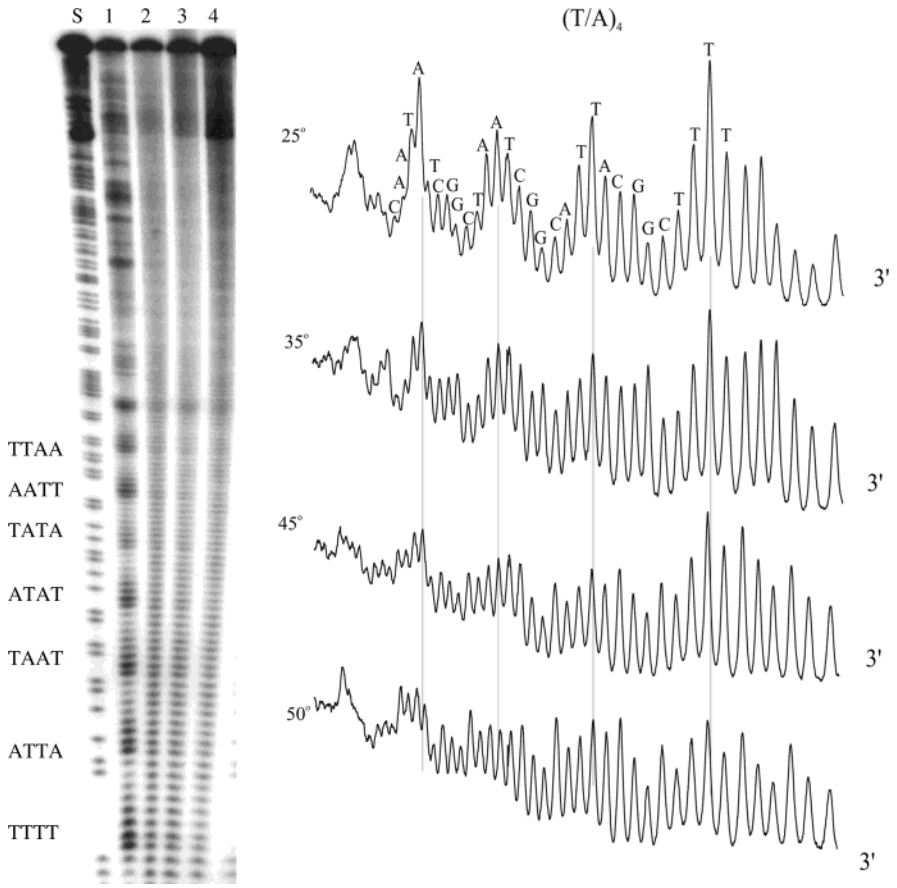

FIGURE 4: Effect of temperature on uranyl cleavage of various $(A/T)_4$. The autoradiograph shows uranyl cleavage at 25, 35, 45, and 50 °C. Sequences of the eight $(A/T)_4$ regions are indicated. Corresponding densitometric scans of an autoradiograph are shown to the right. (The autoradiograph and the scans are from two independent experiments).

characteristic hyperreactivity in the A/T sequences (lane 1 and the upper scan). However, as observed for the curved kinetoplast fragment, the cleavage modulation slowly disappears with increasing temperature, and at 50 °C the cleavage is virtually equal at all nucleotide positions (lanes 2−4 and the lower scans). Surprisingly, this shows that the effect of MPD and temperature on uranyl cleavage hyperreactivity characteristic for AT sequences is not restricted to the curved kinetoplast fragment.

Finally, we asked whether MPD and increased temperature have a similar effect on uranyl cleavage of pure A-tracts when these are present in a curved as compared to a noncurved DNA context. Intensive studies of curved DNA on the basis of A-tracts have focused on the 5′-AAAATTTT sequence, which has been shown to induce abnormal DNA migration, and the 5′-TTTTAAAA sequence, which does not (*31*). When uranyl cleavage of fragments containing these two sequences is performed in the absence of MPD, the regions of A/T base pairs are characterized by local uranyl cleavage maxima in both sequences. However, it is noted that the positions of the maxima are very different in the two sequences. Uranyl cleavage is continuously increasing through the 5′-AT step of the A_4T_4 sequence whereas cleavage is abruptly decreasing at the 5′-TA step of the T_4A_4 sequence (the implications of this will be discussed in a subsequent paper). However, it is clearly demonstrated that MPD modifies the cleavage pattern for both sequences. When cleavage is performed in the presence of 2% MPD, the pattern of uranyl cleavage is changed more dramatically in the curved T_4A_4 sequence than for the noncurved A_4T_4 sequence. But at a concentration of 4% the cleavage is without any particular sequence preference in both cases. Clearly, the uranyl cleavage modulation disappears in both the curved and noncurved A-tract-containing sequences. The effect of MPD on hydroxyl radical cleavage of T_4A_4 and A_4T_4 sequences has previously been analyzed. In accordance with uranyl probing the hydroxyl radical cleavage pattern characteristically for a narrow minor groove disappears in bent A-tracts like A_4T_4 sequences. Since hydroxyl radical cleavage is without any particular modulation in the nonbent T_4A_4 sequences, it was not possible to detect any effect of MPD [*12*, *13*; we have also reproduced these results (data not shown)].

Uranyl photocleavage of A_4T_4 and T_4A_4 at different temperatures was also performed. In Figure 6 cleavage at 25, 35, 45, 55, and 65 °C is represented by scans which represent the cutting frequencies. When cleavage is done at temperatures increasing from 25 to 65 °C, uranyl hyperreactivity in the A/T regions disappears gradually in both the curved and the noncurved DNA at increasing temperatures. However, it is noted that the effect of increasing temperature is observed at a significantly lower temperature for the noncurved T_4A_4 sequence than for the curved A_4T_4 sequence. Comparing uranyl cleavage at 35 and 45 °C illustrates this. This indicates a more resistant structure in the curved DNA. These results therefore suggest that the difference in response to dehydrating agents and temperature between A-tracts present in curved versus noncurved DNA

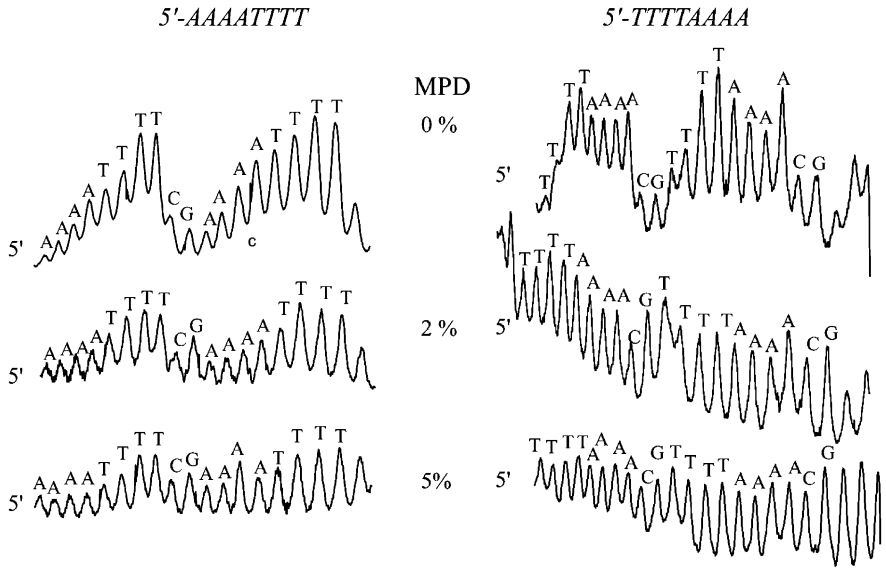

FIGURE 5: MPD effect on uranyl cleavage of A_4T_4 and T_4A_4. Densitometric scans of an autoradiograph from an experiment analyzing uranyl cleavage in the absence or in the presence of 2% or 4% MPD are presented.

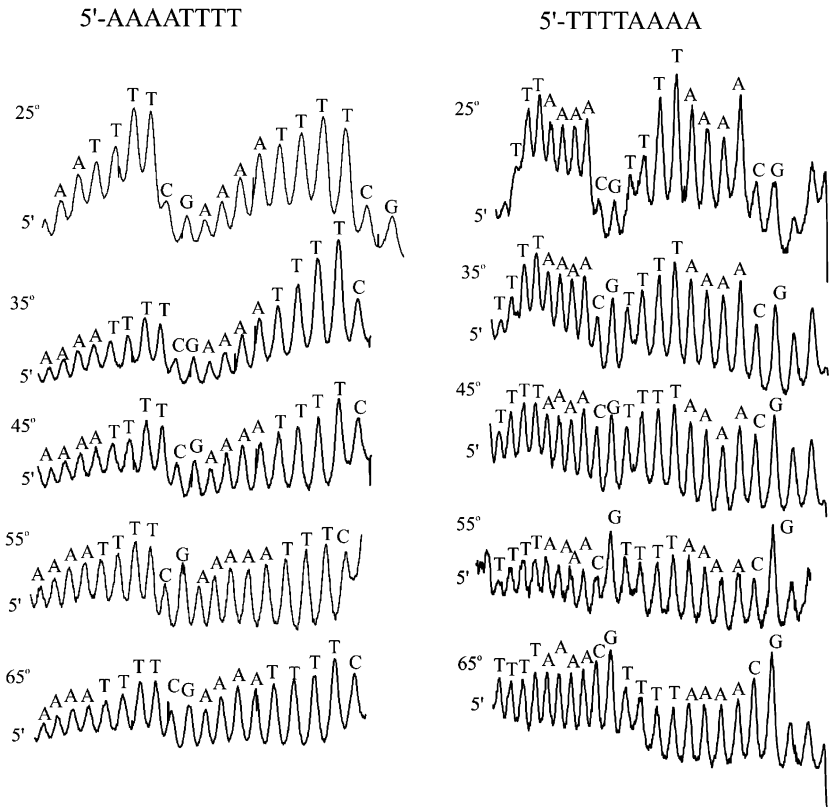

FIGURE 6: Effect of temperature on uranyl cleavage of A_4T_4 and T_4A_4 sequences. Densitometric scans of autoradiographs are presented with temperatures and sequences indicated.

in terms of uranyl sensitivity and thus minor groove width is quantitative rather than qualitative.

DISCUSSION

From the accumulated evidence, it is indisputable that A-tracts in bent DNA have a peculiar conformation distinctly different from that of normal B-DNA and also from that of A-tracts in nonbent DNA. It is also clear that hydroxyl radicals "sense" this conformation, which among its features counts a narrow minor groove, bifurcated hydrogen bonds in the A-T base pairs, high propeller twist, and a distinct spine of hydration in the minor groove. It is, however, still not clear which of these features (if any) are physically the cause of the macroscopic bending. Nonetheless, bending is sensitive to increasing temperature or dehydrating agents (like MPD) and so are correlatively the structural features.

Uranyl photoprobing of A-tract bent DNA as reported here corroborates the above conclusions but also adds a new dimension. The present as well as previous uranyl photoprobing results show that also AT-rich DNA regions as well as A-tracts in nonbent DNA share to a significant extent the above structural features with bent A-tracts. This relates

specifically to the narrowed minor groove and most importantly to the temperature and MPD sensitivity of the conformation. However, the present results most significantly show that the structure is more resistant to both temperature and MPD in bent DNA compared to nonbent DNA. Our results therefore indicate that the rather subtle structural difference of AT regions and A-tracts in nonbent DNA versus A-tracts in bent DNA may be quantitative rather than qualitative; i.e., the structure is more persistent and/or rigid in bent DNA.

The explanation for the discrepancy between the hydroxyl radical and uranyl probing methods may be obtained by considering the mechanism of action of the two reagents. Hydroxyl radicals directly abstract hydrogen atoms of the deoxyriboses (*32*). Consequently, the hydroxyl radical cleavage is strong where the minor groove is wide because the deoxyriboses are more accessible. It has been found that hydroxyl radicals react most strongly with hydrogens at the 5′ position and weakest at the 1′ and 2′ positions (*32*). Therefore, hydroxyl radical cleavage will be dependent on all factors that expose or shield the 5′ H in the DNA structure. Thus, a narrow minor groove in A/T tracts other than pure A-tracts might not be detected by the hydroxyl radical methods due to other structural features of the DNA (e.g., deoxyribose conformation).

Uranyl binds to phosphates of the DNA, and upon irradiation the proximal deoxyriboses are oxidized (*33*). In contrast to hydroxyl radical cleavage, uranyl cleavage is strong where the minor groove is narrow mainly because of the presence of a higher electronegative potential when phosphates of the two strands are closest each other. This results in hyperreactivity possibly via uranyl binding across the narrow minor groove. The cleavage is presumably much less influenced by the sugar conformation. We cannot exclude that the uranyl ion alters the DNA structure upon binding and that the strong cleavage at A/T-rich sequences reflects strong DNA helix deformability/flexibility that facilitates uranyl coordination to the phosphates over the minor groove in these regions. Thus, it may not be possible by this method to differentiate between an existing (“static”) helix structure and a readily inducible helix structure. However, either property of the DNA is highly relevant for biological function and does to a significant degree reflect “two sides of the coin”. Furthermore, this is a general and common dilemma in structural biology, and uranyl photoprobing may indeed eventually shed light on this in a DNA helix context.

The present results therefore also stress that hydroxyl radical and uranyl photoprobing provide different and partly complementary information about DNA structure and dynamics and that detailed interpretation of the data must take the mechanism of action into consideration.

ACKNOWLEDGMENT

We thank Mrs. Neel Louv Jansen for skilled technical assistance.

REFERENCES

1. Koo, H.-S., Wu, H.-M., and Crothers, D. M. (1986) DNA bending at adenine•thymine tracts, *Nature 320*, 501−506.
2. Hagerman, P. J. (1985) Sequence dependence of the curvature of DNA: a test of the phasing hypothesis, *Biochemistry 24*, 7033−7037.
3. Ulanovsky, L. E., and Trifonov E. N. (1987) Estimation of wedge components in curved DNA, *Nature 326*, 720−722.
4. Trifonov, E. N., and Sussman, J. L. (1980) The pitch of chromatin DNA is reflected in its nucleotide sequence, *Proc. Natl. Acad. Sci. U.S.A. 77*, 3816−3820.
5. Dickerson R. E., Goodsell, D. S., and Neidle, S. (1994) “...the tyranny of the lattice...”, *Proc. Natl. Acad. Sci. U.S.A. 91*, 3579−3583.
6. Digabrielle, A. D., Sanderson, M. R., and Steitz, T. A. (1993) A DNA dodecamer containing an adenine tract crystallizes in a unique lattice and exhibits a new bend, *J. Mol Biol. 231*, 1024−1039.
7. Nelson, H. C., Finch, J. T., Luisi, B. F., and Klug, A. (1987) The structure of an oligo(dA)•oligo(dT) tract and its biological implications, *Nature 330*, 221−216.
8. Coll, M., Frederick, C. A., Wang, A. H., and Rich, A. (1987) A bifurcated hydrogen-bonded conformation in the d(A. T) base pairs of the DNA dodecamer d(CGCAAATTTGCG) and its complex with distamycin, *Proc. Natl. Acad. Sci. U.S.A. 84*, 8385−8389.
9. Buckin, V. A., Kankiya, B. I., Bulichov, N. V., Lebedev, A. V., Gukovsky, I. Ya., Chuprina, V. P., Sarvazyan, A. P., and Williams, A. R. (1989) Measurement of anomalously high hydration of (dA)n•(dT)n double helices in dilute solution, *Nature 340*, 321−322.
10. Chuprina, V. P., Heinemann, U., Nurislamov, A. A., Zielenkiewicz, P., Dickerson, R. E., and Saenger, W. (1991) Molecular dynamics simulation of the hydration shell of a B-DNA decamer reveals two main types of minor-groove hydration depending on groove width, *Proc. Natl. Acad. Sci. U.S.A. 88*, 593−597.
11. Sprous, D. S., Zacharias, W., Wood, Z. A., and Harvey, S. C. (1995) Dehydrating agents sharply reduce curvature in DNAs containing A tracts, *Nucleic Acids Res. 23*, 1816−1821.
12. Dlakic, M., Park, K., Griffith, J. D., Harvey, S. C., and Harrington, R. E. (1996) The organic crystallizing agent 2-methyl-2,4-pentanediol reduces DNA curvature by means of structural changes in A-tracts, *J. Biol. Chem. 271*, 17911−17919.
13. Gaununis, R. M., Guo, H., and Tullius, T. D. (1996) Effect of the crystallizing agent 2-methyl-2,4-pentanediol on the structure of adenine tract DNA in solution, *Biochemistry 35*, 13729−13732.
14. Diekmann, S. (1987) Temperature and salt dependence of the gel migration anomaly of curved DNA fragments, *Nucleic Acids Res. 15*, 247−265.
15. Park, Y. W., and Breslauer, K. J (1991) A spectroscopic and calorimetric study of the melting behaviors of a “bent” and a “normal” DNA duplex: [d(GA4T4C)]2 versus [d(GT4A4C)]2, *Proc. Natl. Acad. Sci. U.S.A. 88*, 1551−1555.
16. Chan, S. S., Breslauer, K. J., Hogan, M. E., Kessler, D. J., Austin, R. H., Ojemann, J., Passner, J. M., and Wiles, N. C. (1990) Physical studies of DNA premelting equilibria in duplexes with and without homo dA•dT tracts: correlations with DNA bending, *Biochemistry 29*, 6161−6171.
17. Chan, S. S., Breslauer, K. J., Austin, R. H., and Hogan, M. E. (1993) Thermodynamics and premelting conformational changes of phased (dA)5 tracts, *Biochemistry 32*, 11776−11784.
18. Herrera, J. E., and Chaires, J. B. (1989) A premelting conformational transition in poly(dA)-Poly(dT) coupled to daunomycin binding, *Biochemistry 28*, 1993−2000.
19. Burkhoff, A. M., and Tullius, T. D. (1987) The unusual conformation adopted by the adenine tracts in kinetoplast DNA, *Cell 48*, 935−943.
20. Chuprina, V. P., Fedoroff, O. Y., and Reid, B. R. (1991) New insights into the structure of An tracts and B′-B′ bends in DNA, *Biochemistry 30*, 561−568.
21. Dickerson, R. E., Goodsell, D., and Kopka, M. L. (1996) MPD and DNA bending in crystals and in solution, *J. Mol. Biol. 56*, 108−125.
22. Burkhoff, A. M., and Tullius, T. D. (1988) Structural details of an adenine tract that does not cause DNA to bend, *Nature 331*, 455−457.
23. Price, M. A., and Tullius, T. D. (1993) How the structure of an adenine tract depends on sequence context: a new model for the structure of TnAn DNA sequences, *Biochemistry 32*, 127−136.
24. Nielsen, P. E., Mollegaard, N. E., and Jeppesen, C. (1990) DNA conformational analysis in solution by uranyl mediated photocleavage, *Nucleic Acids Res. 18*, 847−851.
25. Sonnichsen, S. H., and Nielsen, P. E. (1996) Enhanced uranyl photocleavage across the minor groove of all (A/T)4 sequences indicates a similar narrow minor groove conformation, *J. Mol. Recognit. 9*, 219−227.

26. Conner, B. N., Yoon, C., Dickerson, J. L., and Dickerson, R. E. (1984) Helix geometry and hydration in an A-DNA tetramer: IC-C-G-G, *J. Mol. Biol. 174*, 663−695.
27. Johannesson, H., and Halle, B. (1998) Minor groove hydration of DNA in Solution: Dependence on Base composition and sequence, *J. Am. Chem. Soc. 120*, 6859−6870.
28. Jacobson, A., Leupin, W., Liepinsh, E., and Otting, F. (1996) Minor groove hydration of DNA in aqueous solution: sequence-dependent next neighbor effect of the hydration lifetimes in d(TTAA)2 segments measured by NMR spectroscopy, *Nucleic Acids Res. 24*, 2911−2918.
29. Quintana, J. R., Grzeskowiak, K., Yanagi, K., and Dickerson, R. E. (1992) Structure of a B-DNA decamer with a central T−A step: C-G-A-T-T-A-A-T-C-G, *J. Mol. Biol. 225*, 379−395.
30. Goodsell, D. S., Kaczor-Grzeskowiak, M., and Dickerson, R. E. (1994) The crystal structure of C-C-A-T-T-A-A-T-G-G. Implications for bending of B-DNA at T-A steps, *J. Mol. Biol. 239*, 79−96.
31. Hagerman, P. J. (1986) Sequence-directed curvature of DNA, *Nature 321*, 449−450.
32. Balasubramanian, B., Pogozelski, W. K., and Tullius, TD. (1998) DNA strand breaking by the hydroxyl radical is governed by the accessible surface areas of the hydrogen atoms of the DNA backbone, *Proc. Natl. Acad. Sci. U.S.A. 95*, 9738−9743.
33. Nielsen, P. E., Hiort, C., Sonnichsen, S. H., Buchardt, O., Dahl, O., and Norden, B. (1992) DNA binding and photocleavage by uranyl(VI)(UO_2^{2+}) salts, *J. Am. Chem. Soc. 114*, 4967−4975.

BI0343267