JOURNAL OF MAGNETIC RESONANCE **132,** 34–40 (1998)
ARTICLE NO. MN981403

Interresidue Quiet NOEs for DNA Structural Studies

Richard D. Beger, Hari Babu Arthanari, Swarna Basu, and Philip H. Bolton

Chemistry Department, Wesleyan University, Middletown, Connecticut 06459

Received September 25, 1997; revised January 28, 1998

The potential utility of long-range NOEs in DNA has not been exploited since the observed signals have contributions both from the direct magnetization route and from multiple diffusion pathways. The Quiet NOE approach can be used to select for the direct magnetization transfer pathway by suppressing spin diffusion. A single-band Quiet NOE, which allows detection of the direct NOEs between protons in a selected chemical shift window, has been demonstrated on two duplex DNAs, and the NOEs observed can contain important structural information. © 1998 Academic Press

Key Words: **DNA; Quiet NOE; NOE; spin diffusion.**

INTRODUCTION

The determination of the structures of proteins from NMR data often begins with the interpretation of the information present in the NOEs between amide protons. The patterns of the amide–amide NOE connectivities are quite distinct in alpha and beta structures and analysis of these NOEs can often lead to a reasonable start on the picture of the secondary structure of the protein. This sort of information has not been accessible for nucleic acids. Other differences in the approaches used for determination of protein and nucleic acid structures by NMR-based methods is that many proteins have more interresidue and more long-range NOEs per residue than is the case for nucleic acids.

Nucleic acid structure determinations might be aided by having access to additional types of NOE-based information analogous to the amide–amide, alpha–alpha, and long-range NOEs. The patterns in the NOEs between the same atom type on different residues might lead to the same kind of structural analysis that has been useful in the study of proteins. The curvature of DNA, for example, can be difficult to assess on the basis of NMR data as the curvature of DNA is a manifestation of the structure of many base pairs of DNA. The curvature may be associated with patterns in the NOEs between the same atom type on different residues which may be separated by significant distances or more.

As is well known, the intensities of NOEs are roughly proportional to the inverse sixth power of the internuclear distance. Thus, an NOE for a pair of protons 5 Å away from each other will be about 5% that of the NOE of a pair of protons 3 Å away from each other with all other factors equal. Until recently the sensitivity of the spectrometers, the lack of sample, the purity of the samples, and other factors would have made the detection and quantification of small NOEs dubious. However, the quality of currently available spectrometers and samples allow small NOEs to be reliably observed.

The main problem with these weak, long-range NOEs is no longer experimental observation but the way in which to incorporate this information into structure determination methods. For example, there are numerous published spectra in which the NOEs between aromatic protons on adjacent residues are present. However, the NOEs between aromatic protons on adjacent residues have not found much use in structural studies since most of them arise primarily via spin diffusion as indicated in Fig. 1. The transfer of magnetization through the H1′ and H2″ protons can compete with, or overwhelm, the direct transfer NOEs between aromatic protons on adjacent residues. The presence of these diffusion pathways makes the interpretation of the aromatic–aromatic NOEs challenging.

It seemed to us that the aromatic–aromatic NOEs may contain a level of information comparable to that found in the amide–amide NOEs of proteins. Thus, the ability to detect the aromatic–aromatic NOEs in the absence of diffusion could add a new class of information to structural studies. Similarly, the interresidue NOEs involving methyl protons could also be quite informative if they can be detected without contributions from the many competing spin diffusion pathways, some of which are illustrated in Fig. 1. Thus, the interresidue aromatic–aromatic, methyl–methyl and methyl–H2′, H2″ direct NOEs might play a role in nucleic acid studies similar to the complementary roles the amide–amide and alpha–alpha NOEs play in protein structural studies.

Bodenhausen and co-workers have developed an elegant class of experiments for determination of the NOE between any selected pair of nuclei with suppression, or quieting, of the spin diffusion contributions from other nuclei (*1–5*). The ''Quiet'' approach involves selective inversion of the longitudinal magnetization of just the selected pair of nuclei during the NOE mixing period. Quiet experiments have been applied to a number of samples including a DNA (*4*). A

1090-7807/98 $25.00

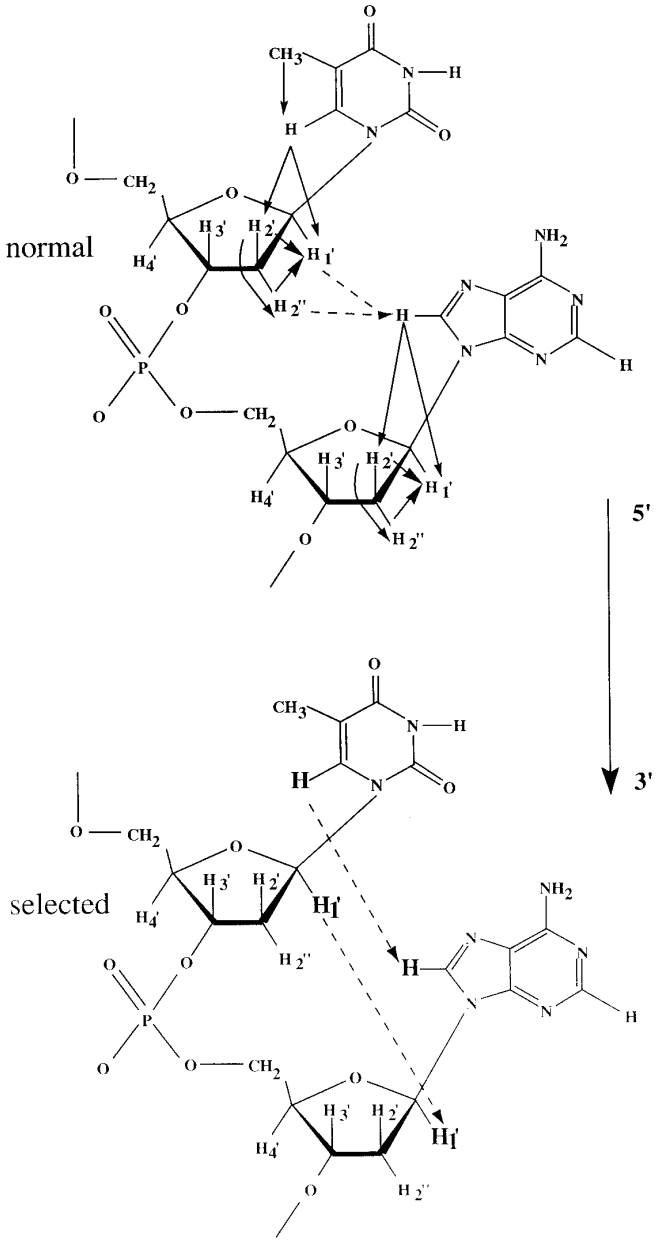

FIG. 1. The top picture indicates some of the intra- and interresidue NOEs that can be observed from DNA samples. The solid lines indicate the intra- and the dashed lines the interresidue connectivities. The figure illustrates that most interresidue connectivities can proceed through multiple spin diffusion pathways. The bottom shows some of the interresidue NOEs that can potentially be observed via Quiet NOESY experiments.

limitation in the original approach is that a separate experiment needs to be carried out for each pair of nuclei. The Quiet approach is distinct from other methods previously used for suppression of spin diffusion effects (*6, 7*).

The original frequency-selective methodology has been extended to allow application to selected frequency bands in a single experiment (*4*). Another such experiment was to examine the amide–amide NOEs of a ^{15}N-labeled protein and to carry out the inversion of the longitudinal magnetization by use of a pulse train which selectively acts on the protons directly attached to a ^{15}N (*5*). This experimental procedure allows the simultaneous observation of amide–amide NOEs with suppression of diffusion effects. This experiment may be particularly useful in investigations of large proteins (*8*) for which spin diffusion makes the interpretation of NOEs challenging (*9*).

This basic idea of quieting an entire class of protons can also be applied to DNAs. The aromatic–aromatic NOEs are well suited to investigation by a homonuclear variation on band-selective Quiet NOEs, as are the methyl to H2′–H2″ NOEs. A prior study investigated the simultaneous inversion of the aromatic and the H2′–H2″ regions so as to allow detection of the Quiet aromatic–H2′/H2″ NOEs (*4*). However, the simpler single-band Quiet NOE offers significant information when applied to the spectral region containing the aromatic protons, which is well isolated. Similarly, the region containing the methyl, H2′, and H2″ protons is also isolated and suitable for selective inversion. Figure 1 illustrates some of the distances that can be interrogated by the single-band Quiet NOEs. The H1′ region also contains H5 resonances and interresidue H5–H1′ NOEs may also be useful since they monitor long-range distances. Since these single-band experiments looked quite promising they have been carried out on two DNA duplexes to examine their utility.

RESULTS AND DISCUSSION

The band selective quiet NOE experiments were carried out on the DNA 5′-($C_1G_2C_3G_4A_5A_6T_7T_8C_9G_{10}C_{11}G_{12}$)-3′ that forms a self-complementary duplex in solution and which is often referred to as the ‘‘Dickerson dodecamer’’ after the group which first carried out the determination of its crystal structure (*31*). The results for the region containing the aromatic–aromatic NOE cross peaks are shown in Fig. 2. In the normal NOESY there are a number of signals in this region. There are NOE cross peaks between T_7H6 and A_6H8 and between T_7H6 and T_8H6, while neither of these cross peaks are present in the Quiet NOE data. By means of contrast, the cross peak between A_5H2 and A_6H2 is present in both the NOE and Quiet NOE data sets. This indicates that the NOEs between T_7H6 and A_6H8 and between T_7H6 and T_8H6 are primarily via spin diffusion, which is consistent with the distances in the crystal structure of this DNA (*19*).

A similar level of discrimination has been found for the cross peaks in the upfield region. The spectral region between about 1 and 3 ppm contains signals primarily from methyl, H2′, and H2″ protons. Of particular interest are the NOEs between the methyl protons and the H2′ and H2″ protons on its own sugar and the methyl and the H2′ and

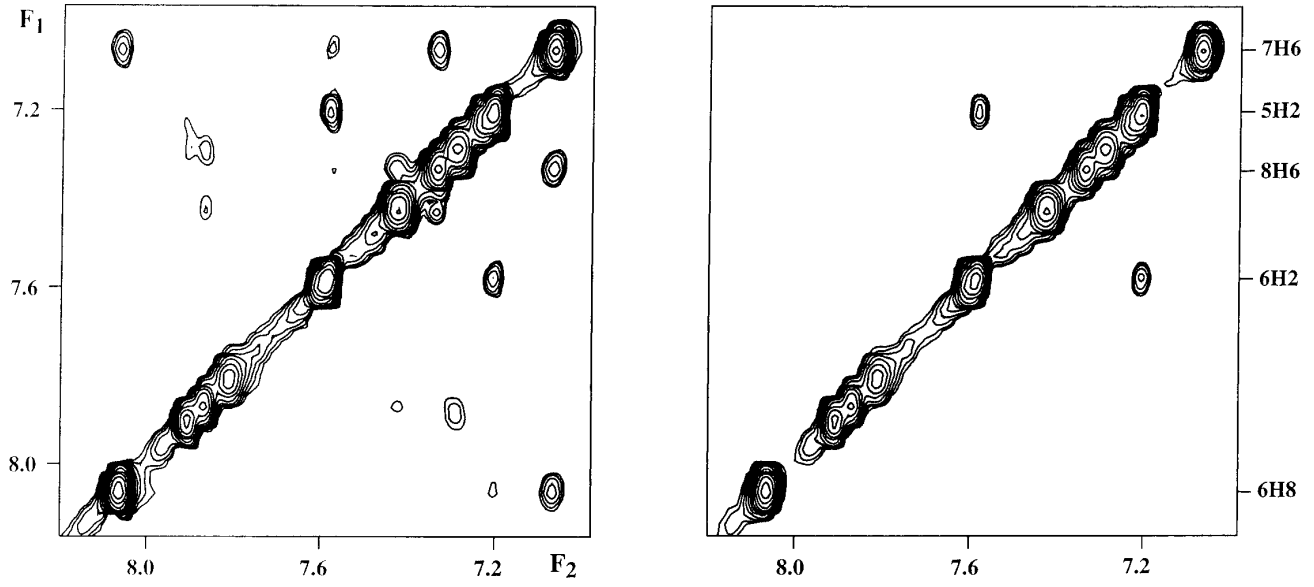

FIG. 2. The spectrum on the left is the aromatic region of the conventional NOESY data for the DNA 5′-($C_1G_2C_3G_4A_5A_6T_7T_8C_9G_{10}C_{11}G_{12}$)-3′ that forms a self-complementary duplex in solution and which is often referred to as the ''Dickerson dodecamer.'' The right contains the same spectral region of the Quiet NOESY data.

H2″ protons on the adjacent residue. The results in Fig. 3 show that in the NOE data the methyl of residue 7 has cross peaks to its own H2′ and H2″ as well as to the methyl of residue 8 and the H2′ and H2″ of residue 7. In the Quiet NOE data there are no intraresidue H2′ or H2″ to T_7Me NOEs. However, there are interresidue NOEs of T_7Me and the H2′ and H2″ protons of residue 6 in the Quiet NOE. Similar results are observed for the methyl of residue 8, which has NOEs to the methyl, H2′ and H2″ of residue 7, and H2′ and H2″ of its own residue. The Quiet NOE of T_8Me only has cross peaks with the methyl, H2′, and H2″ of residue 7.

Thus, the Quiet NOE data is consistent with the intraresidue methyl–H2′/H2″ NOEs arising primarily via dif-

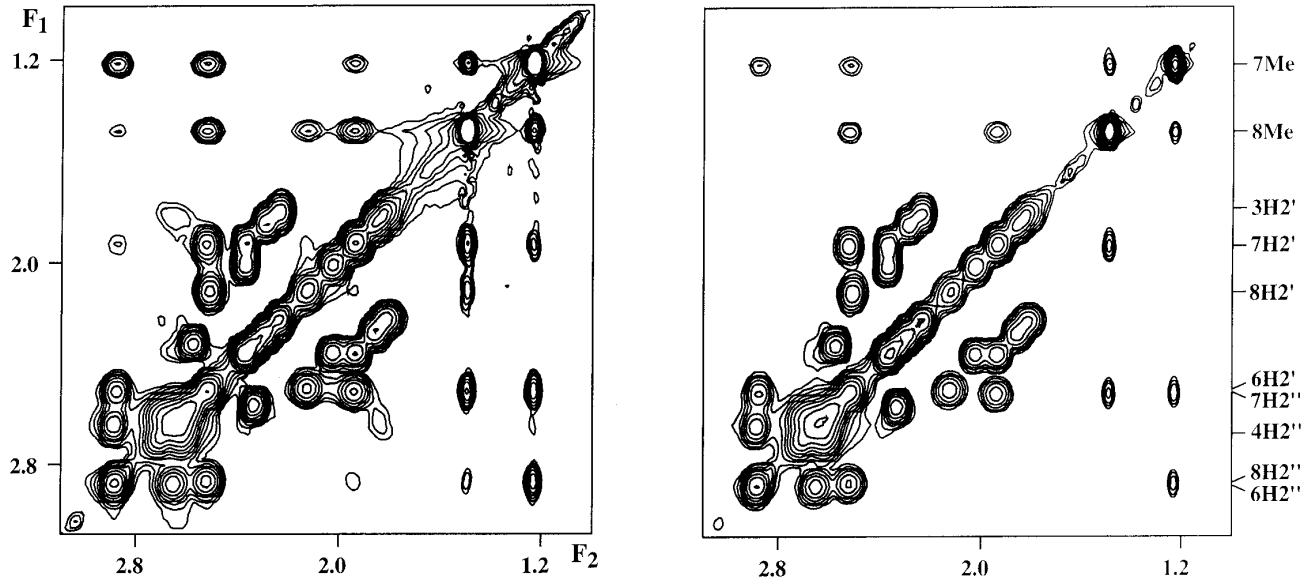

FIG. 3. The spectrum on the left is the upfield region of the conventional NOESY data for the DNA 5′-($C_1G_2C_3G_4A_5A_6T_7T_8C_9G_{10}C_{11}G_{12}$)-3′ that forms a self-complementary duplex in solution and which is often referred to as the ''Dickerson dodecamer''; the right contains the same spectral region of the Quiet NOESY data.

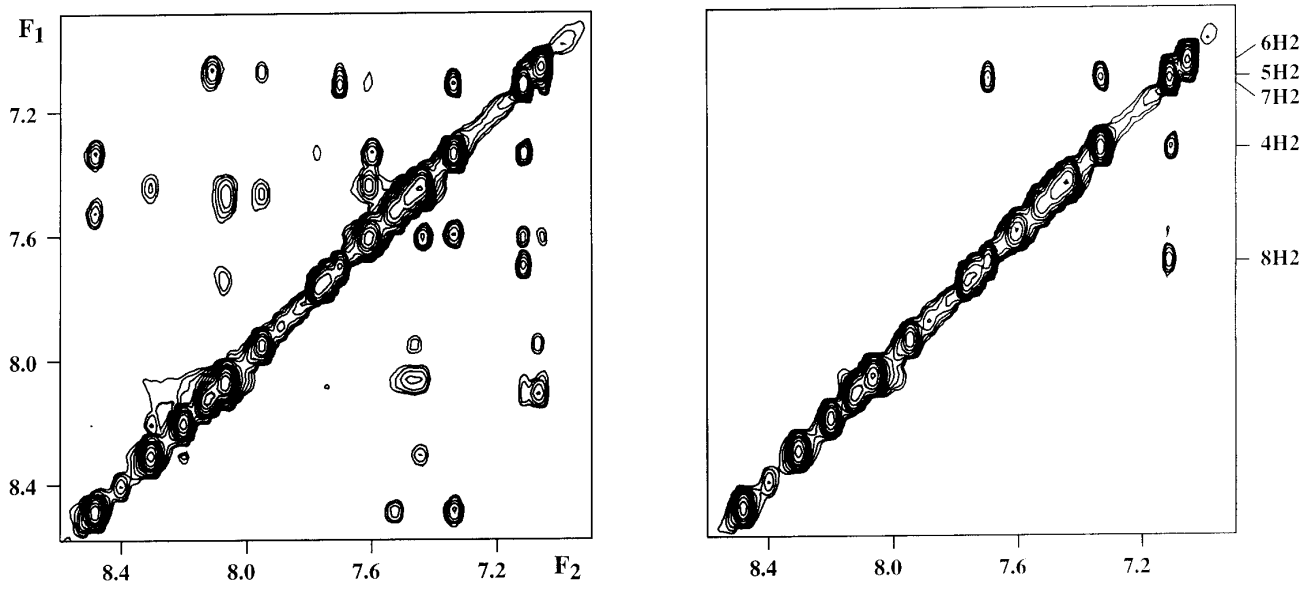

FIG. 4. The spectrum on the left is the aromatic region of the conventional NOESY data for the DNA duplex formed of the sequence 5′-d($C_1G_2C_3A_4A_5A_6A_7A_8T_9G_{10}C_{11}G_{12}$)-3′ paired with the complementary strand 5′-d($C_{13}G_{14}C_{15}A_{16}T_{17}T_{18}T_{19}T_{20}T_{21}G_{22}C_{23}G_{24}$)-3′, and the right contains the same spectral region of the Quiet NOESY data.

fusion, most likely via the H6 and H1′ protons, whereas the interresidue methyl–methyl and methyl–H2′/H2′ NOEs have a significant direct transfer component. Note that in the Quiet NOE data the methyl on residue 7 connects to both the H2′ and H2″ of residue 6 and the methyl on residue 8.

The NMR experiments were also carried out on the DNA duplex of the sequence 5′-d($C_1G_2C_3A_4A_5A_6A_7A_8T_9G_{10}C_{11}G_{12}$)-3′ paired with the complementary strand 5′-d($C_{13}G_{14}C_{15}A_{16}T_{17}T_{18}T_{19}T_{20}T_{21}G_{22}C_{23}G_{24}$)-3′. In this duplex there are five contiguous dA residues on one strand and five contiguous dT on the other. Thus, the Quiet NOE data should report primarily on the dA strand in the aromatic region and the dT strand in the upfield region. The NOE and Quiet NOE data on the aromatic region is shown in Fig. 4. The NOESY data shows that there are a large number of cross peaks in this region while the Quiet NOE data has only two sets of cross peaks. The Quiet cross peaks are the H2, H2 pairs of residues 4 and 5 and of 7 and 8. The chemical shifts of the H2 protons of residues 5, 6, and 7 are nearly degenerate at the field strength used for this experiment. The cross peaks for their H2, H2 pairs appear to be present but will require very high field data to be observed.

The upfield region of the dA tract duplex contains cross peaks between the protons of A_{17} and A_{18} which are quite analogous to those obtained for A7 and A8 for the Dickerson DNA (Fig. 5). In particular, the Quiet NOE data has cross peaks between the H2′ and H2″ protons of residue 16 and the methyl of residue 18 with the methyl of residue 17. The Quiet NOE data also has cross peaks between the H2′ and H2″ protons of residue 17 with the methyl of residue 18. The resolution is not sufficient, at this field strength, to determine the presence of the methyl–methyl NOEs of the subsequent dT residues; however, their H2′, H2″ pattern is consistent with that observed for the other dT residues in both DNAs.

The ROESY experiment might be considered to offer similar information to the Quiet NOE. There is a basic difference, however, between the suppression of signals due to spin diffusion and suppression of the effects of spin diffusion. The ROESY experiment does not suppress relaxation effects from neighboring spins but does suppress the observation of diffusion signals (*1–5*).

The results on the dA tract DNA indicates that the Quiet NOE cross peak between the H2 protons of residues 4 and 5 is larger, by about 30%, than that between residues 7 and 8. This DNA is curved in solution and was found to crystallize such that each unit cell had an ''up'' and a ''down'' form (*32, 33*). A refined solution structure based on NMR data was found to be in generally good agreement with the up structure (*12*). In the down structure the A_4H2, A_5H2 distance is 4.55 Å, in the up structure 4.92 Å, and in the NMR-based structure 4.55 Å. In the down structure the A_7H2, A_8H2 distance is 3.88 Å, in the up 4.31 Å, and in the NMR 5.01 Å. The NMR structure seems to agree with the Quiet NOE data better than either crystal structure in that it gets the rank ordering of the cross peaks correct; the up structure is not far off while the down structure appears to be at a significant difference. However, a more complete comparison will require averaging over the restrained molecular dynamics trajectory used to obtain the NMR structure (*12*).

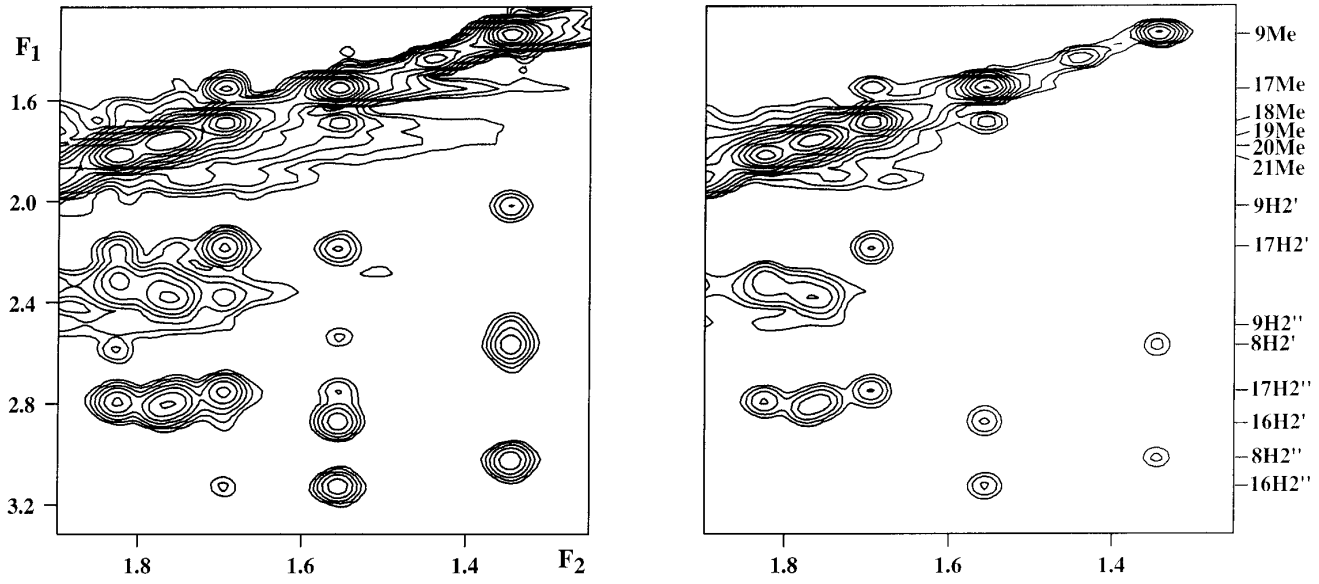

FIG. 5. The spectrum on the left is the upfield region of the conventional NOESY data for the DNA duplex formed of the sequence 5′-d($C_1G_2C_3A_4A_5A_6A_7A_8T_9G_{10}C_{11}G_{12}$)-3′ paired with the complementary strand 5′-d($C_{13}G_{14}C_{15}A_{16}T_{17}T_{18}T_{19}T_{20}T_{21}G_{22}C_{23}G_{24}$)-3′, and the right contains the same spectral region of the Quiet NOESY data.

Figure 6 contains plots of the distance between the methyl of residue *n* and the H2′ of residue $n - 1$ as a function of the helicoidal parameters tilt, roll, and twist for both A- and B-form DNAs. These plots were made to assess the sensitivity of this distance to differences in conformation of the DNA. The plots indicate that this distance can be used to distinguish between A- and B-form DNA. The data also indicates that there is a modest correlation between this distance and tilt with increasing distance correlating with more positive tilt for both A- and B-form DNAs. There also appears to be a modest correlation between this distance and the twist for B-form DNA, with increasing distance correlating with decreasing twist. The plots indicate that this distance is one that can provide a useful constraint for restrained molecular dynamics protocols.

CONCLUSIONS

The single-band Quiet NOE experiment appears to offer a route to a set of very useful NOE information in a quite

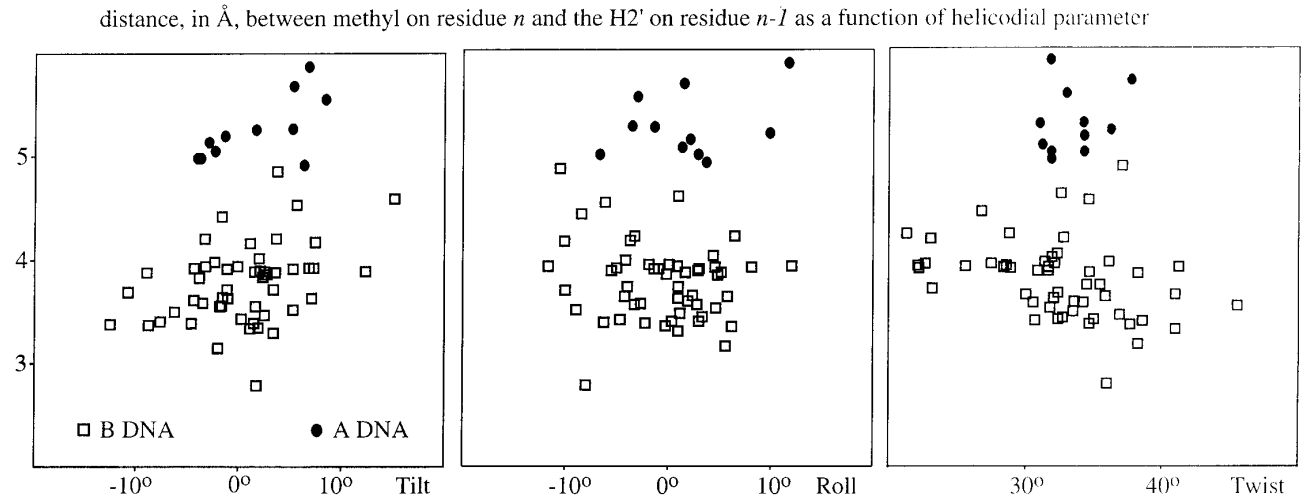

FIG. 6. The plots show the distance between the methyl of residue *n* and the H2′ of residue $n - 1$ as a function of the helicoidal parameters tilt, roll, and twist for A- and B-form DNAs. The distances and helicoidal parameters are from experimentally determined structures.

straightforward experiment. The aromatic–aromatic appear to be most pronounced for adjacent adenosine residues. The upfield NOEs appear to be most interesting for adjacent thymines, though the methyl protons of isolated thymine residues are also informative sites. The overall sensitivity of the Quiet NOE appears to be higher than a comparable ROESY experiment carried out on the same samples. The Quiet NOE data will also allow the incorporation of the NOEs in the aromatic region of the spectra into structure calculations since all of the cross peaks not observed in the Quiet data can be tagged in the refinement protocols as arising from spin diffusion rather than direct pathways. This additional information is now being incorporated into the methods that are being used to determine DNA structures. Single-band selective Quiet NOEs could also be useful in studying unlabeled proteins and other samples.

EXPERIMENTAL

NMR Methods

The DNA samples were the DNA 5′-($C_1G_2C_3G_4A_5A_6$-$T_7T_8C_9G_{10}C_{11}G_{12}$)-3′ that forms a self-complementary duplex and the DNA duplex of the sequence 5′-d($C_1G_2C_3A_4A_5A_6$-$A_7A_8T_9G_{10}C_{11}G_{12}$)-3′ paired with the complementary strand 5′-d($C_{13}G_{14}C_{15}A_{16}T_{17}T_{18}T_{19}T_{20}T_{21}G_{22}C_{23}G_{24}$)-3′, which were prepared as previously described (*11, 12*). The samples were in 100% ^2H_2O and were kept at 27°C during the experiments. All of the NMR results were obtained using a Varian Unityplus 400 spectrometer equipped with a Nalorac IDG400-5 probe with z-gradient capability. Two-dimensional Quiet-NOESY and conventional NOESY experiments were obtained for both DNA duplexes with 64 transients per increment and 300 t_1 increments using the States–Haberkorn method. The acquisition time was 0.173 s. The spectral width was 5000 Hz in both F_1 and F_2. The data was Fourier transformed into 1024 × 8192 points using shifted Gaussians along each dimension. The Quiet-NOESY and NOESY experiments were obtained with a mixing time of 250 ms.

During the Quiet-NOESY a 2.6 ms, 180° Gaussian-shaped pulse was applied in the middle of the mixing time. The transmitter frequency was moved to the center of the desired region for this selective pulse. A Fourier transform of the 2.6 ms 180° Gaussian-shaped pulse is a Gaussian with a full width at half maximum of 880 Hz, which is 2.2 ppm. During the Quiet-NOESY experiments, a 1-ms z-gradient was applied 1 s before the first 90° pulse, right after the second 90° pulse, and 1 ms after the 180° Gaussian-shaped pulse in the middle of the mixing time. During the conventional NOESY, a 1-ms z-gradient was applied 1 s before the first 90° pulse and right after the second 90° pulse, and the last z-gradient was applied at the end of the mixing time.

Structure Analysis Methods

Representative A- and B-form DNA (*13–27*) was chosen from the nucleic acid data bank, NDB, and the internucleotide distances were measured manually using Insight II version 95.0. The distances were taken from the position of the center of the thymine methyl carbon on residue n to the position of the H2′ and H2″ of residue $n - 1$. The helicoidal parameters tilt, twist, and roll for these DNAs were calculated by the use of CURVES 5.1 (*28–30*). Curves is an algorithm for calculating the helicoidal parameters for any nucleic acid segment with respect to a global helical axis. The parameters tilt, roll, and twist are obtained by minimizing a function which represents the variations in helical parameters between successive nucleotides as well as quantifying the kinks and dislocations which exist between successive helical axis segments. The global interbase helical parameters were used in this study.

ACKNOWLEDGMENTS

PHB would like to thank Professor Geoffrey Bodenhausen for many enlightening discussions about the Quiet NOE experiment. This research was supported, in part, by grants GM 51298 from the National Institutes of Health and NP-750 from the American Cancer Society. The 400- and 500-MHz NMR spectrometers were obtained with support from the National Science Foundation grants BIR 93-03077 and BIR-95-12478 and from the Camille and Henry Dreyfus Foundation.

REFERENCES

1. B. Boulat, I. Burghardt, and G. Bodenhausen, *J. Am. Chem. Soc.* **114,** 10679–10679 (1992).
2. I. Burghardt, R. Konrat, B. Boulat, S. J. F. Vincent, and G. Bodenhausen, *J. Chem. Phys.* **98,** 1721–1736 (1993).
3. M. Schwager and G. Bodenhausen, *J. Magn. Reson. Series B* **111,** 40–49 (1996).
4. S. J. F. Vincent, C. Zwahlen, and G. Bodenhausen, *J. Biomolec. NMR* **7,** 169–172 (1996).
5. S. J. F. Vincent, C. Zwahlen, P. H. Bolton, T. Logan, and G. Bodenhausen, *J. Am. Chem. Soc.* **116,** 3531–3532 (1996).
6. J. Fasno, W. M. Westler, J. L. Markley, and S. Macura, *J. Am. Chem. Soc.* **114,** 1523–1524 (1992).
7. W. Massefski and A. G. Redfield, *J. Magn. Reson.* **78,** 150–155 (1988).
8. R. D. Beger and P. H. Bolton, *J. Biomolec. NMR* **10,** 129–142 (1997).
9. A. Kalk and H. J. C. Berendsen, *J. Magn. Reson.* **24,** 343–366 (1976).
10. R. E. Dickerson and H. R. Drew, *J. Mol. Biol.* **149,** 761–775 (1981).
11. J. M. Withka, S. Swaminathan, D. L. Beveridge, and P. H. Bolton, *Science* **255,** 597–599 (1992).
12. M. A. Young, I. Goljer, S. Kumar, J. Srinivasan, D. L. Beveridge, and P. H. Bolton, *Methods Enzymology* **261,** 121–144 (1995).
13. C. M. Nunn and S. Neidle, *Acta Crystallogr.* **53,** 269–274 (1997).
14. D. Rabinovich, T. Haran, M. Eisenstein, and Z. Shakked, *J. Mol. Biol* **200,** 151–161 (1988).
15. D. S. Goodsell, M. L. Kopka, D. Cascio, and R. E. Dickerson, *Proc. Nat. Acad. Sci. USA* **90,** 2930–2934 (1993).
16. D. S. Goodsell, K. Grzeskowiak, and R. E. Dickerson, *Biochemistry* **34,** 1022–1029 (1995).

17. H. Yuan, J. Quintana, and R. E. Dickerson, *Biochemistry* **31,** 8009–8021 (1992).

18. H. C. M. Nelson, J. T. Finch, B. F. Luisi, and A. Klug, *Nature* **330,** 221–226 (1987).

19. H. R. Drew, R. M. Wing, T. Takano, C. Broka, S.Tanaka, K. Itakura, and R. E. Dickerson, *Proc. Nat. Acad. Sci. USA* **78,** 2179–2183 (1981).

20. J. R. Quintana, K. Grzeskowiak, K. Yanagi, and R. E. Dickerson, *J. Mol. Biol* **225,** 379–395 (1992).

21. K. Grzeskowiak, K. Yanagi, and R. E. Dickerson, *J. Biol. Chem.* **266,** 8861–8883 (1991).

22. K. Grzeskowiak, D. S. Goodsell, M. Kaczor-Grezeskowiak, D. Cascio, and R. E. Dickerson, *Biochemistry* **32,** 8923–8931 (1993).

23. M. Eisenstein, F. Frolow, Z. Shakked, and D. Rabinovich, *Nucleic Acids Res.* **18,** 3185–3194 (1990).

24. S. R. Holbrook, R. E. Dickerson, and S. H. Kim, *Acta Crystallogr. Sect. B* **41,** 255–262 (1985).

25. Y. G. Gao, H. Robinson, J. H. V. Boom, and A. H. J. Wang, *Biophys. J.* **69,** 559–568 (1995).

26. Z. Shakked, D. Rabinovich, O. Kennard, W. B. T. Cruse, S. A. Salisbury, and M. A. Viswamitra, *J. Mol. Biol.* **166,** 183–201 (1983).

27. Z. Shakked, G. Guzikevich-Guerstein, D. R. F. Frolow, A. Joachimiak, and P. B. Sigler, *Nature* **368,** 469–474 (1994).

28. R. Lavery and H. Sklenkar, *J. Biomol. Struct. Dynam.* **6,** 63–91 (1988).

29. R. Lavery and H. Sklenkar, *J. Biomol. Struct. Dynam.* **6,** 655–667 (1989).

30. H. S. R. Lavery *in* ''Proceedings of the 6th Conversation in Biomolecular Stereodynamics'' (R. H. Sarma, Ed.), Adenine Press, New York (1990).

31. R. E. Dickerson and H. R. Drew, *J. Mol. Biol.* **149,** 761–775 (1981).

32. A. D. DiGabriele, M. R. Sanderson, and T. A. Steitz, *Proc. Natl. Acad. Sci. USA* **86,** 1816–1820 (1989).

33. A. DiGabriele and T. A. Steitz, *J. Mol. Biol.* **231,** 1024–1039 (1993).