Carbon 13 NMR data were obtained at four magnetic fields for double-stranded DNA samples 120 and 160 nucleotide pairs long. Spectral linewidths are several-fold smaller than predicted for overall rigid rod rotation and increase with increasing field, indicating significant chemical shift dispersion contributions to high field linewidths. Spin-lattice relaxation times are short (e.g. T1 values for CH carbon atoms are approximately 0.5 s at 67.9 MHz), and increase with increasing field. Nuclear Overhauser effects of 0.6 to 0.8 (theoretical maximum = 2.0) were measured for the same protonated and neutral DNA samples at 100.6 MHz. These short T1 values and relatively large nuclear Overhauser effects show that double-stranded DNA undergoes rapid internal motions with effective correlation times of a few nanoseconds. Preliminary data indicate that DNA base carbon motions occur on essentially the same time scale as sugar motions, thus arguing against independent flexibility of the DNA backbone.

Linewidths decreased 2- to 5-fold and nuclear Overhauser effects doubled, upon heat denaturation of DNA, as expected for increased motion. On the other hand, NT1 values of native and denatured DNA were nearly identical, suggesting insensitivity of spin-lattice relaxation times to motions in the nanosecond range in these relatively stiff chains.

The conformational flexibility of DNA and the possibility that double-stranded DNA undergoes rapid internal motions of significant amplitude have recently attracted considerable interest. Magnetic resonance measurements on different nuclei seem particularly well suited for monitoring and describing motions at various DNA sites. High field $^1$H, $^{31}$P, and $^{13}$C spectra of relatively small (140 to 300 np), double-stranded DNA molecules have now been reported (1-8). Jardetzky et al. have also reported protonated sugar and base carbon relaxation behavior and NOE values at 25 MHz (6). The relatively narrow spectral lines observed in spectra of all three nuclei, and significant magnitude of these early NOE measurements, cannot be interpreted in terms of the rotational correlation times of reasonable rigid rod models. They are generally consistent with other motions representing correlation times on the order of a few nanoseconds. Precise descriptions of these motions are presently unspecified, and are not directly obtainable from the simpler theoretical considerations.

The complexity of the double-stranded structure and associated motional modes, and the possible coupling of these modes to produce overall bending or twisting motions, present unusual problems in spectral interpretation. We believe that the unique ability of $^{13}$C NMR parameters obtained at several magnetic fields to probe different parts of the motional autocorrelation functions for many DNA sites is particularly pertinent in this respect. Previously, we reported $^{13}$C spectra of native and heat-denatured, nucleosome core length (approximately 140 np) DNA at 67.7 and 37.7 MHz (4). Here we describe in more detail multifield $^{13}$C data on double- and single-stranded, near nucleosome-core length (120 and 160 np) DNA. Included are linewidths, spin-lattice relaxation times, and nuclear Overhauser enhancements of individual sugar and certain base carbon atoms at 100.6 MHz, and three lower frequencies.

**MATERIALS AND METHODS**

Gram quantities of calf thymus DNA were isolated as follows (9). Frozen calf thymus (500 g, from Pel-Freeze) was ground to a fine powder in a large mortar and pestle while constantly cooling with liquid nitrogen. The powder was suspended in 3 liters of homogenization buffer (0.25 m sucrose, 24 mM KCl, 5 mM MgCl$_2$, 1 mM CaCl$_2$, 4 mM phenylmethylsulfonyl fluoride, 50 mM Tris-HCl (final pH 7.5)) with gentle stirring and allowed to stand for 1 h on ice, then filtered through cheesecloth. Nuclei were pelleted at 700 x $g$ for 10 min and immediately resuspended by gentle shaking in 500 ml of homogenization buffer containing 0.5% Triton X-100. Nuclei were pelleted as above, then lysed by addition of 700 ml of 2 mM CaCl$_2$, 0.02% NaN$_3$, 1 mM phenylmethylsulfonyl fluoride, 1 mM Tris-HCl (final pH 8.0) with gentle stirring and allowed to stand for 1 h on ice, then filtered through cheesecloth. Nuclei were pelleted at 700 x $g$ for 10 min and immediately resuspended by gentle shaking in 500 ml of homogenization buffer containing 0.5% Triton X-100. Nuclei were pelleted as above, then lysed by addition of 700 ml of 2 mM CaCl$_2$, 0.02% NaN$_3$, 1 mM phenylmethylsulfonyl fluoride, 1 mM Tris-HCl (final pH 8.0). Histone H1 was dissociated from the chromatin by slow addition with rapid stirring of an equal volume of 1.2 M NaCl in the above buffer. Chromatin was then digested to about 15% acid solubility at 37 °C with micrococcal nuclease (Sigma, 1 unit/50 mg of DNA). Digestion was terminated by addition of 0.1 volume of 0.1 M Na$_2$EDTA (pH 7.5), then the solution was concentrated to approximately 150 ml by ultrafiltration through an Amicon PM-30 membrane. Oligonucleosomes were partially precipitated by dilution of the mixture with the lysing buffer to a final NaCl concentration of 0.2 M. After standing for 1 h at 0 °C, the oligonucleosomes were pelleted by centrifugation at 17,000 x $g$ for 10 min. The supernatant containing mainly nucleosome...
After extended experiments (>24 h) at elevated temperatures, some tenths of DNA before and after NMR experiments. 1) Digestion with pH, 7.5), at a flow rate of 40 ml/h. Fractions containing nucleosomes (nucleosomes) were separated on a Bio-Gel A-5M column (5 x 100 cm) eluted with 0.6 M NaCl, 10 mM Na2EDTA, 0.02% NaN3, 1 mM Tris-HCl (final pH 7.5), at a flow rate of 40 ml/h. Then digested with 4 mg of Protease K (Merck) at 37 °C for 24 h. Any precipitate was removed by centrifugation at 17,000 x g for 10 min, then the supernatant was extracted three times with phenol:chloroform:isoamyl alcohol (1:1:0.05). Deproteinized DNA was exhaustively dialyzed against 0.1 M NaCl and precipitated with 2 volumes of ethanol. Pellets were dried under vacuum for at least 4 h, then dissolved in the appropriate buffer for NMR studies (usually 39.2 mM Na2HPO4, 14.5 mM NaH2PO4, 2 mM Na2EDTA, 3.1 mM NaN3, final pH 7.2).

Three assays were routinely used to determine single strand contents of DNA before and after NMR experiments. 1) Digestion with S1 nuclease (Sigma) was measured spectrally under conditions where only single-stranded DNA is degraded (10). 2) Thermal denaturation curves were determined using a computer-interfaced Cary 219 spectrophotometer equipped with thermostat probe and programmable temperature regulator. 3) Absorbance measurements were made at four wavelengths before and after heat denaturation as described by Felsenfeld to assess the single strandedness of the initial DNA (11, 12).

DNA lengths were determined by polyacrylamide gel electrophoresis under native and denaturing conditions as described by Maniatis et al. (13). Haell III restriction endonuclease fragments of φX 174 DNA (Bethesda Research Laboratories) and DNA from DNase I digests of calf thymus chromatin were used as markers for the respective gels. Gels were stained with Stains-all or Toluidine blue (Eastman) and scanned with a Gelman densitometer. In the experiments described here, 80% of the DNA was in the range of 160 ± 30 np or 120 ± 20 np and 95% was in the range 160 ± 60 np or 120 ± 40 np. For NMR experiments, DNA was denatured by heating in a water bath at the appropriate temperature, then placed in a pre-equilibrated probe. After extended experiments (>24 h) at elevated temperatures, some discoloration and strand breakage was noted. Discoloration may be partly due to oxidation, but strand breakage probably results from known hydrolytic reactions (14).

Nuclear magnetic resonance (NMR) spectra were obtained at four magnetic field strengths. Low field spectra were obtained at 22.7 MHz on FX-90 spectrometers at the University of California at Los Angeles and California Institute of Technology, and at 37.7 MHz on the in-house design, multinuclear spectrometer at Florida State University, SEM- INOLE (15). High field spectra were obtained at 67.9 MHz on a quadrature detection-modified Bruker HX-270 at Florida State University and at 100.6 MHz on a Bruker WH-400 at the National Science Foundation Regional Facility at the University of South Carolina. All double-stranded DNA spectra were obtained at 32 ± 2 °C using, at higher fields, two level decoupling (16) to prevent radiofrequency heating of samples. Conventional Helmholz coil probes were used in all the superconducting instruments except at 37.7 MHz, where a side-spinner solenoid coil probe (17) was employed. T1 values were obtained from three parameter fits (18) to data from fast inversion recovery pulse sequences (19). NOE values were obtained from gated decoupling experiments using single 90° pulses separated by wait periods of at least 5 times the T1 values for the carbon atoms being measured. Linewidths were corrected for the total line broadening by subtracting the linewidth of the internal standard, ethanol (double-stranded DNA), or by subtraction of the applied digital broadening (single-stranded DNA).

RESULTS

Carbon 13 NMR spectra of 120 np length, fully double-stranded DNA fragments were obtained at four magnetic fields, the highest being 100.6 MHz (Fig. 1). All observed linewidths were significantly smaller than those calculated from overall rotational correlation times for a rigid rod corresponding to this DNA length. Furthermore, only the linewidths determined at the lower fields appear to be controlled strictly by dynamics (Fig. 2). At 67.9 MHz, and especially at 100.6 MHz, dispersion of shielding for the individual carbon types significantly broadens the resonance bands. All of the sugar carbon atoms experience similar chemical shift dispersion of 1.4 to 1.9 ppm, except for C2', which shows a lesser increase in linewidth corresponding to a shift dispersion of approximately 1.0 ppm. In contrast, it is interesting that the thymidine methyl carbon resonance is little affected by shift dispersion at high fields. The sugar carbon C5' linewidth is approximately 40% less at lower fields than are the other sugar carbon linewidths. This is consistent with a higher mobility of this exocyclic carbon.

Heat denaturation of DNA causes significant sharpening of the sugar carbon lines by factors of 2 to 5 (Fig. 1 and Table 1). The largest linewidth decrease is observed for C5'. Base

---

**Fig. 1.** 13C NMR spectra of (a) double-stranded and (b) single-stranded 120-np DNA, obtained at 100.6 MHz (scales approximately identical). (a) 80 mg/ml, 32 °C, 8000 scans, 20 Hz digital broadening, and scan interval 1 s; (b) 80 mg/ml, 65 °C, 3500 scans, 20 Hz digital broadening, and scan interval 1.5 s.
Carbon 13 NMR Studies of DNA

**Fig. 2.** Linewidths of double-stranded 160 np (60 mg/ml) as a function of magnetic field, 22.8 to 100.6 MHz. O, C4', C1' band (unresolved); △, C3'; □, C5'; ●, C2'; ■, thymidine methyl. Bars indicate the mean deviations of three different spectra at 67.9 MHz and two different spectra at 37.7 and 100.6 MHz.

**TABLE I**

| Carbon atoms | Native* | Denatured† |
|--------------|---------|------------|
| Sugar        |         |            |
| C2'          | 262 ± 21| 102 ± 11   |
| C5'          | 154 ± 37| 31 ± 8     |
| C3'          | 248 ± 23| 60 ± 17    |
| C4',C1'      | 266 ± 14| 125 ± 30   |
| Base         |         |            |
| T(Me)        | 83 ± 3  | 42 ± 14    |
| C3           | 42 ± 14 |            |
| T4,C4        | 74 ± 4  |            |

*In Hz; estimated precision of three experiments (± S.D.).
†Measured at 32 °C.
‡Measured at 85 °C.

**TABLE II**

| Carbon atoms | 160 np DNA* | 120 np DNA† |
|--------------|-------------|-------------|
|              | 37.7 MHz    | 67.9 MHz    | 100.6 MHz   | 67.9 MHz    | 100.6 MHz   |
| C2'          | 0.20        | 0.27 ± 0.01 | 0.41 ± 0.01 | 0.28 ± 0.03 |
| C5'          | 0.22 ± 0.03 | 0.37 ± 0.03 | 0.53 ± 0.06 | 0.32 ± 0.01 |
| C3'          | 0.21 ± 0.01 | 0.63 ± 0.06 | 0.50 ± 0.01 |              |
| C4',C1'      | 0.21 ± 0.04 | 0.63 ± 0.08 | 0.41 ± 0.02 |              |
| T(Me)        | 0.90        | 1.6 ± 0.02  |              | (0.55)      |
| C5           |             |             |              |             |
|              | 0.32        | 0.45        | 0.34         | 0.24        |
|              | 0.12        | 0.32        | 0.33         | 0.28        |
|              | 0.21 ± 0.04 | 0.57        | 0.63 ± 0.05  | 0.44 ± 0.07 |
|              | 0.21 ± 0.04 | 0.66        | 0.59 ± 0.07  | 0.47        |
| C5           | 1.0         | 1.3         | 1.0          | 0.65        |
| C4,T4       | 1.7         |             | 1.0          | 0.65        |

*160-np DNA is essentially 100% double-stranded by assays described in the text.
†Native DNA measurements at 32 °C.
‡Denatured DNA measurements at 85 °C.
§120-np DNA was 15% single-stranded by assays described in the text.

**DISCUSSION**

The data presented, although insufficient to fully model DNA behavior, provide some interesting insights into the motional dynamics of double- and single-stranded DNA. The protonated carbon NOEF values, along with the small linewidths and T1 values, argue strongly for the existence of some kind of uniform internal motion in the double-stranded DNA with a correlation time on the order of 1 ns (6). Overall rotation of the DNA chain, treated as a rigid rod, cannot account for this rapid correlation time; only some sort of internal motion can do this. The unexpectedly equivalent NT, and NOEF data for sugar and protonated base carbon atoms indicates that both structures undergo similar dynamics. These results argue against major conformational flexibility for the sugar residues by themselves.

Upon heat denaturation there is a predictable increase in NOEF values, and decrease in linewidths, for all protonated carbon atoms. This is expected from the macroscopically observable decrease in chain stiffness. In contrast, spin lattice relaxation time measurements at 67.9 and 100.6 MHz appear to be insensitive to changes in motion in the time frame observed by NMR, since heat denaturation had little effect on the measured NT; values. These T1; values have been determined accurately, considering the formidable task in-

**TABLE III**

| Carbon atoms | NOEF Native DNA* | NOEF Denatured DNA† |
|--------------|------------------|---------------------|
| Sugar        |                   |                     |
| C2'          | 0.77              | 1.2                 |
| C5'          | 0.64              | 1.4                 |
| C3'          | 0.67              | 1.4                 |
| C4',C1'      | 0.57              | 1.3                 |
| Base         |                   |                     |
| T(Me)        | 0.93              | 1.2                 |
| C5 (protonated) | 0.50            | 1.1                 |
| T5 (nonprotonated) | 0.27         | 0.56                |

* Measured at 32 °C.
†Measured at 85 °C.
Carbon 13 NMR Studies of DNA

Fig. 3. Dipolar $T_1$ for a CH carbon as a function of an effective correlation time for molecular tumbling, $\tau$, at 3.5 and 6.3 Tesla. (A) isotropic or pseudoisotropic tumbling characterized by an exponential autocorrelation function $B(\tau)$; (B) complex behavior resulting from a nonexponential decay of $G(\tau)$. This requires that motion be represented by a set of $\tau$ values, or distribution around some mean $\tau$.

volved. This insensitivity of $T_1$ values may reflect three possibilities:

1. The NT$_1$ values may correspond to $\tau_{\text{eff}}$ values more or less symmetrically disposed about the $T_1$ minimum (Fig. 3a).

2. The motional dynamics may reflect a nonexponential autocorrelation function, producing a flattened dependence of NT$_1$ on the rate of molecular reorientation (Fig. 3b) (21).

3. The NT$_1$ values may have a complex dependence on composite overall and internal motions resulting in little change of the observed parameters.

It is possible that explanations 2 and 3 both contribute to our observations. The key to proper interpretation of these data lies in use of both $T_1$ and NOE parameters, obtained over dispersed magnetic fields, and thus probing the autocorrelation function at various times (22, 23). Such experiments are planned, along with theoretical calculations to attempt to fully characterize the DNA dynamics.

Acknowledgments—We thank the staff and the director of the National Science Foundation regional facility, Dr. Paul D. Ellis, for their assistance.

REFERENCES

1. Davanloo, P., Armitage, I. M., and Crothers, D. M. (1979) Biopolymers 18, 663-680
2. Klevan, L., Armitage, I. M., and Crothers, D. M. (1979) Nucleic Acids Res. 6, 1607-1616
3. Hogan, M. E., and Jardetzky, O. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 6341-6345
4. Rill, R. L., Hilliard, P. R., Jr., Bailey, J. T., and Levy, G. C. (1980) J. Am. Chem. Soc. 102, 418-429
5. Early, T. A., and Kearns, D. R. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4165-4169
6. Hogan, M. E., and Jardetzky, O. (1980) Biochemistry 19, 3460-3468
7. Bolton, P. H., and James, T. L. (1979) J. Phys. Chem. 83, 3359-3366
8. Bolton, P. H., and James, T. L. (1980) Biochemistry 19, 1388-1392
9. Rill, R. L., Shaw, B. R., and Van Holde, K. E. (1980) Methods Cell Biol. 18, 69-104
10. Vogt, V. M. (1960) Methods Enzymol. 85, 248-255
11. Felsenfeld, G., and Hirschman, S. Z. (1965) J. Mol. Biol. 13, 407-427
12. Hirschman, S. Z., and Felsenfeld, G. (1966) J. Mol. Biol. 16, 347-368
13. Maniatas, T., Jeffrey, A., and Van de Sande (1975) Biochemistry 14, 3787-3794
14. Kochetkov, N. K., and Budowskii, E. I. (1972) in Organic Chemistry of Nucleic Acids (Todd, L., and Brown, D. M., translation eds) Part B, p. 522, Plenum Press, New York
15. Levy, G. C., Rosanske, R. C., Wright, D., and Terpstra, D. (1978) Abstracts of the Experimental NMR Conference, Blacksburg, VA
16. Levy, G. C., Peat, T. R., and Rosanske, R. C. (1975) J. Magnetic Res. 18, 205
17. Bailey, J. T., Rosanske, R. C., and Levy, G. C. (1981) Rev. Sci. Instrum., in press
18. Kowalewski, J., and Levy, G. C. (1977) J. Magnetic Res. 26, 533-536
19. Canet, D., Levy, G. C., and Peat, I. R. (1975) J. Magnetic Res. 18, 199-204
20. Levy, G. C., and Edlund, U. (1975) J. Am. Chem. Soc. 97, 5031-5032
21. Schaefer, J. (1975) Macromolecules 6, 882-888
22. Levy, G. C., Azelson, D. E., Schwartz, R., and Hochmann, J. (1978) J. Am. Chem. Soc. 100, 410-424
23. Shindo, H. (1980) Biopolymers 19, 509-522