molecules it is expected that the extreme narrowing limit holds. Our experiments with the two isotopes of chlorine provide a test for whether exchange is slow. If the line width were controlled by slow exchange, transverse relaxation should be essentially independent of the isotopic identity. The ratio of ^{35}Cl and ^{37}Cl line widths should then be unity.[12] On the other hand, if exchange is fast, the ratio of line widths should go as the square of the ratio of quadrupole moments ($1.26^2 = 1.6$).[9,12,13] Indeed, except for the lowest concentration, the ratio of line widths is in the range 1.6-1.9. Thus, throughout the range of concentrations, exchange between free and associated perchlorate is fast. A possible alternative explanation, that the line broadening derives from exchange between environments with a very large chemical shift difference, is excluded by the observation of only a 38 ppm chemical shift difference (Table IV) between the species. We may conclude that each perchlorate ion must visit a silyl cation many times on the time scale of transverse relaxation, even at 0.584 M.[14]

Conclusions

Triphenylsilyl and trimethylsilyl perchlorates exist in an equilibrium between free silylenium ions and an associated form that may be covalent or ion-paired. The chlorine resonances change from a broad peak of line width in excess of 1000 Hz above 0.1 M to a sharp peak of line width less than 30 Hz below 0.005 M. The chemical shift responds in like manner, moving from a value of about δ -30 for the associated form to about δ 5 for the free ion. The $^{35}Cl/^{37}Cl$ line width ratio of about 1.6 requires that exchange between free and associated forms be fast. The equilibrium constant between free and associated forms and the percentages of individual species may be calculated from a least-squares analysis of the data. For both trimethylsilyl and triphenylsilyl, the free ion is present at the 50% level at about 0.03 M and at the 98% level at 0.005 M. By 0.5 M, free Me_3Si^+ has dropped to 20%. The amount of water in solution both by direct measurement and from the least-squares calculation is below 0.004 M and can have no palpable effect on the observations.

Experimental Section

All NMR spectra were recorded on a Varian XLA-400 spectrometer (^{35}Cl at 39.17 MHz, ^{37}Cl at 32.61 MHz). Chlorine chemical shifts are reported with respect to external dilute $HClO_4$ in D_2O. The solvent sulfolane was dried over BaO initially and distilled. It was then dried over CaH_2, again distilled, and stored over CaH_2. The silyl perchlorates were prepared by the reaction of the appropriate silane with trityl perchlorate, as described previously.[5] Dilutions were carried out by removing a portion of the sample and replacing it with the same amount of solvent. Measurements for ^{35}Cl and ^{37}Cl were carried out on the same solutions in consecutive experiments in order to achieve identity of concentration and temperature. All experiments were repeated and found to be reproducible.

(13) Harris, R. K. *Nuclear Magnetic Resonance Spectroscopy*; Pitman: London, 1983; p 232.
(14) We appreciate useful comments of a reviewer regarding the isotopic ratios of chlorine line widths.

Solid-State ^2H NMR Investigation of Internal Motion in 2′-Deoxythymidine

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Abstract: ^2H NMR spectroscopy is used to characterize the dynamics of 2′-deoxythymidine in the solid state. Three different specifically deuteriated nucleosides were prepared, with labels at the methyl group, at the C6 position, and at the 5′-methylene group, respectively. The ^2H NMR quadrupole echo line shapes and spin-lattice (T_1) relaxation times were analyzed to determine the mechanism and rate of motion of the labeled moieties. Only the methyl group undergoes large-amplitude motion, which consists of 3-fold jumps about the C_3 symmetry axis with a room-temperature correlation time $\tau_c \sim 3.0$ ps. The apparent activation energy (E_a) for this process is 5.9 kJ/mol. The data also indicate that the entire pyrimidine base undergoes a rapid, small-amplitude motion, which we have modeled as a ±9° libration about the equilibrium position. At 298 K, the libration occurs with correlation time $\tau_c \sim 1.0$ ps. The motion of the pyrimidine base also affects the methyl group, but the added motion causes only a small variation in the room-temperature methyl jump correlation time, $\tau_c \sim 4.2$ ps. The librational motion makes only a small contribution to the spin-lattice relaxation of the methyl deuterons, and this likely does not contribute significantly to the unusually low value of the apparent activation energy that is observed. The 5′-methylene group also appears to be undergoing a similar libration (±10°) as the pyrimidine base but with a much longer correlation time, $\tau_c \sim 200$ ns at room temperature. From the models of motion and the experimental line shapes, we are able to determine the static values of the asymmetry parameter (η) and the quadrupole coupling constant for deuterons at the various sites. The static asymmetry parameter was assumed to be zero for the methyl and methylene deuterons, while $\eta_{static} \sim 0.03$ for the deuteron at the 6-position on the pyrimidine base. The static quadrupole coupling constants are 159, 174, and 179 kHz for the methyl, methylene, and base deuterons, respectively.

The realization that proteins, DNA, and RNA are highly dynamic structures has focused interest on the role of internal motion in the function of these biomolecules.[1] Solid-state ^2H NMR spectroscopy is a powerful method for investigating these dynamic processes. The rate and type of motion of various deuteriated moieties may be deduced from analysis of the powder pattern line shapes and the spin-lattice (T_1) relaxation times of ^2H NMR spectra.[2-6]

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(1) (a) Gurd, F. R. N.; Rothgeb, T. M. *Adv. Protein Chem.* **1979**, *33*, 73-165. (b) Karplus, M.; McCammon, J. A. *CRC Crit. Rev. Biochem.* **1981**, *9*, 293-349. (c) Holbrook, S. R.; Kim, S.-H. *J. Mol. Biol.* **1984**, *173*, 361-388. (d) Kearns, D. R. *CRC Crit. Rev. Biochem.* **1984**, *15*, 237-290.

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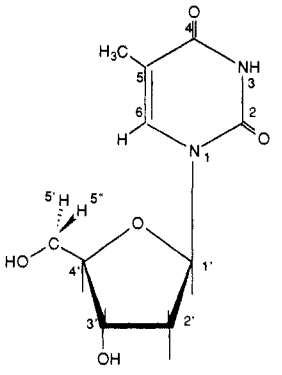
As a basis for understanding the internal motions in the macromolecule, it is important to characterize the dynamics of the constituent monomers in the crystalline or amorphous solid state and to determine the static values of the deuterium quadrupole coupling constant (e^2qQ/h) and asymmetry parameter (η) of deuterons at various positions in these compounds. In this paper, we report the results of a solid-state ^2H NMR study of 2′-deoxythymidine labeled at several different positions on the pyrimidine base and at the 5′-methylene group on the deoxyribose sugar.



2′-deoxythymidine

Experimental Section

Labeled Compounds. We have prepared the following deuteriated compounds: 2′-deoxythymidine-*methyl*-d_3, 2′-deoxythymidine-6-d_1, and a 1:1 mixture of the two isomers of 2′-deoxythymidine-5′-d_1. The selective incorporation of deuterium labels was accomplished as follows.

Deoxythymidine-*methyl*-d_3 was prepared with a modification of the procedure of Kinoshita and co-workers.[7] Activated platinum was prepared by bubbling deuterium gas (Matheson) through a stirred suspension of 50 mg (0.22 mmol) of PtO_2 (Aldrich) and 2 mL of 99% D_2O (Cambridge Isotopes) in a 10-mL round-bottomed flask. After 2 h of purging, the fluids were pipetted off, and 1 mL of fresh D_2O was added. A solution containing 50 mg (2.06 mmol) of 2′-deoxythymidine (Aldrich) dissolved in 2 mL of D_2O was added to the flask, and the mixture was purged with deuterium gas for an additional 20 min. The flask was sealed and stirred at 75–80 °C. After 72 h, the suspension was filtered, and the solution, lyophilized. Chromatography on silica gel, with $CHCl_3$–MeOH (97:3, v/v) as the eluant, followed by rotary evaporation resulted in white crystals (42.5 mg, 85% yield). The purity of the labeled compound and the extent of deuterium incorporation were determined by high-resolution ^1H NMR spectroscopy at 500 MHz. There was greater than 95% exchange at the methyl position and less than 5% exchange at the H6 base position; no impurities could be detected. Labile deuterons were reexchanged by lyophilizing from doubly distilled water three additional times.

Deoxythymidine-6-d_1 was prepared by the procedure of Rabi and Fox.[8] The nucleoside was first dissolved in D_2O and lyophilized to exchange the labile N–H and sugar hydroxyl protons. The preexchanged deoxythymidine (968 mg, 4 mmol) was dissolved in 12 mL of DMSO-d_6 (MSD Isotopes), and 4 mL of a solution containing 10% NaOD in D_2O was added. The mixture was sealed in a tube and heated in an oven at 140 °C for 45 h. The resulting brown mixture was diluted with water and neutralized. Activated-charcoal chromatography (1:1 (w/w) charcoal–cellulose powder in water) followed by evaporation and subsequent trituration in cold ethanol resulted in a white crystalline product (710 mg, 73% yield). High-resolution 500-MHz ^1H NMR spectroscopy showed that the compound was identical with authentic deoxythymidine except for 90% deuterium exchange at the 6-position. Labile deuterium was reexchanged by lyophilization from doubly distilled water three additional times.

A 1:1 mixture of the two isomers of deoxythymidine-5′-d_1 was prepared via a strategy involving protection–deprotection[9] and oxidation–reduction.[10]

When established methods were used, 2′-deoxythymidine was protected at the 5′-position with the acid labile 4,4′-dimethoxytrityl group,[9,11] converted to the 3′-*O*-acyl-5′-(dimethoxytrityl) species,[9,12] and deprotected[13] to yield 3′-*O*-acylthymidine in 80% overall yield.

The 3′-*O*-acylthymidine was converted to the desired product by oxidation to the 5′-aldehyde with a modification of the method of Pfitzner and Moffat,[10,14] followed by sodium borodeuteride reduction and deprotection.[15] To a stirred solution of 3′-*O*-acylthymidine (477 mg, 1.7 mmol) in 10 mL of anhydrous dimethyl sulfoxide (Aldrich) at 25 °C was added 1.05 g (5.1 mmol) of dicyclohexylcarbodiimide (Aldrich) and 81 mg (0.84 mmol) of methylphosphonic acid (Aldrich). After 5 h at 25 °C, a solution of 428 mg (3.4 mmol) of oxalic acid dihydrate (Aldrich) in 3 mL of methanol was added. The mixture was stirred for an additional 30 min at 25 °C and filtered, and the filtrate was concentrated under vacuum. The resultant solid was dissolved in 100 mL of brine and 50 mL of ethyl acetate. The two layers were separated, and the aqueous phase was extracted with two additional 50-mL portions of ethyl acetate. The combined organic extracts were washed with brine (2 × 50 mL), dried over Na_2SO_4, and concentrated under vacuum to yield 500 mg of a gummy white solid. The solid was dissolved in a solution of 8.5 mL of water and 0.5 mL of 15% NaOH in water, treated with 340 mg (8.5 mmol) of $NaBD_4$ (Aldrich), and stirred for 30 min at 25 °C. The mixture was diluted with 5 mL of methanol, stirred for 35 min at 25 °C, neutralized by the addition of 1 N HCl (7.0 mL), stirred for an additional 12 h at 25 °C, and filtered. The filtrate was concentrated under vacuum to yield a granular white solid. The crude reaction product was converted to the corresponding 3′,5′-*O*-acyl derivative,[9,12] purified by flash chromatography (2% methanol, 98% CH_2Cl_2), deprotected in methanolic ammonia,[15] and recrystallized from ethyl acetate to yield the desired product as a white crystalline solid (128 mg, 31%). High-resolution 500-MHz ^1H NMR spectroscopy revealed a 1:1 mixture of the two isomers of deoxythymidine-5′-d_1. Each isomer had greater than 98% deuterium exchange at one of the 5′ protons. There was a small amount of impurities (~7% level) present in the final sample. However, solid-state ^2H NMR spectroscopy revealed the presence of only one species, indicating that the impurity was not deuteriated and did not significantly perturb the dynamic environment of the desired specifically labeled nucleoside.

Solid-State NMR Spectroscopy. Solid-state ^2H NMR spectra were obtained at 76.76 MHz on a home-built NMR spectrometer[16] with a quadrupolar echo pulse sequence.[17] Typically, the delay between pulses was 50 μs, and the dwell time was 900 ns. The 90° pulse length was less than 3.1 μs. Data acquisition was initiated prior to the top of the solid echo. The time-domain data were left-shifted to the echo maximum prior to Fourier transformation. No apodization was applied to the experimental spectra of the deuteriomethyl compound, and 1500–2000-Hz Lorentzian line broadening was applied to the ^2H NMR spectra of the other compounds.

Deuteron spin–lattice relaxation times (T_1) were determined by an inversion–recovery pulse sequence for the deuteriomethyl compound and a saturation–recovery pulse sequence for the other compounds. The magnetization recovery was obtained from either the height of the quadrupole echo or the height of the singularity of the powder pattern. In the latter instance, contributions by both quadrupolar transitions were considered so that the T_1 value determined was the weighted average of two orientations. The data were analyzed using a linear least-squares analysis in the case of the methyl-labeled and methylene-labeled nu-

(2) (a) Torchia, D. A. *Annu. Rev. Biophys. Bioeng.* **1984**, *13*, 125–144. (b) Opella, S. J. *Methods Enzymol.* **1986**, *131*, 327–361.
(3) (a) Kinsey, R. A.; Kintanar, A.; Oldfield, E. *J. Biol. Chem.* **1981**, *256*, 9028–9036. (b) Gall, C. M.; DiVerdi, J. A.; Opella, S. J. *J. Am. Chem. Soc.* **1981**, *103*, 5039–5043.
(4) (a) Keniry, M. A.; Kintanar, A.; Smith, R. L.; Gutowsky, H. S.; Oldfield, E. *Biochemistry* **1984**, *23*, 288–298. (b) Colnago, L. A.; Valentine, K. G.; Opella, S. J. *Biochemistry* **1987**, *26*, 847–854.
(5) Beshah, K.; Olejniczak, E. T.; Griffin, R. G. *J. Chem. Phys.* **1987**, *86*, 4730–4736.
(6) Hiyama, Y.; Roy, S.; Guo, K.; Butler, L. G.; Torchia, D. A. *J. Am. Chem. Soc.* **1987**, *109*, 2525–2526.
(7) Kinoshita, T.; Schram, K. H.; McCloskey, J. A. *J. Labelled Compd. Radiopharm.* **1982**, *19*, 525–534.
(8) Rabi, J. A.; Fox, J. J. *J. Am. Chem. Soc.* **1973**, *95*, 1628–1632.
(9) (a) Smith, M.; Rammler, D. H.; Goldberg, I. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1962**, *84*, 430–440. (b) Rammler, D. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1962**, *84*, 3112–3122.
(10) Pfitzner, K. C.; Moffatt, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 5661–5670.
(11) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316–1319.
(12) Höfle, G.; Steglich, W.; Vorbrüggen, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 569–583.
(13) Adams, S. P.; Kavka, K. S.; Wykes, E. J.; Holder, S. B.; Galluppi, G. R. *J. Am. Chem. Soc.* **1983**, *105*, 661–663.
(14) Craig, J. W.; Sternberg, E. D.; Jones, G. H.; Moffatt, J. G. *J. Org. Chem.* **1986**, *51*, 1258–1264.
(15) Neilson, T.; Werstiuk, E. S. *Can. J. Chem.* **1971**, *49*, 493–499.
(16) (a) Gladden, J. A.; Drobny, G., to be submitted for publication. (b) Klevit, R. E.; Drobny, G. *Biochemistry* **1986**, *25*, 7770–7773.
(17) Davis, J. H.; Jeffery, K. R.; Bloom, M.; Valic, M. I.; Higgs, T. P. *Chem. Phys. Lett.* **1976**, *42*, 390–395.

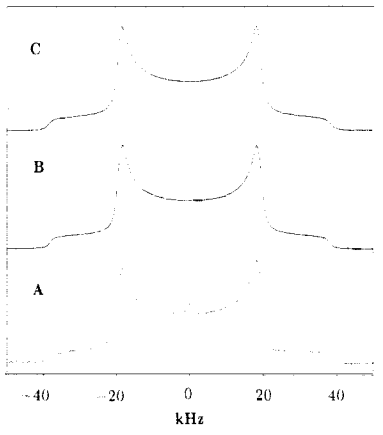


Figure 1. (A) Solid-state ^2H NMR spectrum of 2'-deoxythymidine-*methyl*-d_3 at 298 K, obtained at a 76.76-MHz resonance frequency with a quadrupole echo pulse sequence. A 42-mg sample required 500 scans, with a recycle time of 5 s to ensure thermal equilibrium. The 90° pulse length was 3.1 μs, and the delay between pulses was 50 μs. A total of 1024 complex data points were collected at a dwell time of 1 μs. (B) Calculated spectrum based on a rigid-lattice model. The best visual fit simulation yielded the values $\eta_{eff} = 0.04$ and $(e^2qQ/h)_{eff} = 51$ kHz. (C) Calculated spectrum based on the two-axis motional model described in the text. The simulation used the values $\eta_{static} = 0.0$ and $(e^2qQ/h)_{static} = 159$ kHz with a methyl jump correlation time $\tau_c = 4.2 \times 10^{-12}$ s and an axis libration correlation time $\tau_c = 0.78 \times 10^{-12}$ s.

cleosides and a nonlinear least-squares analysis[18] in the case of the base-labeled deoxythymidine.

Spectra at low temperatures were obtained with liquid-nitrogen boil-off as the cooling gas; temperatures were constant (and accurate) to within ±1 °C. The temperature was measured with a copper–constantan thermocouple.

Correlation times (τ_c) were calculated from the corresponding T_1 values with computer programs that utilized the expressions for two-site and three-site jump models of motion given by Torchia and Szabo.[19] Correlation times for the motional model of the methyl group, which included a 3-fold jump about the C_3 symmetry axis and an uncorrelated small-amplitude 2-fold jump of the symmetry axis, were determined with an expression (Alam et al., unpublished results) based on the correlation function of Torchia and Szabo for two-axis motion[19]

$$C_{aa'}(t) = \Gamma_{aa'}^{axis}(t) \sum_{b=-2}^{2} d_{ba}^{(2)}(\Theta)\, d_{ba'}^{(2)}(\Theta)\, [d_{0b}^{(2)}(\beta)]^2\, \Gamma_{bb}^{Me}(t)$$

where β is the angle between the z axis in the principle axis system and the methyl symmetry axis, Θ is the angle describing the orientation of the methyl symmetry axis in the crystal fixed frame, and the $d_{mn}^{(2)}$ are elements of the reduced Wigner rotation matrix.[20] The correlation function for the two-site model, $\Gamma_{aa'}^{axis}$, was simplified by the approximation that the two jump sites had equal probabilities. The three-site correlation function, $\Gamma_{bb'}^{Me}$, was simplified by retaining only the diagonal terms. In all correlation time calculations, the proper spatial averages of the functions were used depending on whether the T_1 value was determined from the height of the quadrupole echo or the height of the line-shape singularity.

Simulations of the ^2H NMR line shapes were obtained with a program described by Greenfield and co-workers,[21] which can accommodate various motional models. Values for e^2qQ/h and η were determined from the calculated spectrum (obtained with the appropriate motional model) on the basis of a visual fit to the experimental line shape. Simulations included a 1500–2000-Hz Lorentzian line broadening to match the apodization of the experimental spectra and 1000-Hz Gaussian line broadening to account for other line-broadening mechanisms such as proton dipolar coupling.

Results

Methyl Group Motion. The ^2H NMR spectrum of deoxythymidine-*methyl*-d_3 is shown in Figure 1A, together with a rigid-lattice simulation of the line shape (Figure 1B). The simulation yields the effective (motionally averaged) values of the asymmetry parameter, $\eta_{eff} = 0.04$, and the quadrupole coupling constant, $(e^2qQ/h)_{eff} = 51 \pm 1$ kHz. The value of the effective coupling constant indicates that there must be fast ($\tau_c \leq 10^{-8}$ s) reorientation of the methyl group about the C_3 symmetry axis.[4-6] The motion about this axis most likely involves either rotational diffusion or discrete 3-fold jumps. Either mechanism for fast reorientation of the methyl group about the C_3 axis causes the reduction of the quadrupole coupling constant to approximately one-third of the static value. If this were the only motion affecting the methyl deuterons, then $(e^2qQ/h)_{static} \approx 154$ kHz. This value of the static quadrupole coupling constant is somewhat smaller than that of methyl groups in other compounds[5,22,23] and may reflect an additional rapid, small-amplitude motion of the thymidine methyl group. The nonzero value of the asymmetry parameter is also somewhat unusual for methyl groups[5,22] although a value of $\eta_{eff} = 0.07$ has been reported for the deuteriomethyl group in thymine.[6] An effective nonzero asymmetry parameter may be induced by an additional fast, small-amplitude, 2-fold jump of the C_3 axis or by a variation of the static quadrupole coupling constant as a function of methyl orientation about the C_3 axis (vide infra).

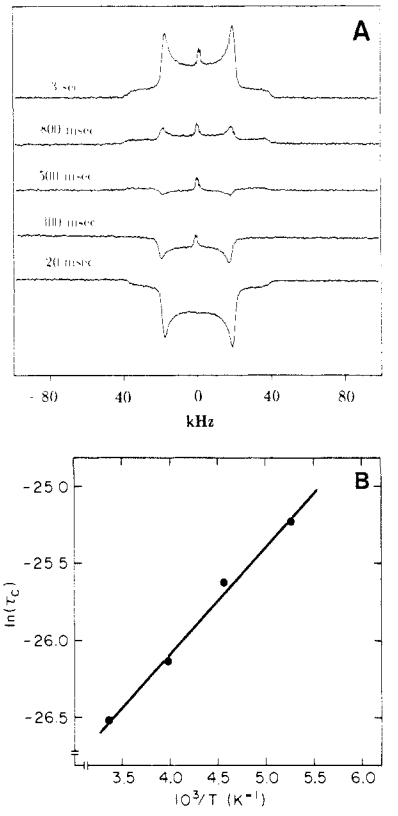


Figure 2. (A) Representative partially relaxed inversion-recovery ^2H NMR spectra of deuteriomethyl-labeled deoxythymidine at 251 K. The delay times are given to the left of each spectrum. The spectrometer settings are as described in Figure 1. (B) Arrhenius plot presentation of the spin–lattice relaxation time (T_1) data. The correlation times were calculated as described in the text, assuming that only the 3-fold methyl jump contributed to the relaxation. A least-squares fit of the data yields the apparent activation energy for the methyl jump $E_a = 5.9$ kJ/mol.

In order to distinguish between rotational diffusion or 3-fold jumps about the C_3 axis, it is necessary to investigate the spin–lattice (T_1) relaxation behavior of the methyl deuterons in some detail. The inversion-recovery ^2H NMR spectra of deoxythymidine-*methyl*-d_3 at 251 K are shown in Figure 2A. Inspection of these spectra reveals that the T_1 is anisotropic across the powder pattern. For example, in the spectrum acquired with delay time 300 ms the outer edges (shoulders) of the powder pattern are

(18) De Fontaine, D. L.; Ross, D. K.; Ternai, B. *J. Magn. Reson.* **1975**, *18*, 276–281.
(19) Torchia, D. A.; Szabo, A. *J. Magn. Reson.* **1982**, *49*, 107–121.
(20) Brink, D. M.; Satchler, G. R. *Angular Momentum*; Oxford University: London, 1962; p 24.
(21) Greenfield, M. S.; Ronemus, A. D.; Vold, R. L.; Vold, R. R.; Ellis, P. D.; Raidy, T. E. *J. Magn. Reson.* **1987**, *72*, 89–107.
(22) Barnes, R. G. In *Advances in Nuclear Quadrupole Resonance*; Smith, J. A. S., Ed.; Pergamon: New York, 1974; Vol. 1, pp 335–355.
(23) Rinne, M.; Depireux, J. Reference 22; pp 357–389.

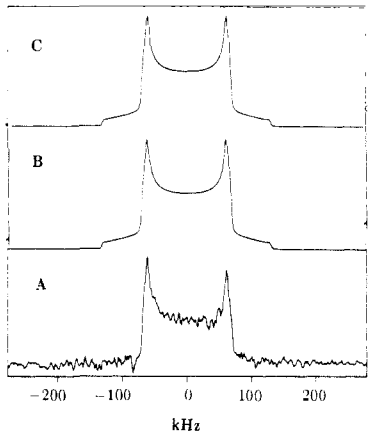


Figure 3. (A) Solid-state ^2H NMR spectrum of 2'-deoxythymidine-6-d_1 at 298 K. The spectrum was obtained as described in Figure 1 except the dwell time was 900 ns. A 20-mg sample required 3900 scans with a 40-s delay between scans. (B) Calculated rigid-lattice spectrum yielding the values $\eta_{eff} = 0.07$ and $(e^2qQ/h)_{eff} = 175$ kHz. (C) Calculated spectrum assuming a small-amplitude (±9°) librational motion of the base as described in the text. The simulation used $\eta_{static} = 0.03$ and $(e^2qQ/h)_{static} = 179$ kHz with a librational correlation time $\tau_c = 0.78 \times 10^{-12}$ s.

nulled, and the singularities are inverted; while in the spectrum acquired with delay time 500 ms the shoulders are upright, and the singularities are slightly inverted. Clearly, the shoulders of the powder pattern have a much shorter T_1 than the singularity. The anisotropy of the spin–lattice relaxation time is consistent with a 3-fold jump motion of the methyl group.[19]

Once the mechanism of motion has been determined, it is possible to calculate the correlation time of the motion from the value of the T_1 as described by Torchia and Szabo.[19] The ^2H NMR spin–lattice relaxation time of deuteriomethyl–deoxythymidine was measured at several temperatures ranging from 191 to 298 K. The T_1 values ranged from 327 ms at the lowest temperature to 1.12 s at room temperature. In all instances, the magnetization recovery was exponential. The correlation time (τ_c) for the 3-fold jump motion was calculated from each value of the T_1 with the computer program described in the Experimental Section. The data are presented in an Arrhenius plot (Figure 2B), where we have assumed that the jump is a single activated process so that $\tau_c = \tau_0 \exp(E_a/RT)$. The plot yields an apparent activation energy, $E_a = 5.9$ kJ/mol, and a preexponential factor, $\tau_0 = 2.8 \times 10^{-13}$ s. The value of the apparent activation energy is somewhat lower than what has been reported in the literature for the 3-fold jump motion of other methyl groups.[4,5] The correlation time for methyl 3-fold jumps in deoxythymidine at room temperature is $\tau_c \sim 3.0$ ps.

Motion of the Pyrimidine Base. The ^2H NMR spectrum of deoxythymidine-6-d_1 is shown in Figure 3A together with a rigid-lattice simulation of the line shape (Figure 3B). The simulation yields effective values of the asymmetry parameter, $\eta_{eff} = 0.07$, and a quadrupole coupling constant, $(e^2qQ/h)_{eff} = 175 \pm 2$ kHz. The powder pattern line shape is consistent with the pyrimidine ring being essentially immobile on the ^2H NMR time scale. That is, there is no large-amplitude motion of the thymine base with $\tau_c \leq 10^{-3}$ s. However, we cannot rule out the possibility of a fast, small-amplitude motion of the ring such as a jump between two orientations about the equilibrium position. Such a motion would cause a slight decrease of the quadrupole coupling constant and a slight increase of the asymmetry parameter from the respective static values.

The T_1 of deoxythymidine-6-d_1 was measured at room temperature, and the magnetization recovery was found to be exponential. The T_1 value of 38 ± 3 s is too small for the pyrimidine base to be completely rigid on the ^2H NMR time scale. A fast, small-amplitude libration of the base could account for the relatively efficient relaxation of the 6-deuteron. The possibility that the relaxation of the 6-deuteron is caused by dipolar coupling to the methyl protons was also considered. However, our rough calculations show that, for a methyl group reorienting at such a fast rate ($\tau_c \sim 3.0$ ps), the T_1 would be on the order of 5000 s, which does not agree with the experimentally observed value.

We now consider the ^2H NMR spectral data of the methyl-labeled and base-labeled deoxythymidine together. A fast, small-amplitude motion of the pyrimidine base would also affect the ^2H NMR line shape and T_1 of the deuteriomethyl moiety. A model of motion that can account for the line shape and spin–lattice relaxation data for both labeled compounds would have the base jumping rapidly ($\tau_c \leq 10^{-8}$ s) about the N1–C4 axis between two orientations that are ±9° from the equilibrium position, while the methyl group undergoes fast 3-fold jumps about the C_3 symmetry axis independent of the base motion.

A ±9° libration of the pyrimidine base is sufficient to account for the observed asymmetry parameter of the methyl deuterons ($\eta_{eff} = 0.04$) if we assume that the static asymmetry parameter of the methyl deuterons is zero, as has been reported for many aliphatic deuterons.[22] Moreover, the proposed motional model would require $(e^2qQ/h)_{static} = 159$ kHz for the methyl deuterons, which is in good agreement with reported values of the quadrupole coupling constant of methyl deuterons in other compounds.[22] A calculated ^2H NMR spectrum based on these values of the static asymmetry parameter and quadrupole coupling constant and the motional model is shown in Figure 1C. The agreement with the experimental line shape and the rigid-lattice simulation is excellent.

A calculated ^2H NMR spectrum of deoxythymidine-6-d_1, which takes into account the proposed fast libration of the base is shown in Figure 3C. This simulation yields the static values of the asymmetry parameter, $\eta_{static} = 0.03$, and the quadrupole coupling constant, $(e^2qQ/h)_{static} = 179$ kHz. These values are in remarkable agreement with those reported for deuterons in aromatic systems.[22] Again, the agreement of the experimental and calculated spectra is excellent.

The correlation time of the base libration was calculated from the T_1 of the 6-deuteron with the 2-fold jump computer program described above. Using a static quadrupole coupling constant of 179 kHz and the libration angle ±9°, we obtain the room-temperature correlation time for the base libration, $\tau_c \sim 0.8$ ps. The experimental line shape is inconsistent with the motion occurring with the other possible correlation time ($\tau_c \approx 10^{-6}$ s) from the so-called slow side of the T_1 curve.

Next, we consider the effect of the motional model on the spin–lattice relaxation behavior of the methyl deuterons. We have derived an expression for the spin–lattice relaxation rate that explicitly takes into account both the 3-fold jump of the methyl group and the small-amplitude 2-fold jump of the symmetry axis. When a static quadrupole coupling constant of 159 kHz for methyl deuterons and the libration angle and rate listed above are used, a room-temperature correlation time for the 3-fold methyl jump, $\tau_c \sim 4.2$ ps, is calculated. This is not substantially different from the value calculated previously when only the 3-fold methyl jump was considered. Unfortunately, we can only compare the one value at room temperature because the rate of base libration at other temperatures cannot be estimated. However, if the small-amplitude libration is assumed to have a small apparent activation energy, then the calculated methyl correlation time at each temperature will be larger than that which was calculated previously, assuming only a 3-fold methyl jump for the motional model. Our calculations show that these differences are quite small over the temperature range studied. Thus, the contribution of the small-angle libration to spin–lattice relaxation probably has little effect on the low apparent activation energy of the 3-fold methyl jump that was measured in Figure 2B.

Motion of the 5'-Methylene Group. The ^2H NMR spectrum of deoxythymidine-5'-d_1 is shown in Figure 4A together with a rigid-lattice simulation of the line shape (Figure 4B). The fit of this calculated spectrum to the experimental powder pattern is quite poor. Note especially that there is too much intensity in the center of the calculated spectrum. Nevertheless, the simulation yields effective values of the asymmetry parameter, $\eta_{eff} = 0.05$, and the quadrupole coupling constant, $(e^2qQ/h)_{eff} = 166 \pm 2$ kHz. The powder pattern line shape is consistent with there being no

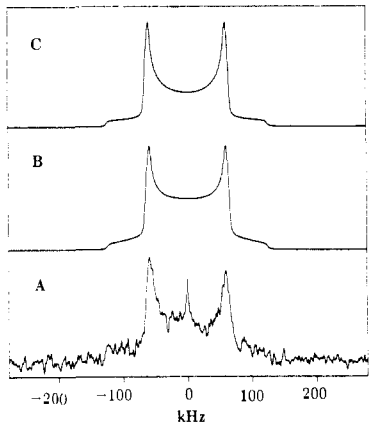


Figure 4. (A) Solid-state ^{2}H NMR spectrum of a 1:1 mixture of the two isomers of 2'-deoxythymidine-5'-d_1 at 298 K. The spectrum was obtained as described in Figure 3. A 15-mg sample required 1500 scans with a 15-s recycle delay. (B) Calculated spectrum based on the rigid-lattice model. The fit of this simulation is poor but yields $\eta_{eff} = 0.05$ and $(e^2qQ/h)_{eff} = 166$ kHz. (C) Calculated spectrum assuming a small-amplitude (±10°) libration of the methylene group as described in the text. The simulation used $\eta_{static} = 0.0$ and $(e^2qQ/h)_{static} = 174$ kHz with a librational correlation time $\tau_c = 2.0 \times 10^{-7}$ s.

large-amplitude motion that is fast on the NMR time scale ($\tau_c \leq 10^{-3}$ s). The value of the quadrupole coupling constant is somewhat smaller than that reported for aliphatic deuterons and, as noted earlier, the nonzero value of the asymmetry parameter is also unexpected.[22]

The T_1 of the 5'-methylene-deuteriated deoxythymidine was measured at room temperature, and the magnetization recovery was found to be exponential. The T_1 value of 2.7 s suggests that there is a fairly rapid, small-amplitude motion of the methylene group that is responsible for the efficient relaxation. If the static value of the asymmetry parameter is assumed to be zero, then a motion that consists of a libration of the methylene group between two orientations about the equilibrium position can account for the nonzero value of the effective asymmetry parameter. In this model, the C–D bond vector jumps between two sites that are tilted ±10° from the equilibrium position. Such a motion would have to occur with a correlation time $\tau_c \approx 10^{-7}$ s or $\tau_c \approx 10^{-11}$ s to account for the observed T_1 at room temperature. We found that a simulation with a correlation time $\tau_c = 2.0 \times 10^{-7}$ s for the 2-fold ±10° libration fits the experimental line shape best. The calculated ^{2}H NMR spectrum is shown in Figure 4C. Note especially the improved fit of the center of the powder pattern. The simulation yields a value of the static quadrupole coupling constant, $(e^2qQ/h)_{static} = 174 \pm 2$ kHz. This value is high in the range of what has previously been reported for methylene groups[23] but nevertheless is still reasonable.

In the above discussion, it was tacitly assumed that the quadrupole coupling constants of the two label positions are equal. In principle, this assumption is not necessarily valid since the two deuterons are diastereotopic. Nevertheless, if the coupling constants are different, the difference must be less than ~5 kHz or the spectrum in Figure 4A would have appeared to be the superposition of two resolved powder patterns. Such a small difference would have little effect on the rate or amplitude of the methylene motion that has been determined.

Discussion

The ^{2}H NMR spectra of the various selectively deuteriated deoxythymidines in this study indicate that there is no fast, large-amplitude, internal motion of the molecule, with the exception of the 3-fold jump of the methyl group about the C_3 symmetry axis. However, we cannot rule out the presence of large-amplitude motions of the deoxyribose ring proper (such as a puckering motion), since we had no compounds that were labeled at the strategic positions (e.g. at the 2'-methylene). Roy et al. have reported a preliminary solid-state ^{2}H NMR study of deoxyguanosine and deoxythymidine that were biosynthetically deuteriated at the 2'-methylene group.[24] Their results suggested that there are small-amplitude motions of this moiety on a time scale of $\leq 10^{-6}$ s. Further studies are necessary to elucidate the motion of the sugar ring in deoxynucleosides.

The ^{2}H NMR spectral parameters reported here do suggest the presence of rapid, small-amplitude motions of the various moieties of the nucleoside. We have chosen a very simple model of fast small-angle jumps between two sites to account for the data. In particular, such a motional model could account for the nonzero value of the asymmetry parameter of aliphatic deuterons while also explaining the reduced values of the quadrupole coupling constant and the relatively efficient spin–lattice relaxation.

Since we were unable to directly determine the static values of the asymmetry parameter and quadrupole coupling constant of our compounds, our analysis relies on empirical comparison to model compounds. The studies of these model compounds[22,23] clearly demonstrate that deuterons in aromatic systems have coupling constants in the neighborhood of 178 kHz and asymmetry parameters $\eta \sim 0.04$, while deuterons in aliphatic compounds in general have smaller coupling constants (~168 kHz) and asymmetry parameters $\eta = 0$. Theoretical considerations suggest that the state of hybridization of the carbon in the C–D bond can explain these effects quite well.[22]

We note that our simple two-site libration is not the only model that can account for the experimental data. In particular, models involving jumps over a larger number of sites (e.g. jumps over four sites with unequal probabilities) can also cause a nonzero effective asymmetry parameter while reducing the quadrupole coupling constant and contributing to relaxation. In the case of the methylene-labeled compound, a slight difference in the quadrupole coupling constants of the diastereotopic deuterons could account for the nonzero η_{eff}. Then, the motion of the methylene group could possibly be more isotropic, but the amplitude and rate of the motion would not be significantly different. In the absence of any additional evidence, however, we prefer the simpler model of a two-site libration.

Another model than can account for the nonzero η_{eff} is that the static quadrupole coupling constant is not the same at all the sites visited by the (symmetric) motion.[6,22] In general, such a situation will generate an asymmetry in the averaged interaction so the effective asymmetry parameter will be nonzero. Such a model has been invoked by Hiyama et al.[6] to account for their observed η_{eff} of 0.07 for the deuteriomethyl moiety on thymine. We cannot discount the possibility that a similar situation exists in deoxythymidine. However, unlike the previous study of thymine, we have ample evidence that there is rapid, small-amplitude motion of the pyrimidine base in deoxythymidine; and as we have already mentioned, such a motion could easily account for the nonzero value of the asymmetry parameter of the methyl deuterons.

In summary, we have obtained ^{2}H NMR spectra and spin–lattice relaxation times of several deoxythymidines that were selectively deuteriated on the methyl group, on the base, and on the 5'-methylene group of the sugar. Analysis of the spectral parameters allowed us to make several observations about the internal motions of these isotopically labeled moieties. In addition to the rapid 3-fold jumps of the methyl group; there are fast, small-amplitude librations in the molecule. A simple model of two-site jumps between orientations that are ≈9° from the equilibrium position could account for the data and allowed us to extract the static values of the spectral parameters.

The results of this study provide a basis for understanding the internal motion of deoxynucleosides when they are incorporated in DNA. We have incorporated deoxythymidine-6-d_1 into the self-complementary dodecamer d(CGCGAATTCGCG) at the T7 position, and we have initiated solid-state ^{2}H NMR studies of this molecule (Kintanar et al., unpublished results). The incorporation of the methyl-labeled and methylene-labeled deoxynucleosides into DNA oligomers of defined sequence is in progress. We feel that ^{2}H NMR studies of these synthetic, deuteriated

(24) Roy, S.; Hiyama, Y.; Torchia, D. A.; Cohen, J. S. *J. Am. Chem. Soc.* **1986**, *108*, 1675–1678.

oligomers will help to characterize the internal motion of these biomolecules and will provide important insights into the possible functional role of such flexibility.

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The ^{18}O Isotope Shift in ^{13}C Nuclear Magnetic Resonance Spectroscopy. 12. Position of Bond Cleavage in the Acid-Catalyzed Hydrolysis of Sucrose[1]

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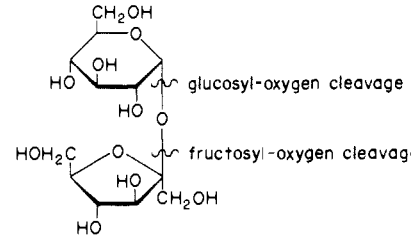
Abstract: The ^{18}O isotope-induced shift in ^{13}C NMR spectroscopy was used to elucidate the point of bond cleavage in the acid-catalyzed hydrolysis of sucrose. Sucrose was hydrolyzed in the presence of $H_2{}^{18}O$, and the incorporation of ^{18}O into the several anomeric products was simultaneously assayed as a function of time. While the equilibrium ratio of ^{18}O was detected in the fructose anomers throughout the course of the hydrolysis, the percentage incorporation of ^{18}O into the glucose anomers slowly increased to the equilibrium ratio. Independent experimental evidence was obtained with ^1H NMR; the ratio of β-glucose to α-glucose was observed to increase from an initially detected value of less than 2:3 to the equilibrium value of greater than 3:2 during the hydrolysis reaction. The data from the NMR experiments were quantitated and compared to theoretical models based on hydrolysis mechanisms involving fructosyl–oxygen bond cleavage and glucosyl–oxygen bond cleavage. The results clearly indicate fructosyl–oxygen bond cleavage under the reaction conditions employed.

The acid-catalyzed hydrolysis of sucrose has a rich history. It was perhaps the earliest catalytic reaction to be observed.[2] It was the first reaction to be studied kinetically[3] and was one of the first reactions to be studied as a function of temperature.[4] Arrhenius developed the equation bearing his name using data from early sucrose hydrolysis experiments.[5] Subsequently, debate arose concerning whether, in fact, the hydrolysis of sucrose followed Arrhenius' original formulation. In 1947 Moelwyn-Hughes summarized the results of several investigators, stating "There no longer remains any doubt that the Arrhenius energy of activation for the inversion [hydrolysis] of cane sugar shows a decrease as the temperature is raised."[6] Recently this conclusion has been vigorously challenged.[7]

There is also controversy regarding the detailed mechanism of the hydrolysis reaction. The reaction is generally considered to follow an A-1 mechanism in which a fast preequilibrium protonation is followed by a unimolecular, rate-determining heterolysis of a carbon–oxygen bond, although general acid catalysis of the hydrolysis has also been proposed.[8] The rate constant of the specific acid-catalyzed reaction has been shown to vary with sucrose concentration, the acid concentration, and the concentration of the supporting electrolyte.[9] The site of protonation is generally considered to be the bridge oxygen, although protonation of the fructosyl-ring oxygen has also been proposed.[10] Finally, the identity of the bond that cleaves in the rate-determining step to form the reaction products has not been satisfactorily demonstrated,[11] despite the several proposals that have been published (see Discussion).



The question of the site of bond cleavage in sucrose remains unresolved primarily because of uncertainties inherent in the experimental techniques previously employed. The ^{18}O isotope-induced shift in ^{13}C NMR[12] has been used to study a number of hydrolysis reactions. In favorable cases, this technique may be used to assay simultaneously the rate of hydrolysis, the position of bond cleavage, and any subsequent oxygen-exchange reactions of the products. This technique appeared to be uniquely suited to resolve the question of the site of bond cleavage in sucrose. In this paper we describe the results of our investigation using the ^{18}O isotope shift in ^{13}C NMR to elucidate the point of bond cleavage in the acid-catalyzed hydrolysis of sucrose; independent

(1) Paper 11: Röhm, K. H.; Van Etten, R. L. *Eur. J. Biochem.* **1986**, *160*, 327–332.
(2) (a) Clément, N.; Desormes, C.-B. *Ann. Chim. Phys.* **1806**, *59*, 329–339. (b) Sabatier, P.; Reid, E. E. *Catalysis in Organic Chemistry*; Van Nostrand: New York, 1923; p 326.
(3) Wilhelmy, L. *Ann. Phys. Chem. (Leipzig)* **1850**, *81*, 413–433.
(4) (a) Urech, F. *Ber.* **1883**, *16*, 762–766. (b) Spohr, J. *Z. Phys. Chem. (Leipzig)* **1888**, *2*, 194–217.
(5) Arrhenius, S. *Z. Phys. Chem. (Leipzig)* **1889**, *4*, 226–248.
(6) Moelwyn-Hughes, E. A. *The Kinetics of Reactions in Solution*; Clarendon: Oxford, 1947; p 59.
(7) (a) Buchanan, S.; Kubler, D. G.; Meigs, C.; Owens, M.; Tallman, A. *Int. J. Chem. Kinet.* **1983**, *15*, 1229–1234. (b) Ward, R. J. *Int. J. Chem. Kinet.* **1985**, *17*, 11–15.
(8) Hammett, L. P.; Paul, M. A. *J. Am. Chem. Soc.* **1934**, *56*, 830–832.
(9) Leininger, P. M.; Kilpatrick, M. *J. Am. Chem. Soc.* **1938**, *60*, 2891–2899.
(10) Szejtli, J.; Henriques, R. D.; Castiñeira, M. *Acta Chim. Acad. Sci. Hung.* **1970**, *66*, 213–227.
(11) Oon, S. M.; Kubler, D. G. *J. Org. Chem.* **1982**, *47*, 1166–1171.
(12) Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 252–253.

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