shown that it breaks in lyophilized Cu_2Zn_2SOD and Cu_2Cu_2SOD.[24]

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Deuterium NMR Spectra and Librational Motions of the Base Pairs in Oriented Calf Thymus DNA

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The different forms of solid DNA are usually distinguished by X-ray crystallography[1,2] and single-crystal X-ray studies have also been used to evaluate the degree of librational motion in di- and polynucleotides.[3] Similar information cannot be obtained for polynucleotides from X-ray fiber diffraction patterns, and in such cases NMR is quite useful. Phosphorus NMR studies, in particular, have linked the rate and extent of backbone motion to the degree of hydration of random[4,5] and aligned[6-8] samples of polynucleotides, but so far only limited use has been made of deuterium NMR.[4,9] Results reported here for oriented samples of Li^+ and Na^+ DNA deuterated in the adenine and guanine 8-positions demonstrate how deuterium NMR spectra of oriented samples can be used to characterize structural and dynamic properties of the bases in solid DNA.

Quadrupole echo deuteron spectra (38.4 MHz) are shown in Figure 1 for two samples (A, Na salt; B, Li salt) of oriented high molecular weight calf thymus DNA (Worthington Biochemicals). The 8-positions of adenine and guanine were deuterated at 63 °C in a D_2O buffer (pD 7.0), and oriented samples were prepared by the wet-spinning technique.[10] The samples were equilibrated with H_2O over saturated salt solutions[11] to a relative humidity of 66% for Li-DNA and 75% for Na-DNA. Spectra were recorded with the helix axes oriented parallel (top) and perpendicular (bottom) to the magnetic field, B_0. The Li-DNA spectra (Figure 1B) are characteristic of a sample with C-D bonds distributed uniformly at right angles to a common (helix) axis: at $\beta = 0°$ (top right) we observe two transitions at $\nu = \pm(69.3 \pm 0.7)$ kHz, while at $\beta = 90°$ (bottom right) we obtain a "cylindrical powder pattern" with singularities at $\pm(59.8 \pm 0.6)$ kHz and $\pm(130 \pm 2)$ kHz. The Na-DNA spectra (Figure 1A) are more complex: broad peaks at ±46 kHz as well as narrow features near ±69 kHz are predicted for a sample with base pairs tilted ~70° relative to the helix axis and a distribution of helix orientations. The relatively high intensity of the narrow doublet suggests the presence of minor amounts of B form, but except for that all four line shapes may be simulated by assuming a ±10-12° Gaussian distribution of the helix axes. X-ray diffraction patterns of these samples support our interpretation of the NMR spectra.

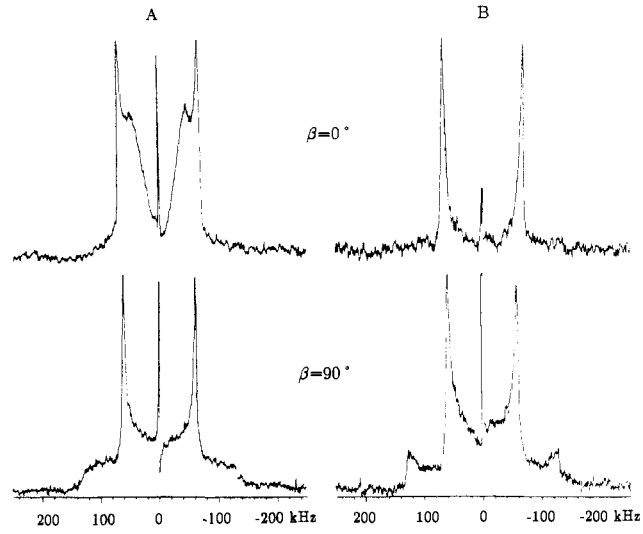

Figure 1. Deuterium NMR spectra obtained at 38.4 MHz of ca. 140-mg samples of the Na salt (A) and Li salt (B) of oriented calf thymus DNA. β is the angle between the DNA helix axis and the magnetic field. The spectra of Li-DNA at 66% relative humidity (B top and bottom) show that this sample is pure B form, while the spectra in Figure 1A of Na DNA at 75% relative humidity are characteristic of the A form, with possibly a small contribution from the B form. The sharp central peak is due to natural abundance deuterium in the water used to hydrate the samples. The spectra were obtained by using from 10 000 to 200 000 quadrupole echo pulse sequences with 2.5-μs $\pi/2$ pulses and 400-ms repetition times.

A more quantitative analysis of the B-form Li-DNA spectra in Figure 1B may be performed by considering the effect of librational motion upon the quadrupole Hamiltonian. For this purpose we used a two-step transformation[12] of the deuteron quadrupole coupling tensor (principal axis system is defined with the z axis along the C-D bond and the x axis in the purine plane), first through Euler angles (ϕ=0°,θ,χ=90°) to an intermediate frame with the z axis along the helix axis and then through angles (α,β,γ) to the laboratory frame with the z axis along B_0. We assume that the base pairs undergo librations in two planes: through $\pm\phi_0$ in the molecular plane about the base normal and through $\pm\theta_0$ in a plane perpendicular to the base pair. Assuming further that the C-D bonds are uniformly distributed within a region $-\phi_0 \leq \phi \leq \phi_0$ about $\langle\phi\rangle = 0°$ and $-\theta_0 \leq \theta \leq \theta_0$ about $\langle\theta\rangle = 90°$, we obtain the following expression for the motionally averaged deuteron transition frequencies:

$$\nu = \pm\tfrac{3}{4}(e^2qQ/h) \times [\tfrac{1}{2}(3\cos^2\beta - 1)f(\eta,\theta_0) - \tfrac{1}{2}\sin^2\beta\cos 2\gamma\, g(\eta,\theta_0,\phi_0)] \quad (1)$$

where

$$f(\eta,\theta_0) = \frac{1}{4}\left(1 - \eta - (3+\eta)\frac{\sin 2\theta_0}{2\theta_0}\right) \quad (2)$$

$$g(\eta,\theta_0,\phi_0) = \frac{1}{4}\frac{\sin 2\phi_0}{2\phi_0}\left(3(\eta-1) - (3+\eta)\frac{\sin 2\theta_0}{2\theta_0}\right) \quad (3)$$

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(12) The use of a two-step transformation in the description of this type of librational motion is, while exact for B DNA, only approximately correct for the A form, where a three-step transformation is required to describe librations in two mutually perpendicular planes.

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The form of eq 1 used here emphasizes that f and g function as the effective interaction strength and asymmetry parameter, respectively.

The "static" quadrupole coupling constant e^2qQ/h and asymmetry parameter η were determined from spectra of 8-deuterated adenosine and guanosine,[13] whose spin-lattice relaxation times exceed 5 s and therefore serve as suitably rigid reference materials. By inserting $e^2qQ/h = 179 \pm 1$ kHz and $\eta = 0.06 \pm 0.01$ as well as the transition frequencies for $\beta = 0°$ ($\nu = 69.3 \pm 0.7$ kHz) and $\beta = 90°$, $\gamma = 90°$ ($\nu = 59.8 \pm 0.6$ kHz) into eq 1-3, we estimate the librational amplitudes $\phi_0 = 13 \pm 2°$ and $\theta_0 = 10 \pm 2°$ for the B-form Li-DNA (Figure 1B). These amplitudes, while necessarily model-dependent, are rather insensitive to the precise form of the librational potential.

The deuteron spectra of dehydrated Li-DNA do not exhibit such signs of motional narrowing and $T_1 > 5$ s. In contrast, we find that $T_1 = 80$ ms at 38 MHz for Li-DNA at 75% relative humidity. The analysis presented above demonstrates that the much shorter T_1 is due to motions of limited amplitude but with significant spectral density in the Larmor frequency range. Preliminary relaxation measurements at 76 MHz show that T_1 is frequency-dependent and the determination of individual spectral densities of motion using techniques developed previously for liquid crystals[13] is currently in progress in our laboratory.

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A Catalytic Tin System for Trapping of Radicals from Cyclization Reactions. Regio- and Stereocontrolled Formation of Two Adjacent Chiral Centers

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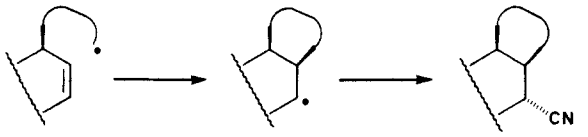
The successful transfer of a cyano group to the radical formed by a cyclization reaction (**1** → **2** → **3**)[1] owes its importance, inter



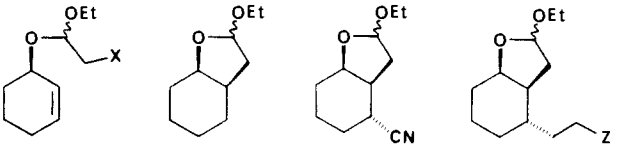
1 **2** **3**

alia, to the fact that together with the bromo acetal cyclization or its variants,[2] it becomes an important component of a method for positioning substituents on adjacent ring carbons with complete regiochemical and high stereochemical control (vide infra).

One of the requirements for successful trapping is minimization of hydrogen atom transfer to the cyclized radical **2**. This is particularly difficult when efficient hydrogen atom donors such as tin hydrides are used as a source of the tin radicals employed to generate the initial radical **1**.[3] Our original design of the cyano-trapping method,[1] therefore, avoided tin hydrides in favor of hexaphenyldistannane photolysis to produce the requisite tin radicals. This was successful in demonstrating the feasibility of the cyclization-trapping scheme, but it had several drawbacks, such as the formation of UV absorbing, insoluble, polymeric tin species. We therefore turned again to the possibility of using tin hydrides as a source of tin radicals.

We now report that we have succeeded in defining conditions that make practical the cyclization-cyano-trapping reaction, as well as other cyclization-trapping operations.

As we have reported previously,[1] even in the presence of an excess of *tert*-butyl isocyanide, the bromo acetal **4**, X = Br, cyclizes



4 **5** **6** **7**

to **5** under otherwise typical cyclization conditions (0.1 M tributylstannane; AIBN; refluxing benzene) without yielding any appreciable amount of the cyano-trapping product **6**. It was, however, encouraging to find that very slow addition of tributylstannane did produce the desired cyano compound **6**, albeit in low yield.

Production of tin hydride in low concentration, as well as drastic reduction of the quantity of tin species required for the conversion of **4** to **6**, might be achieved by a sodium borohydride-catalytic tributyltin halide system.[4] We therefore noted with interest Giese's report that this system, using photolytic initiation in ethanol, was effective in adding radicals derived from alkyl iodides to electrophilic olefins.[5] Unfortunately, cyano-trapping failed under Giese's conditions, even with an excess of *tert*-butyl isocyanide. Only the simple cyclization product **5** could be obtained from **4**, X = Br or I.[6,7] In spite of this initial setback, success was eventually achieved by using *tert*-butanol as solvent and sodium cyanoborohydride as reducing agent.

The following experiment is typical: A mixture of 44 mg of iodo acetal **4**, X = I,[8,9] 19 mg (2 equiv) of sodium cyanoborohydride, 3 mg (0.1 equiv) of AIBN, 247 mg (20 equiv) of *tert*-butyl isocyanide, and 5 mg (0.1 equiv) of Bu_3SnCl in 4 mL of degassed *tert*-butyl alcohol was prepared and immediately refluxed for 4 h under argon.[10] Workup and purification[11] gave the

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(6) For simple radical cyclization, the catalytic tin process is obviously a convenient alternative to the use of stoichiometric tin hydrides.
(7) Significantly, the *tert*-butyl isocyanide was unaffected under these conditions.
(8) Iodo acetals are preferred because bromo acetals often lead to incomplete reaction in cyclization-trapping experiments using catalytic tin. Presumably, this reflects their difference in rates of reaction with tin radicals, which may also react with radical traps. Cf.: Carlsson, D. J.; Ingold, K. U. *J. Am. Chem. Soc* **1968**, *90*, 7047. Ingold, K. U.; Lusztyk, J.; Scaiano, J. C. *J. Am. Chem. Soc.* **1984**, *106*, 343. Saegusa, T.; Kobayashi, S.; Ito, Y.; Yasuda, N. *J. Am. Chem. Soc.* **1968**, *90*, 4182.
(9) Mixed Iodo acetals are very easily prepared (>90% yield) by dropwise addition of a solution of ethyl vinyl ether (1.3 equiv) in methylene chloride to a stirred heterogeneous mixture of *N*-iodosuccimimide (1.05 equiv) and a primary or secondary alcohol in methylene chloride at -20 °C. Even the tertiary alcohol 1-vinylcyclohexanol gives 70% yield by this method. For a related procedure, see: Ueno, Y.; Chino, K.; Watanabe, M.; Moriya, O.; Okawara, M. *J. Am. Chem. Soc.* **1982**, *104*, 5564.
(10) The yield was the same on 10 times this scale. It is important to point out that *tert*-butyl alcohol and excess *tert*-butyl isocyanide can be distilled after completion of the reaction and reused without affecting the cyclization-trapping yields.

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