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Determination of the DNA Sugar Pucker Using ^{13}C NMR Spectroscopy†

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ABSTRACT: Solid-state ^{13}C NMR spectroscopy of a series of crystalline nucleosides and nucleotides allows direct measurement of the effect of the deoxyribose ring conformation on the carbon chemical shift. It is found that 3′-endo conformers have 3′ and 5′ chemical shifts significantly (5–10 ppm) upfield of comparable 3′-exo and 2′-endo conformers. The latter two conformers may be distinguished by smaller but still significant differences in the carbon chemical shifts at the C-2′ and C-4′ positions. High-resolution solid-state NMR of three modifications of fibrous calf thymus DNA shows that these trends are maintained in high-molecular-weight DNA and confirms that the major ring pucker in A-DNA is 3′-endo, while both B-DNA and C-DNA are largely 2′-endo. The data show that ^{13}C NMR spectroscopy is a straightforward and useful probe of DNA ring pucker in both solution and the solid state.

Solid-state NMR has recently been added to the panoply of spectroscopic methods available for the determination of the structure of biological macromolecules. Application of solid-state NMR to these problems has usually followed one of two rather different approaches. The first approach uses cross-polarization/magic-angle spinning (CP-MAS) to eliminate the broadening effect of the anisotropic interactions that are intrinsic to NMR in the solid state, thus producing high-resolution spectra of rare spin-$^1/_2$ nuclei in solid or solidlike biological samples. This allows measurement of isotropic chemical shifts, which can then be related to molecular conformation. An example of the successful use of this strategy is the recent determination of the chromophore structure of the membrane protein bacteriorhodopsin largely using solid-state NMR (Harbison et al., 1983, 1984, 1985; deGroot et al., 1989). The second approach utilizes the *anisotropy* of the interactions observable in solid-state NMR spectra to determine the orientation of the principal axis system of the chemical shift, dipolar, or quadrupolar tensors relative to some sample reference frame; since the orientation of the principal axes of these NMR interactions can readily be related to the local molecular frame, this approach can be used directly to determine the orientation and structure of biological materials (Cross & Opella, 1985; Lewis et al., 1985). These two approaches have also been recently combined using a new two-dimensional CP-MAS experiment (Harbison & Spiess, 1986; Tang et al., 1989).

While it has been widely used to investigate other systems, application of solid-state NMR to DNA has been rather limited. ^{31}P studies have been used to observe the structure and dynamics of the phosphodiester backbone of DNA (Shindo et al., 1981, 1985; diVerdi & Opella, 1981; Nall et al., 1981). More recently, deuterium NMR has been used to probe specifically deuterated wet-spun DNA samples, allowing observation of the DNA bases (Vold et al., 1986; Brandes et al., 1988). However, high-resolution solid-state NMR methods have not extensively been applied to DNA. This is curious, given the great success of high-resolution solution NMR studies of DNA oligomers. Proton NMR studies, employing two-dimensional sequential assignment techniques (Feigon et

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al., 1982) and cross-relaxation measurements, have given structural information for both typical and unusual DNA oligomers (Ragagopal & Feigon, 1989). ^{31}P and ^{13}C have also been sporadically applied to DNA; the latter has been used to detect changes in hydrogen bonding and conformation in DNA oligomers (Lankhorst et al., 1985; Borer et al., 1988).

One of the major conformational variables in DNA is the puckering of the deoxyribose ring. Fiber diffraction (Arnott & Hukins, 1972) and high-resolution X-ray studies of DNA oligomers (Shakked et al., 1981; Drew et al., 1981; Wang et al., 1979) have shown that, in the major modifications of crystalline and fibrous DNA, the ring pucker is usually either 3′-endo or 2′-endo. These variations are not merely coincidental; because of its relationship with the torsion angles within the deoxyribose ring, the ring pucker is inextricably linked to the helix geometry. Since ^{13}C chemical shifts are often highly conformation-dependent, one might expect them to be sensitive to ring pucker; this has not hitherto been demonstrated, probably because the A form of DNA has not previously been observed by solution ^{13}C NMR. In the solid, however, all of the major forms of DNA are accessible, either as fibers or as crystalline oligomers, or both. In addition, crystalline nucleosides and nucleotides possessing a variety of ring puckers are available. Solid-state NMR has the great virtue that it allows one to determine chemical shifts for crystalline materials for which the X-ray structure is known, permitting correlation of the NMR parameters directly with structural features, without the frequently incorrect assumption that the molecular conformations in the solid state and solution are identical. We have obtained chemical shifts for a series of nucleosides and nucleotides with different deoxyribose ring conformations and will show that the ^{13}C chemical shifts are related in a direct and straightforward way to the ring pucker. CP-MAS of fibrous DNA samples allows us then to visualize directly the ring conformation of the major forms of DNA, confirming the results of fiber diffraction studies but showing the existence of considerable heterogeneity of ring conformation in the native polymer.

Materials and Methods

All of the nucleosides and nucleotides used in this work were purchased from Sigma Chemical Co. (St. Louis, MO). These substances were crystallized by slow evaporation from aqueous solution at room temperature, except for the anhydrous (triclinic) modification of 5-bromodeoxycytidine, which was prepared from the monohydrate (obtained as above) by heating in vacuo at 80 °C for 12 h.

Calf thymus DNA was purchased from Sigma. The A-DNA samples were prepared by precipitating the DNA (in a 1 mg/mL solution in 0.4 M aqueous NaCl) with ethanol. The DNA was centrifuged out, equilibrated with a solution of 0.08 M NaCl in 80% ethanol, and then dried and stored at 79% relative humidity for several weeks. After packing in the sample rotor, the sample was reequilibrated at the same humidity for several days, before the rotor was sealed. C-DNA samples were obtained by incubating similar samples in a similar way at 30% relative humidity.

B-DNA samples had a lithium counterion. Lithium was exchanged for sodium by dissolving the commercial sodium–DNA in 0.4 M LiCl solution and dialyzing three times against 2.5 M LiCl. It was then precipitated in alcohol, dried, and stored at 66% humidity, before packing in MAS rotors as above.

All DNA samples were subjected to elemental analysis (Galbraith Laboratories). The Na–DNA samples used for A- and C-DNA had an excess NaCl content of 0.47 mol/mol of base pairs. Water content of the A-DNA samples was 22.35% by Karl Fischer test. The Li–DNA samples had 0.98 mol of LiCl/mol of base pairs and a Karl Fischer water content of 29.9%. These salt and water contents correspond to those known in previous studies to favor A- and B-DNA, respectively (Rupprecht & Forslind, 1970).

Solid-state NMR spectra were obtained by using a home-built NMR spectrometer operating at a field of 7.1 T (301.42-MHz ^{1}H frequency). Nucleoside and nucleotide spectra were obtained by using cross-polarization and magic-angle spinning, in a home-built probe incorporating Doty Scientific stator and rotors. Typical sample quantities were 250 mg. Spinning speeds were 4–4.5 kHz. Proton and ^{13}C rotating-frame frequencies were 55–60 kHz during both cross-polarization and decoupling. Recycle delays were optimized for each sample but were typically 16 s for materials that possessed water of crystallization and several minutes for anhydrous materials. It was found also that brominated and iodinated derivatives tended to have shorter proton relaxation times, possibly as a result of level crossings produced by the sample rotation acting on the large quadrupolar tensors of these nuclei. Typical cross-polarization contact times were 1 ms; usually 128–512 transients were averaged for each spectrum. Spectra for the DNA samples were obtained under similar conditions, but it was necessary to reduce the sample amounts to limit dissipative power losses; typical repetition times for the DNA were 3–4 s and were limited by sample heating rather than by relaxation.

Spectra of the model compounds and of DNA were assigned by comparison with solution chemical shifts. This method could possibly produce ambiguities in the deoxyribose region of the spectrum between the 3′ and 5′ carbons, and between C-1′ and C-4′, because of similar chemical shifts. The 5′ carbons were distinguished from the 3′ by their faster dipolar evolution in the delayed decoupling experiment (Opella & Frey, 1979), while the 1′ carbons were significantly broadened in comparison with the 4′ carbons as a result of dipolar coupling with the directly bonded quadrupolar ^{14}N nucleus. All shifts were referenced to external tetramethylsilane.

Results

Figure 1 shows a representative ^{13}C CP-MAS spectrum of a deoxyribonucleoside (deoxyadenosine), obtained at a spinning speed of 4.03 kHz. All 10 isotropic ^{13}C resonances are resolved for the 10 chemically distinct carbons in this crystal. The resonances were assigned as described above. The base carbon signals are quite distinct from those of the deoxyribose moiety; they have higher chemical shifts and larger shielding anisotropies, which at this magnetic field and spinning speed are manifest in the presence of a single pair of fairly weak rotational sidebands. The sugar carbons, in contrast, have no discernible sidebands. Some broadening of the base carbon signals by directly bonded ^{14}N is noticeable, particularly for C-6; in addition, the dexoxyribose C-2′ signal is slightly broader than the other sugar lines. This is probably a result of inadequate decoupling power (Garroway et al., 1981).

In Figure 2 we compare the deoxyribose region of the ^{13}C spectra of two deoxycytidine derivatives, deoxycytidine hydrochloride, whose crystal structure shows it to have a 3′-endo sugar pucker (Subramanian & Hunt, 1970), and deoxycytidine 5′-phosphate, whose pucker is 3′-exo (Viswamitra et al., 1971). In addition to the five sugar carbon signals, resonances from the base C-5 are seen; this position is significantly upfield shifted in base-protonated cytidine derivatives such as the two compounds shown here. Large upfield shifts are noted at the C-3′ and C-5′ positions in the 3′-endo compound.

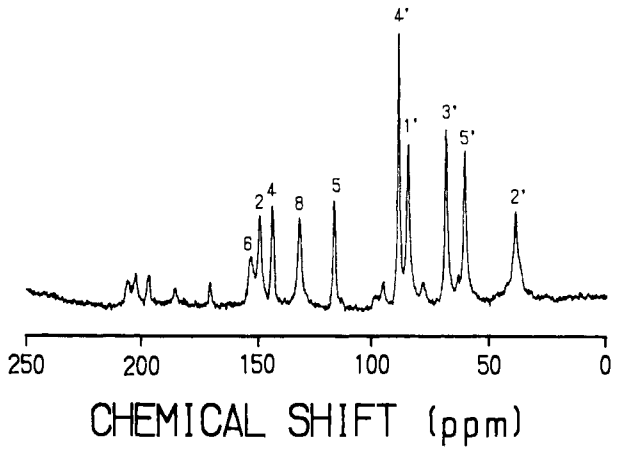

FIGURE 1: Cross-polarization/magic-angle spinning (CP-MAS) spectrum of deoxyadenosine monohydrate, obtained at a spinning speed of 4.03 kHz. The 10 isotropic resonances are assigned; the remaining weak peaks are rotational sidebands arising from incomplete averaging of the chemical shielding anisotropy by the sample rotation. The spectrum is typical of that of a 3′-exo nucleoside.

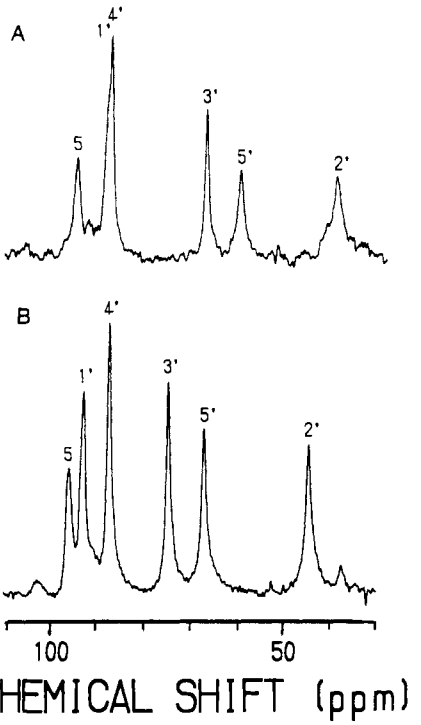

FIGURE 2: Comparison of the CP-MAS spectra of two deoxycytidine derivatives, deoxycytidine hydrochloride (A), which is 3′-endo, and deoxycytidine 5′-phosphate (B), which is 3′-exo. Only the upfield region of the spectrum, containing the sugar resonances, is plotted.

Figure 3 shows a more direct comparison: between two crystallographic forms of the same compound (5-bromodeoxycytidine), the monoclinic variant, which is hydrated and contains three distinct molecules per unit cell, all of which have a 3′-endo sugar pucker (Low et al., 1981a), and the anhydrous triclinic form, which has four molecules per unit cell, in a variety of different ring puckers, none of which is 3′-endo (Low et al., 1981b). The ^{13}C chemical shifts for the three very similar molecules of the monoclinic form are almost identical, and the presence of chemically distinct species is only manifest in the line widths and in shoulders on several of the peaks. The triclinic form shows two and three clearly resolved resonances for the C-3′ and C-5′ carbons, respectively. The C-3′ resonances have intensity ratios of approximately 3:1; it is tempting to speculate that the weaker resonance corresponds with

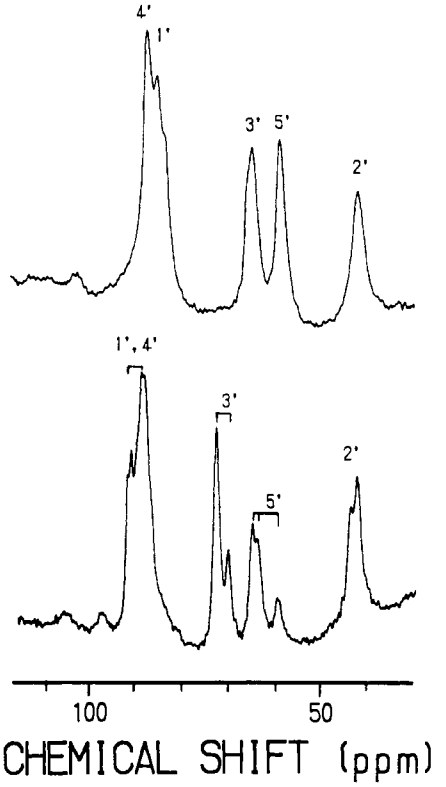

FIGURE 3: Comparison of the CP-MAS spectrum of two crystalline forms of the same substance (5-bromodeoxycytidine). (Top) The monoclinic modification, which has three molecules per asymmetric unit, all of which are 3′-endo. (Bottom) The triclinic modification, with four molecules per asymmetric unit, none of which is 3′-endo.

molecule D in the crystal structure, which has the unusual O-4′-endo pucker. We should note parenthetically that the quadrupolar bromine atom has profound effects on the base resonances of this compound, rendering the directly bonded C-5 carbon invisibly broad and significantly broadening the next-nearest-neighbor C-6 and C-4 carbons. Similar effects are seen in the other brominated and iodinated nucleosides studied and have been noted previously in brominated polymers (Eckert et al., 1987).

In Figure 4 we show the upfield portion of the spectra of DNA at three different ambient relative humidities and electrolyte contents, which produce respectively the A, B, and C forms of DNA. The identity of these three forms was confirmed by examining the ^{31}P NMR spectra, which are quite characteristic (Shindo et al., 1981). The spectra of the A and C forms were obtained with approximately 100 mg wet weight of Na–DNA; that of the B form with approximately 30 mg of Li–DNA. These comparatively small sample amounts were used because of the deleterious effects of the materials on the probe Q, presumably a result of the high dielectric strength and ionic conductivity of fibrous DNA. This effect was particularly pronounced for B-DNA. Larger samples caused unacceptable lengthening of the proton 90° pulse and great difficulties matching the high-frequency circuit impedance to 50 Ω. The A and C spectra were averaged for 12 h and that of B-DNA for 48 h. The spectra contain an extra upfield resonance compared with those in Figures 1–3; this is due to the thymine methyls. The larger line widths of the DNA spectra, particularly of the B and C forms, compared with those of the crystalline nucleosides studied, are presumably due to sequence and conformational heterogeneity.

In Table I we list the ^{13}C chemical shifts for the deoxyribose carbons in all of the nucleosides and nucleotides studied and

Table I: ^{13}C Chemical Shifts of Model Compounds[a]

compound	C-1′	C-2′	C-3′	C-4′	C-5′	pucker	ref
2′-deoxycytidine hydrochloride	87.2	38.7	66.7	87.2	59.5	3′-endo	*b*
5-bromo-2′-deoxycytidine monohydrate (three molecules)		42.0 (3)	64.8 (3)		58.9 (3)	3′-endo	*c*
2′-deoxycytidine 5′-phosphate monohydrate	92.9	44.6	74.7	87.5	67.1	3′-exo	*d*
5-iodo-2′-deoxycytidine	90.0	44.3	72.0	90.0	61.5	3′-exo	*e*
2′-deoxyadenosine monohydrate	88.6	42.5	72.3	92.7	64.3	3′-exo	*f*
5-bromo-2′-deoxyuridine	88.0	41.3	72.8	88.0	64.1	2′-endo	*g*
5-iodo-2′-deoxyuridine	84.2	38.7	73.4	88.3	62.8	2′-endo	*h*
5-bromo-2′-deoxycytidine, triclinic (four molecules)		42.4 (3)	72.8 (3)		65.2 (2)	3′-exo	*i*
		43.8 (1)			64.1 (1)	2′-endo	
						1′-exo	
			70.5 (1)		59.9 (1)	O-4′-endo	

[a] All shifts are given in ppm relative to external tetramethylsilane, are uncorrected for bulk magnetic susceptibility, and are expected to be accurate to ±0.3 ppm. The numbers in parentheses are the number of molecules contributing to that resonance, when there is more than one chemically equivalent molecule per unit cell. References refer to the X-ray structure. [b] Subramanian and Hunt (1970). [c] Low et al. (1981a). [d] Viswamitra et al. (1971). [e] Ghiassy et al. (1981). [f] Watson et al. (1965). [g] Iball et al. (1966). [h] Camerman and Trotter (1965). [i] Low et al. (1981b).

Table II: ^{13}C Chemical Shifts of DNA Sugar Carbons[a]

modification	C-1′	C-2′	C-3′	C-4′	C-5′
A form	81.6	38.7	68.2	83.6	60.9
B form	84.4	36.3	77.3	84.4	66.2
C form	83.9	37.3	75.5	81.3	65.4

[a] Average chemical shifts in ppm for three principal forms of DNA, prepared as fiber samples as described in the text. All chemical shifts are given relative to tetramethylsilane and are uncorrected for susceptibility effects.

correlate these shifts with the sugar pucker, recalculated by us using published X-ray coordinates. In Table II we give the ^{13}C shifts of the deoxyribose carbons of the three forms of DNA shown in Figure 4.

Discussion

Nature of the Ring Pucker in Crystalline Nucleosides, Nucleotides, and DNA. The originally proposed structure of B-DNA (Crick & Watson, 1954) included a puckered deoxyribose ring. Spencer (1959) pointed out that steric considerations in cyclopentane rings favor one of the atoms being displaced out of plane, on either the exo or the endo side. Deoxyribose structures are thus frequently described in terms of which atom is out of plane, and on which side. In real structures, however, no more than three atoms can occupy the same plane, and therefore, any precise description of a five-membered ring must specify the puckers of two ring atoms. In fact, between any two "pure" single-atom puckers of adjacent ring atoms one can conceive of a continuum of two-atom puckered conformations through which one can distort the first pucker, via a "twisted half-chair", to give the second. It is found that for many cyclopentane derivatives and several five-membered rings of biological interest (such as proline), there exists a very shallow potential surface for such distortion, leading to dynamic disorder which is readily observable via diffraction, ELDOR (Margulis et al., 1973), and solid-state NMR (Sarkar et al., 1986). Curiously enough, this does not appear to be a complication in the present work. All of the crystalline nucleosides and nucleotides studied have deoxyribose rings that approximate closely to "pure" single-atom puckers, with displacements of the four coplanar ring atoms of no more than 0.01 nm from the best plane between them. Furthermore, the proton and carbon T_1 values of virtually all of the model compounds studied are rather long, tending to rule out any rapid flexing of the five-membered rings. Nor is there any evidence of slower motions, which would be manifest as broadening of the CP-MAS spectra (Suwelack et al., 1981). While nucleosides and nucleotides do crystallize in different ring conformations, in no single crystalline derivative studied by us is there any evidence for dynamic or

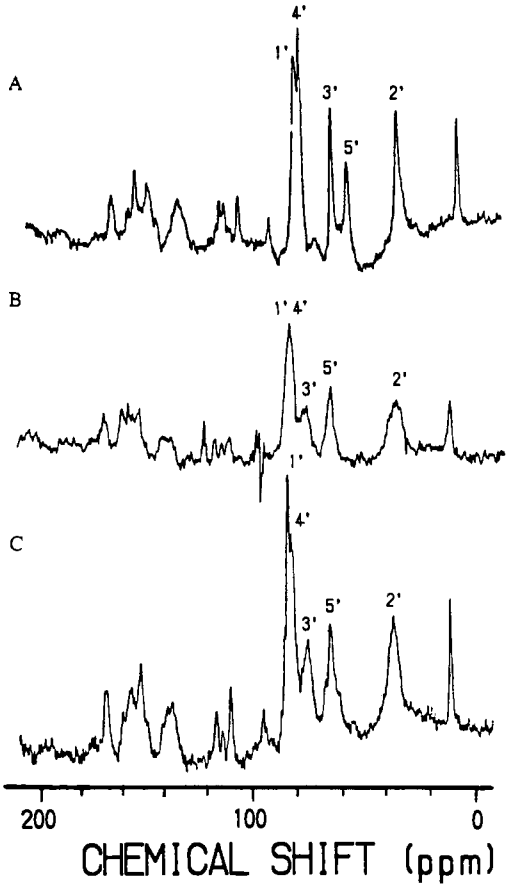

FIGURE 4: CP-MAS spectra of three of the major modifications of fibrous DNA (A) Sodium–DNA in the A form, obtained at 79% relative humidity. (B) Lithium–DNA in the B form, obtained at 66% relative humidity. (C) Sodium–DNA in the C form, obtained at 30% relative humidity.

static disorder involving multiple conformations. This may be a result of the more extensive hydrogen bonding of the deoxyribose ring compared with prolines or cyclopentanes. This bonding probably tends to fix the ring into a single static conformation.

Origin of the 3′-Endo Shift. As the data in Table I and the spectra shown in Figures 2 and 3 show, there is a large and consistent upfield shift at the C-3′ position in 3′-endo conformers and a significant shift also at the C-5′ position. The average chemical shifts of all of the 3′-endo conformers are 65.3 ± 1.4 and 59.1 ± 0.4 ppm for the 3′ and 5′ carbons respectively, in contrast to the averages of 72.7 ± 2.2 and 63.8 ± 3.9 ppm for all of the non-3′-endo derivatives. The shifts thus form two nonoverlapping ranges which can be used as

unambiguous indicators of the ring pucker. While there are chemical differences between many of the derivatives studied, and these differences may be expected to perturb the ^{13}C chemical shifts (as will be discussed below), these differences are wholly inadequate to account for the magnitude or the consistency of the shifts observed. Particularly convincing are the data for the two crystalline forms of 5-bromodeoxycytidine. The hydrated crystalline modification of this substance contains three chemically inequivalent molecules in nearly identical 3′-endo conformations (Low et al., 1981a) while the anhydrous triclinic modification has four inequivalent molecules in a variety of conformations, all of which are non-3′-endo (Low et al., 1981b). The shifts in this compound follow the general trend and cannot be attributed to chemical differences.

The most plausible explanation of the large and consistent upfield shift of the 3′ and 5′ resonances in 3′-endo derivatives relative to other conformers attributes these shifts to steric interactions between the axial 3′ proton and the bulky substituents on the endo side of the deoxyribose ring. Since this side of the ring accommodates both the base and the 5′ carbon, it is likely to be much more crowded than the exo side. In particular, in derivatives with the 3′-endo geometry and a gauche–gauche conformation about the C-4′–C-5′ bond, there exists a close contact between the axial 3′ proton and the oxygen on C-5′. Such large steric interactions are well-known to cause sizable upfield shifts (up to 10 ppm) in ^{13}C NMR, and this effect has been well documented in both solution and the solid state (Harbison et al., 1985). The physical origin of the shift is electrostatic repulsion of the σ-bonding electrons away from the contact and toward the carbon, where they contribute to an increase in the diamagnetic shielding (Grant & Cheney, 1968). In this specific case the steric interaction involves an electronegative oxygen, presumably via its lone-pair oribtals, and thus the upfield shift is likely to be augmented. In contast, the axial proton in the 3′-exo conformation has no significant close contacts, and therefore, the steric effect is likely to be greatly diminished or absent.

A complementary effect might be expected for the C-5′ carbon, and in fact, significant upfield shifts are observed for this resonance in C-3′-endo conformers. The interpretation of the C-5′ shift is somewhat complicated by the existence of conformational isomerism about the C-4′–C-5′ bond. For example, 5-iododeoxycytosine, a 3′-exo nucleoside that has the more unusual and sterically hindered gauche–trans configuration about this bond (Ghiassy et al., 1981), has a C-5′ shift significantly upfield of the other non-3′-endo model compounds, although it does not quite fall in the range of the 3′-endo shifts. This point is important, since this "extended" conformation has recently also been detected in a crystalline oligonucleotide (Haran et al., 1987).

Conformation-Dependent Shifts in the Non-3′-endo Derivatives. Most of the non-3′-endo derivatives examined are either 2′-endo or 3′-exo. Examination of the C-2′ and the C-4′ chemical shifts of these compounds shows that these resonances are sensitive to this difference; the ^{13}C chemical shifts at both positions in 2′-endo derivatives are significantly upfield of those of the 3′-exo compounds. The differences are smaller than those observed at the 3′-position, and as a result any conclusions drawn from them must be more tentative; nonetheless, their consistent nature leads us to believe that they may be a useful means of distinguishing between these two forms. They are likely also to have a steric origin; however, the contacts between the axial 2′ proton and the endo substituents are not as close as those of the 3′ proton, and therefore, the shifts are smaller.

Table III: Predicted ^{13}C Chemical Shifts of DNA Sugar Carbons[a]

conformation	C-2′	C-3′	C-4′	C-5′
3′-endo	39.4	67.6	83.6	61.4
3′-exo	42.0	75.3	88.4	67.1
2′-endo	38.2	75.4	84.5	65.7

[a] Calculated from the averaged chemical shifts of the nucleosides in Table I, corrected for the effect of phosphate esterification as described in the text.

Other Influences on the ^{13}C Chemical Shifts. So far in our discussion we have ignored chemical differences between our nucleotides and nucleosides, and between these low-molecular-weight compounds and DNA. These differences are of two major types: in phosphate substitution and in base substitution. We shall consider these separately.

Comparison of the solution chemical shifts of nucleosides with those of the analogous nucleotides, based on data in the Sadtler Index (1985), indicates that changing a free –OH group to a phosphate ester linkage has a rather small effect on the carbon chemical shifts of nucleosides. For example, the chemical shifts of adenosine 5′-monophosphate are 1.8 ppm downfield of that of free adenosine at the C-5′ position, 1.8 ppm upfield at the C-4′ position, and 0.2 ppm downfield at the C-3′ position. Very similar results are obtained for guanosine. These effects are small and cannot be responsible for the large C-3′ shifts we observe in our model compounds. We must however apply corrections of this magnitude to our nucleoside data in order that the chemical shifts we obtain be strictly comparable with those of DNA.

Comparison of the chemical shifts of nucleosides formed from different DNA bases (Sadtler, 1985; Chandrasegaran et al., 1985) suggests that the influence of the base on the ^{13}C chemical shifts of the deoxyribose moiety is small and is confined largely to the carbon immediately adjacent to the base (C-1′). Thus, base substitution cannot be responsible for the large effects noted here. Parenthetically, however, we note that we do in fact detect large shift changes at the C-1′ position which may be related to the torsion angle between the deoxyribose and ring planes; these effects have not as yet been fully analyzed.

^{13}C Chemical Shifts of DNA. In order to compare nucleoside data with DNA, we must calculate the effect on the ^{13}C chemical shifts of nearby carbons of esterifying a primary or secondary alcohol. A good first approximation is to use the shift induced by converting nucleosides to the corresponding phosphate esters. On the basis of the shift differences between adenosine and guanosine and their corresponding 5′-monophosphates, we estimate the effect of esterification by a phosphate ester to be a downfield shift of 1.6 ppm at the carbon immediately adjacent to the ester linkage, an upfield shift of 1.8 ppm on the next-nearest-neighbor carbon, a 0.7 ppm downfield shift at the γ carbon, and negligible shifts further away. Using these corrections and averaged data from Table I, we have assembled in Table III the expected chemical shifts in DNA for each of the three major ring conformers. If we now compare these predicted values with the spectrum in Figure 4A and the experimental data in Table II, it is clear that the values for A-DNA are very close to those expected for a 3′-endo conformer, as is predicted from fiber diffraction studies (Arnott & Hukins, 1972). The ^{13}C lines for A-DNA are also rather narrow, as might be expected from the highly crystalline nature of this form. In addition to the 3′-endo lines, close inspection of Figure 4A also shows minor resonances that are in the positions expected for a 2′-endo conformer; these correspond to a population of 10–15% non-3′-endo sugars in our fiber samples. Spectra of other samples suggest that the

intensity of non-3′-endo signal depends considerably on sample preparation, humidity, and salt content; a more extensive study of the influence of these variables will be published elsewhere. However, the observation of a proportion of non-3′-endo conformer is in qualitative agreement with the results of Brandes et al. (1988), who detected approximately 25% of "B-like" conformer in the deuterium NMR spectra of similarly prepared samples. Our results suggest that it is very difficult to prepare fibrous native DNA in the A form entirely free of B-DNA.

At very high humidities (above 95%) our A-DNA samples enter the B form. B-DNA can also be produced by using lithium–DNA at lower humidities, and ^{13}C spectra of the two types of sample appear nearly identical. B-form DNA has drastic effects on the probe tuning and the quality factor; we find that more than 30 mg of B-DNA in our probe makes it impossible to tune and match the high-frequency channel. As a result, the signal to noise of the B-DNA spectra in Figure 4B is significantly worse than that of A-DNA, despite the longer acquisition time. The deoxyribose shifts of B-DNA are consistent with a 2′-endo conformer. The lines are significantly broader in B-DNA than in A-DNA; poor sensitivity has made it impossible to determine if this broadening is homogeneous in origin, a result of the slow motions known to exist in fibrous B-DNA (Shindo et al., 1985); or inhomogeneous, arising from heterogeneity in the deoxyribose conformer. However, the data explicitly rule out any significant fraction of A-DNA in the fibrous B-DNA samples. The chemical shifts obtained by us for fibrous B-DNA are very similar to those previously observed for DNA oligomers in solution [e.g., Leupin et al. (1987)].

Dehydration of A-DNA leads to so-called C-DNA. The ^{13}C spectra of C-DNA are similar to those of B-DNA. In this case the line widths of C-DNA are definitely inhomogeneous and therefore presumably result from heterogeneous sugar conformations. The width of the C-2′ resonance in particular may suggest a range of sugar puckers in the sample between C-2′-endo and C-3′-exo. Additionally, there are indications of a pair of sharp peaks with chemical shifts characteristic of the 3′-endo conformation straddling the main C-5′ peak, suggesting the persistence of a small proportion of "A-like" DNA even at very low humidities. These peaks and the overall broader lines in C-DNA are consistent with the relatively disordered nature of this form, which has been inferred from fiber diffraction (Franklin & Gosling, 1953).

Significance of These Findings. These results suggest that ^{13}C NMR spectroscopy should be a straightforward and unequivocal method of determining sugar pucker in DNA. While these results were obtained by using solid-state NMR techniques, the findings may well have considerable value in the determination of the conformation of DNA oligomers in solution. Until now, deoxyribose ring conformation in solution has been deduced primarily via proton NMR spectroscopy, either by using proton cross-relaxation rates or by analysis of coupling constants (Orbons & Altona, 1986; Rinkel & Altona, 1987; Widmer & Wüthrich, 1987). The former method is fraught with danger, in that the strong distance dependence of cross-relaxation rates means that in the presence of multiple conformers in rapid exchange erroneous results are likely to be obtained. However, several elegant studies have shown that an analysis of combinations of the three-bond proton J-coupling constants within the deoxyribose moiety, using Karplus-type relationships, can be used to determine ring pucker and the proportions of rapidly exchanging conformers (Rinkel & Altona, 1987). Because of the sensitivity advantages of proton NMR, the latter method has obvious signal-to-noise advantages over the present one and also avoids the necessity of assigning the ^{13}C spectrum. On the other hand, it does require resolution of J-coupled multiplets and accurate measurement of the coupling constants. These tasks become rapidly more difficult as the length of the DNA oligomer increases, both on account of increased spectral clutter and as a result of dipolar broadening. Moreover, problems of sensitivity and assignment of rare-spin nuclei such as ^{13}C have recently been alleviated by the development of indirect detection methods (Bax et al., 1983; Griffey et al., 1985). Additionally, the overall dispersion of ^{13}C spectra is much higher than that of protons; at 11.4 T, the difference in NMR frequency at the C-3′ position between the 3′-endo and 2′-endo conformers is approximately 1 kHz. In comparison, J-coupling differences between the conformers are of the order of a few hertz. For this reason, small changes in the populations of ring conformers are likely to be more easily detected by ^{13}C NMR. Finally, the ^{13}C chemical shift changes, like proton coupling constants, can be expected to average in a simple linear fashion in the presence of exchanging conformers. In summary, the present method is less sensitive than solution ^{1}H NMR, and for small oligomers, where the proton spectra are well resolved, ring pucker can most readily be determined by using coupling constants. However, for larger oligomers, polymeric DNA fragments, and particularly fibrous or crystalline DNA (where well-resolved proton spectra are in any case unobtainable), analysis of the ^{13}C chemical shifts may well be the most useful way to study the deoxyribose ring conformation. We can specifically predict from our data that Z-DNA oligomers, which can be readily produced in solution, should show upfield chemical shifts at the 3′- and 5′-positions for half of the deoxyribose residues.

The results also show that CP-MAS is an excellent means of probing the heterogeneity of fiber DNA samples, since it equally weights crystalline and amorphous material. In fiber diffraction, in contrast, disordered material contributes predominantly to background scattering and tends to be ignored. It is hoped that this new method will allow us in the future to probe the effects of base sequence, ligand binding, and environmental conditions on DNA conformation and heterogeneity. Such studies are now in progress.

Registry No. Deoxyribose, 533-67-5; 2′-deoxycytidine, 951-77-9; 5-bromo-2′-deoxycytidine, 1022-79-3; 2′-deoxycytidine 5′-phosphate, 1032-65-1; 5-iodo-2′-deoxycytidine, 611-53-0; 2′-deoxyadenosine, 958-09-8; 5-bromo-2′-deoxyuridine, 59-14-3; 5-iodo-2′-deoxyuridine, 54-42-2.

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