Appendix C

In the product approximations (eq 17 and A9), the sums that must be calculated to evaluate the time correlation functions are quite lengthy. London and Avitable[27,29] have described an algorithm that can be used to reduce the computation time based on the symmetry relations for the reduced Wigner matrices and the symmetry associated with the potential for the rotational motion. To demonstrate what is involved, we show how to reduce the sum required for the C_6 relaxation in the product approximation. In order to use the algorithm, the sum in eq A9 is written in the form

$$\sum_{\substack{abcde\\ b'c'd'e'}} \langle d_{ab}^{2} d_{ab'}^{\prime 2} e^{i[b(\phi_1(t)+\pi)-b'(\phi_1(0)+\pi)]}\rangle \ldots \times$$
$$\langle d_{de}^{2} d_{d'e'}^{2} e^{i[e(\phi_4(t)+\pi)-e'(\phi_4(0)+\pi)]}\rangle Y_e^{2} Y_e^{*2} =$$
$$\sum_{\substack{abcde\\ b'c'd'e'}} [(-1)^{a-a'} d_{ab}^{2} d_{ab'}^{2}][(-1)^{b-b'} d_{bc}^{2} d_{b'c'} \langle e^{i(b\phi_1(t)-b'\phi_1(0))}\rangle] \times$$
$$[(-1)^{c-c'} d_{cd}^{2} d_{c'd'}^{2} \langle e^{i(c\phi_2(t)-c'\phi_2(0))}\rangle] \times$$
$$[(-1)^{d-d'} d_{de}^{2} d_{d'e'}^{2} \langle e^{i[d\phi_3(t)-d'\phi_3(0)]}\rangle] \times$$
$$[(-1)^{e-e'} d_{e0}^{2} d_{e'0}^{2} \langle e^{i[e\phi_4(t)-e'\phi_4(0)]}\rangle \cos([e-e']\phi_{mol}) (5/4\pi) \quad (C1)$$

We have suppressed all the β angles and used the fact that

$$R_e[Y_e^{2}(\theta_{mol},\phi_{mol}) Y_{e'}^{2*}(\theta_{mol},\phi_{mol})] = [d_{e0}^{2} d_{e'0}^{2} \cos([e-e']\phi_{mol})(5/4\pi)] \quad (C2)$$

since only the real part contributes to the sum.

The four-dimensional arrays are introduced

$$N_{ii'jj'} = (-1)^{i-i'} d_{ij}^{2} d_{i'j'}^{2} \quad (C3)$$

and used to express the sum, eq C1, as

$$\frac{5}{4\pi} \sum_{\substack{abcde\\ b'c'd'e'}} N_{aabb'}[N_{bb'cc'}\langle e^{i[b\phi_1(t)-b'\phi_1(0)]}\rangle][N_{cc'dd'}\langle e^{i[c\phi_2(t)-c'\phi_2(0)]}\rangle] \times$$
$$[N_{dd'ee'}\langle e^{i[d\phi_3(t)-d'\phi_3(0)]}\rangle][N_{ee'00}\langle e^{i[e\phi_4(t)-e'\phi_4(0)]}\rangle] \cos[(e-e')\phi_{mol}] \quad (C4)$$

Since not all the terms in this sum are distinct,[27,30] it is convenient to combine equivalent terms by use of the additional four-dimensional array,[27,30]

$$M_{ii'jj'} = N_{ii'jj'} + (i-\delta_{j-j'})(-1)^{j+j'} N_{ii-j'j} + (1-\delta_{jj'})[N_{iij'j} + (1-\delta_{j-j'})(-1)^{j+j'} N_{ii'-j-j'}] \quad (C5)$$

In terms of this array the required time correlation function can be rewritten

$$\langle Y_0^{2}(\theta(t)\phi(t)) Y_0^{2*}(\theta(0)\phi(0))\rangle = \left(\frac{1}{4\pi}\right) e^{-t/t_0} \sum_{a=0}^{2} \sum_{\substack{bcde\\ =0}}^{2} \sum_{b'=-b}^{b} \sum_{c'=-c}^{c} \sum_{d'=-d}^{d} \sum_{e'=-e}^{e} [C_a M_{aabb'}] \times [M_{bb'cc'}] \langle e^{i[b\phi_1(t)-b'\phi_1(0)]}\rangle][M_{cc'dd'}\langle e^{i[c\phi_2(t)-c'\phi_2(0)]}\rangle] \times [M_{dd'ee'}\langle e^{i[d\phi_3(t)-d'\phi_3(0)]}\rangle] \times [M_{ee'00}\langle e^{i[e\phi_4(t)-e'\phi_4(0)]}\rangle] \cos([e-e']\phi_{mol}) \quad (C6)$$

where $C_0 = 1$ and $C_1 = C_2 = 2$. The time correlations required to compute this sum, $\langle e^{i(m\phi(t)-m'\phi(0))}\rangle$, can be obtained either from the trajectory or from simplified models. In order to evaluate the time correlation of the spherical harmonics for atom C_6 at 100 points in time, the symmetry relations have been used to reduce the sum from 195 000 000 terms (eq C1) to 1 970 000 terms (eq C6). Equation C6 was verified by comparison with eq C1 for the first few time increments. The cost of computing the correlation functions for a chain much longer than heptane becomes prohibitively expensive, even when the summation is simplified as described here.

Paramagnetic Doping as an Aid in Obtaining High-Resolution ^{13}C NMR Spectra of Biomolecules in the Solid State

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Abstract: Introduction of a paramagnetic impurity is shown to be an effective method in obtaining ^{13}C cross-polarization magic angle spinning (CP–MAS) spectra of biomolecules in the solid state. When doped with Cu^{2+} as a paramagnetic impurity, ^{13}C spectra of imidazole, uracil, thymine, and cytosine·H_2O could be easily obtained. But their spectra in the pure compounds were far too difficult to obtain because of the long proton spin–lattice relaxation times. It is also confirmed that a moderately low amount of paramagnetic species (Cu^{2+} molar concentration $\lesssim$ 0.4% used in the present study) does not produce any observable shift or broadening of the ^{13}C resonances, thereby preserving the quality of the spectra. The spectra so obtained exhibit features peculiar to the solid state. The solid state ^{13}C CP–MAS spectrum of imidazole reveals three distinct ^{13}C resonances, as against two in solution, because of the freezing out of tautomerism.

Sensitivity enhancement by cross-polarization (CP)[1,2] and resolution enhancement by dipolar decoupling[2] and magic angle spinning (MAS)[3,4] have made it possible to obtain high-resolution, natural-abundance ^{13}C NMR spectra in the solid state.[5] The main advantage of using CP–MAS technique rests with the fact that the repetition time of the experiment, while signal averaging, is essentially determined by a shorter proton spin–lattice relaxation time (T_1^H) compared to a much longer carbon spin–lattice relaxation time (T_1^C). But T_1^H can be very long (greater than 100 s) in the extreme correlation limits, viz., $\omega_{OH}\tau_C >> 1$ (very slow motion) and $\omega_{OH}\tau_C << 1$ (very fast motion), rendering a CP–MAS experiment to be of low sensitivity. Therefore it becomes necessary to device means by which T_1^H can be reduced and a ^{13}C spectrum easily obtained with a gain in detection sensitivity.

The utility of introducing a paramagnetic impurity into a host lattice with a subsequent reduction in T_1^H is well known.[6,7] With

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the assumption that the transfer of energy within the lattice from a region of high spin temperature to a region of low spin temperature is a spin-diffusion process caused by spin flip-flop between neighbor nuclei, it has been shown that $(1/T_1) \propto NaD$, where N is the number of paramagnetic nuclei per unit volume, a is the distance between nuclei, and D is the diffusion constant. The effect of paramagnetic doping in a proton-rich sample containing ^{13}C at natural abundance is as follows: the paramagnetic impurity should be very effective in reducing the T_1 of the proton and carbon-13 which are located very close to the paramagnetic center because of the very large magnetic moment and the short spin-lattice relaxation time of the electron. Spin-diffusion is very efficient among protons, in proton-rich small molecules and even in larger biomolecules such as DNA,[8] as they form a strongly coupled spin system. Thus protons located far away from the paramagnetic center are able to communicate with those closer to the paramagnetic center because of the strong spin-spin interaction, and the overall T_1 for the proton spin system is reduced. But for carbons at natural abundance (1.1%), spin-spin interaction is very weak and spin-diffusion will be inefficient; only those carbons which are closer to the paramagnetic center are affected while those located far away are not influenced. Thus one can create this preferential effect at moderately low concentration of paramagnetic impurity, and, with a dramatically reduced T_1^H, make full use of CP-MAS to obtain ^{13}C spectra that are unaffected by paramagnetic doping. Also, it has been confirmed that introduction of a small amount of paramagnetic impurity does not change the space group symmetry or the crystal structure of host molecules except for the slight deformation in the local structure around the paramagnetic center.[9] Hence spectral characteristics of the host molecule will remain undisturbed by paramagnetic doping.

In this paper we explore the applications of this possibility in obtaining high-resolution ^{13}C spectra of some biomolecules in the solid state. The following molecules were chosen for the present study: L-alanine, imidazole, cytosine·H_2O, uracil, and thymine. L-Alanine has a very short T_1^H because of reorientational motion of CH_3 and NH_3^+ groups[10] and so it is easy to obtain a CP-MAS ^{13}C spectrum without the need for Cu^{2+} doping. This spectrum can then be compared with a spectrum taken on a Cu^{2+} doped sample to see the effect of a paramagnetic induced shift or broadening. Imidazole is known to be a planar molecule[11] and its T_1^H is expected to be very long. The pyrimidine bases, viz., cytosine, uracil, and thymine, also have long T_1^H values,[8] and they, together with imidazole, are biologically important.

Experimental Section

^{13}C CP-MAS spectra were obtained at room temperature on a Bruker CXP-200 FT NMR spectrometer operating at 200 MHz for ^1H and 50.3 MHz for ^{13}C. A matched single contact Hartmann-Hahn spin-locked CP was established by using applied radio frequency fields of 15 G for protons and 60 G for ^{13}C. A Delrin spinner was used and its spinning frequency was estimated to be 4.5 kHz from the location of spinning sidebands. There is a strong ^{13}C signal due to Delrin (appearing at around 40 ppm relative to benzene), but fortunately this does not interfere with any of the ^{13}C resonances of interest in the biomolecules. ^{13}C spectra in the solution state at neutral pD were obtained on a Bruker WH-400 FT NMR spectrometer, using proton noise decoupling. The solution ^{13}C spectra were obtained at room temperature for L-alanine and imidazole and at 60 °C for cytosine·H_2O, uracil, and thymine because of a low room temperature solubility in D_2O. T_1^Hs were measured on a Bruker B-KR 321s pulsed NMR spectrometer by employing $\pi-\tau-\pi/2$ ($T_1^H < 2$ s) and $n(\pi/2)-\tau-\pi/2$ ($T_1^H > 2$ s) pulse sequences. The undoped samples were degassed and used for T_1^H measurement. Cu^{2+} was introduced as a paramagnetic impurity into the host lattice by recrystallization from an aqueous solution containing cupric chloride. The Cu^{2+} content in the samples was determined by atomic absorption spectroscopy on a Perkin-Elmer 305A atomic absorption spectrometer.

Table I. Proton Spin-Lattice Relaxation Times (T_1^H) and Cu^{2+}/Host Molecule Molar Ratio (R)

compound	T_1^H, s undoped	T_1^H, s Cu^{2+} doped	R, %
L-alanine	5.6×10^{-2}	3.6×10^{-2}	5.9×10^{-2}
imidazole	1.6×10^{3}	2.8×10^{1}	4.4×10^{-3}
cytosine·H_2O	1.1×10^{1}	1.5×10^{-1}	4.1×10^{-1}
uracil	1.1×10^{2}	0.2×10^{1}	3.8×10^{-2}
thymine	2.3×10^{1}	1.8×10^{1}	2.6×10^{-3}

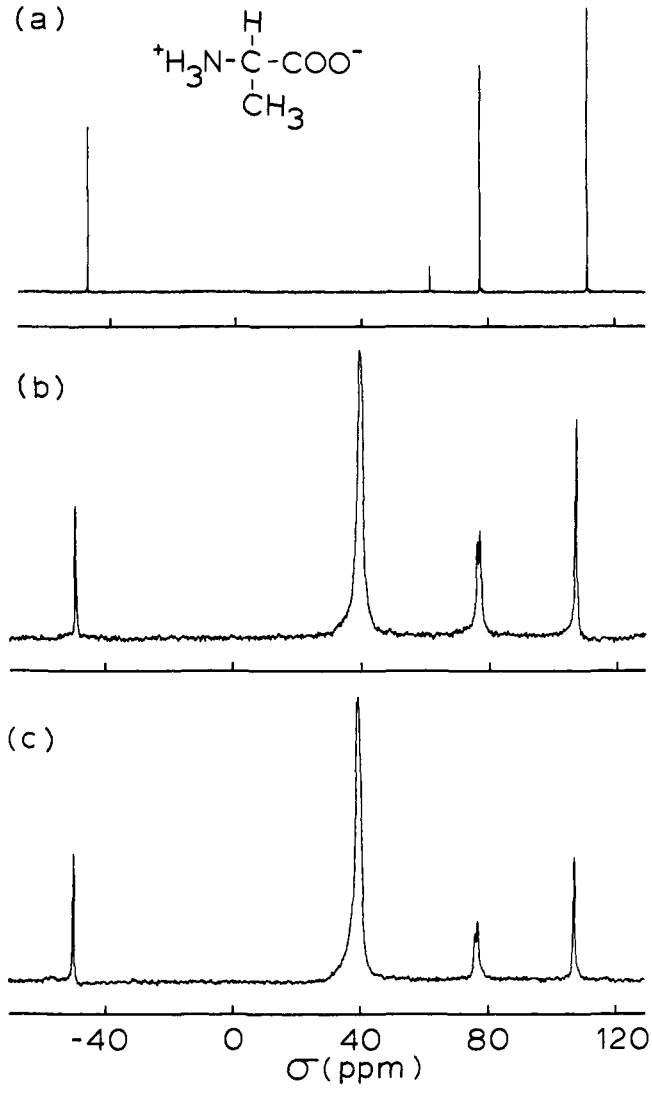

Figure 1. High-resolution ^{13}C spectra of L-alanine: (a) solution spectrum; (b) CP-MAS spectrum of undoped sample; and (c) CP-MAS spectrum of Cu^{2+} doped sample. Chemical shifts are with reference to benzene. The additional ^{13}C signal at 61 ppm in Figure 1a is due to dioxane used as an internal reference. The splitting of $^{\alpha}C$ resonance is due to the ^{14}N quadrupole effect (see text). The signal at 40 ppm in Figures 1b and 1c is due to the spinner (Delrin).

Results and Discussion

The results of relaxation time measurements (T_1^H) and the mole percentage of the Cu^{2+} content in the chosen samples are presented in Table I. It is readily seen that Cu^{2+} doping has reduced T_1^H to a great extent, especially in imidazole and uracil. Also, the measured relaxation times in Cu^{2+} doped samples are consistent with the experimentally determined Cu^{2+} concentration.

Figures 1b and 1c show the CP-MAS spectra obtained in undoped and Cu^{2+} doped L-alanine, respectively. The spectra taken with undoped L-alanine in the solution state is shown in

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Table II. Carbon-13 Chemical Shifts[a]

compd	carbon positions				
L-alanine[b]	COO^-	α_C		β_C	
	−49.4 (−49.4)	76.9, 77.7 (76.9, 77.7)		108.0 (108.0)	
	−47.9	77.0		111.5	
imidazole[c]		C-2	C-4, C-5		
		−8.7	1.2	12.5	
		−7.6	6.5	6.5	
	C-2	C-4	C-5	C-6	CH_3
cytosine·H_2O	−32.3	−41.5, −39.4	33.2	−16.0	
	−31.5	−39.9	32.7	−15.7	
uracil	−23.1	−42.7	28.3	−18.8	
	−25.3	−39.5	26.6	−15.7	
thymine	−28.3	−36.6	15.0	−9.7	114.7
	−25.3	−39.7	17.7	−11.3	116.6

[a] Shifts given in parts per million relative to benzene (benzene is 128.5 ppm down field from Me_4Si) and positive values indicate higher field. Chemical shifts determined in the solid state are shown on the first row against each compound and those measured in solution are shown on the second row. [b] Values in parentheses for L-alanine denote the chemical shifts measured in the undoped sample. [c] An exact assignment of C-4 and C-5 in imidazole has not been made (see text).

Figure 1a for comparison. The chemical shifts are given in Table II. Inspection of Figures 1b and 1c and Table II clearly shows that introduction of Cu^{2+} has produced no observable shift or broadening of any of the ^{13}C resonances in the spectra for the Cu^{2+} doped sample. It is also seen that the solution and solid state spectra follow each other very closely and in the correct order.

It is known that the presence of a paramagnetic impurity causes a shift in the NMR resonance position.[12] The resonance shift for a dilute paramagnetic solid is given by[13]

$$\frac{H}{H_0} = \frac{\langle S \rangle}{H_0} g\beta \left(\frac{3\cos^2\theta - 1}{r_{e-c}^3} \right) + \frac{\langle S \rangle}{H_0} A \qquad (1)$$

where $\langle S \rangle$ is an averaged electron spin polarization experienced by nuclear spins because of rapid electronic relaxation and is given by

$$\langle S \rangle = -\frac{g\beta H_0 S(S+1)}{3kT} \qquad (2)$$

Here r_{e-c} is the electron–nuclear (^{13}C) distance, θ is the angle between the vector connecting the electron and nuclear spin and the external magnetic field H_0, and A is the isotropic hyperfine coupling constant. It is important to note that both dipolar and contact shifts are proportional to $\langle S \rangle$ and the shifts increase linearly with the inverse temperture T. In eq 1 the first term represents the dipolar interaction between the electron and ^{13}C and the second term takes into account the shifts produced by isotropic hyperfine interactions and the pseudocontact shift. The dipolar contribution will be significant only for ^{13}C nuclei located very close to the paramagnetic center as an inverse cube electron–carbon-13 distance, r_{e-c}, is envisaged in eq 1. Since the isotropic part of the shift arises from electron spin delocalization through the covalent bond, most carbons may not show this shift except for those very close to the paramagnetic impurity.

Another important consideration in paramagnetic systems is the spectral line broadening.[12] For systems with $T_1^C \gg T_2^C$, which is appropriate for our study, the T_2^C process dominates the line broadening. Here T_2^C is essentially determined by electron–carbon-13 dipolar interaction modulated by electron spin relaxation time (T_{1e}, T_{2e}) and has spectral densities at ω_C, ω_e, and ω_r.[12,14] Here the additional $J(\omega_r)$ comes due to MAS at a frequency ν_r = $\omega_r/2\pi$.[14] The strength of this relaxation interaction wiill be determined by electron–carbon-13 distance r_{e-c}. The ^{13}C T_2 values and the resonance line widths ($\Delta\nu = 1/\pi T_2$) are therefore calculable from a knowledge of r_{e-c}, T_{1e}, and T_{2e}.

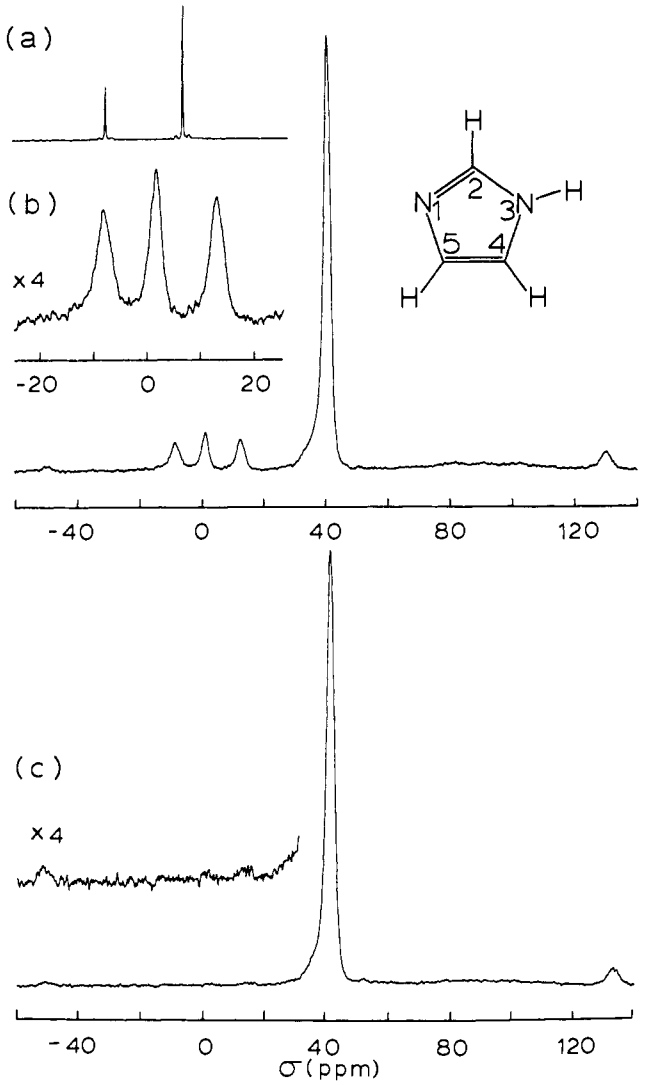

Figure 2. High-resolution ^{13}C spectra of imidazole: (a) solution spectrum; (b) CP–MAS spectrum of Cu^{2+} doped sample (1000 scans; 10 s recycle time; 1 ms mixing time); and (c) CP-MAS spectrum of undoped sample (conditions same as in b). Chemical shifts are with reference to benzene.

Theoretical estimates of ^{13}C resonance shift and line broadening have been made for cytosine·H_2O which has the largest Cu^{2+}/host molecule molar ratio (Table I). The average Cu–Cu distance was calculated to be 33 Å and the percentage of ^{13}C nuclei having r_{e-c} > 7.5 Å was estimated to be 95.1%. The shift due to the dipolar term in eq 1 was estimated to be 10 ppm for r_{e-c} = 7.5 Å. This shows that only 5% of carbons will cause any appreciable shift, and even this minor contribution will be removed by MAS. Also, the pseudocontact shift was estimated to be less than 0.7 ppm for r_{e-c} = 7.5 Å.[12] This shift, which is not removed by MAS, is therefore an order of magnitude smaller than the dipolar shift and is negligible. Similarly, only 5% of carbons will experience a T_2 broadening of about 113 Hz (calculated using r_{e-c} = 7.5 Å, T_{1e} (Cu^{2+}) = 2×10^{-8} s and T_{2e} (Cu^{2+}) = 2×10^{-8} s)[12] while the remaining 95% which contribute to the observed signal intensity will not experience significant broadening. On the other hand, for uracil, which has the most convenient T_1^H value for the CP–MAS experiment, it was estimated that 99.4% of carbons may not experience any significant shift or broadening of the ^{13}C resonances. These simple theoretical calculations are well supported by experimental measurements of ^{13}C chemical shifts and ^{13}C resonance line widths in undoped and Cu^{2+} doped L-alanine

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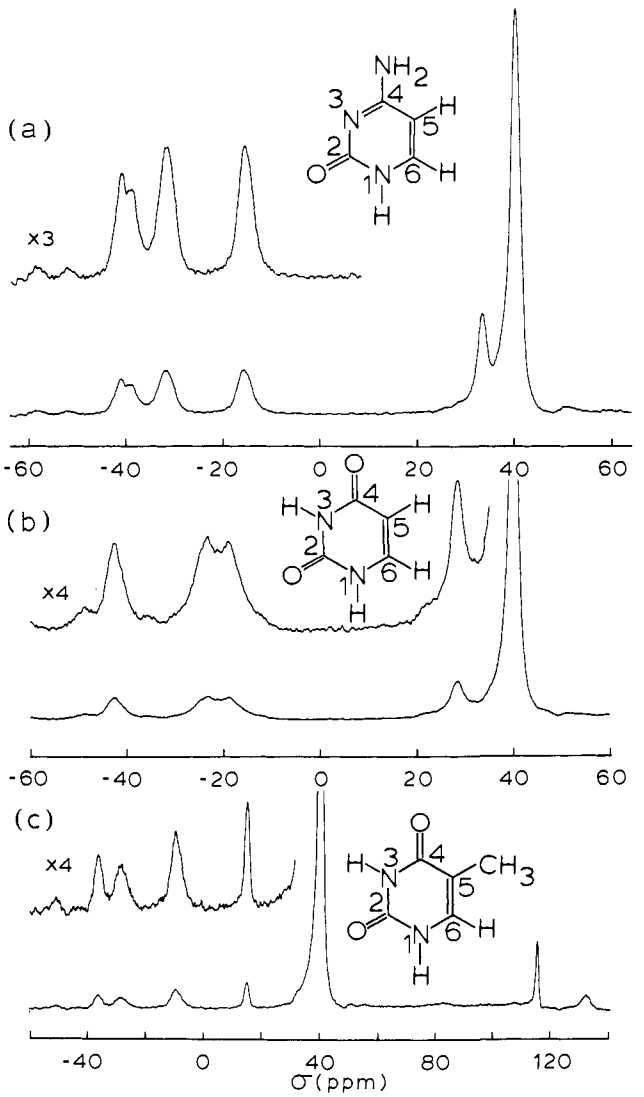

Figure 3. CP-MAS ^{13}C spectra of purimidine bases: (a) cytosine·H_2O; (b) uracil; and (c) thymine. All doped with Cu^{2+}. Conditions are the same as in Figure 2 except scans = 1000 for cytosine·H_2O, 2500 for uracil, and 1155 for thymine. Chemical shifts are with reference to benzene.

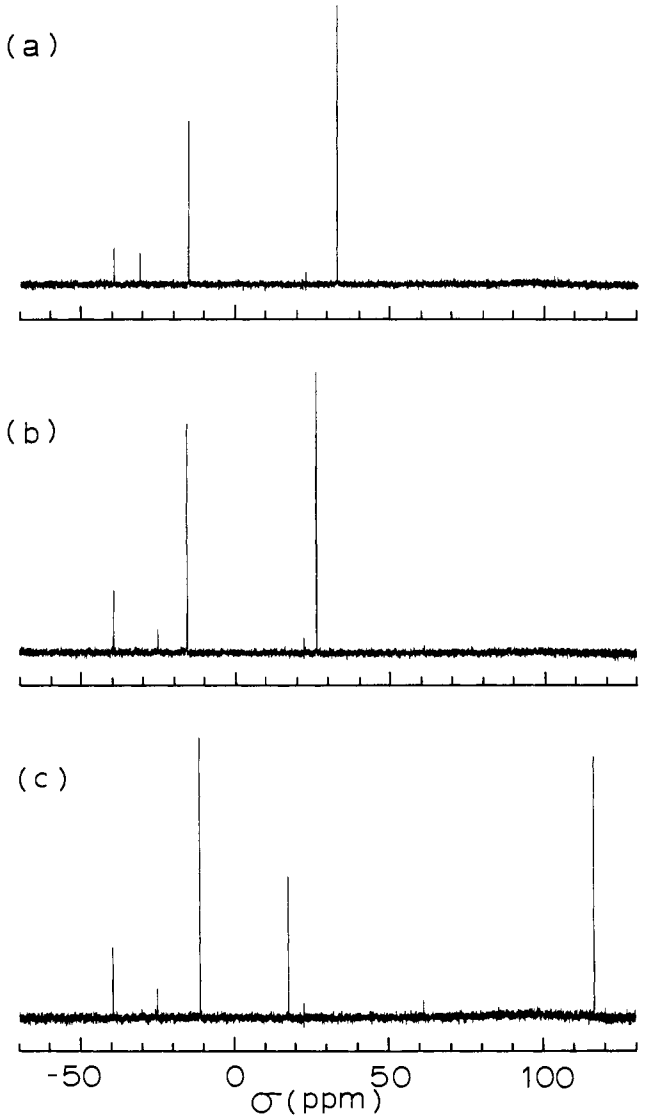

Figure 4. Solution ^{13}C spectra of pyrimidine bases in D_2O at neutral pD: (a) cytosine·H_2O; (b) uracil; and (c) thymine. Chemical shifts are with reference to benzene.

(Table II). The slight decrease in ^{13}C signal intensity, noticed in Figure 1c, is presumably due to the T_2 broadening effects which are not reduced by MAS.

Figure 2 shows the dramatic effect of Cu^{2+} doping in obtaining CP-MAS spectra in imidazole. Without the presence of Cu^{2+} no ^{13}C resonance could be observed because of a $T_1^H > 1000$ s (Figure 2c). But with the introduction of Cu^{2+}, a satisfactory ^{13}C CP-MAS spectrum could be obtained within an hour of signal averaging under identical spectrometer conditions (Figure 2b). In solution, at neutral pD, the magnetic equivalence of C-4 and C-5 arises from rapid (on the NMR time scale) tautomeric equilibrium between 1H and 3H forms, giving rise to a single resonance for C-4 and C-5 with an intensity twice that of C-2 (Figure 2a).[15] However, in the solid state the tautomeric exchange is presumably frozen, leading to the loss of molecular symmetry and to the appearance of a three-line spectrum.

It is now clearly established that for a solid spinning at the magic angle the resonance of a carbon bonded to nitrogen-14 will be either split or broadened.[16-18] This is caused by ^{14}N quadrupole interaction via the dipolar interaction between carbon and nitrogen-14 since ^{14}N quadrupole interaction is of comparable size to Zeeman interaction. When the magic angle spinning frequency ν_r is small compared with ^{14}N quadrupole interaction, adiabatic approximation can be used to determine the line shape of the carbon bonded to nitrogen. The resulting line shape of the carbon bonded to nitrogen-14 will depend upon the quadrupole coupling constant (e^2Qq), its sign, the strength of the dipolar interaction between carbon and nitrogen, the strength of the static magnetic field, and the spinning frequency.[16-18]

For imidazole, it is seen that the ^{13}C resonances are not sharp, but are broadened to different extents (Figure 2b). The broadening of ^{13}C resonances in imidazole comes from the fact that all the three carbons are bonded to nitrogens. Following the solution spectrum, we assign the lowest field peak to be C-2. As C-2 is bonded to two nitrogens it should experience a large broadening, which is indeed observed (Figure 2b). ^{14}N *NQR* results of imidazole show that the e^2Qq/h value of the protonated nitrogen is ~2 MHz smaller than the nonprotonated nitrogen (NH, 1.424 MHz; N, 3.291 MHz).[19] Such a significant difference in the value of e^2Qq for these nitrogens will be reflected noticeably in the relative line broadening of the resonances of the carbon to

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which each is bonded. This feature is also observed in Figure 2b, where the signal at 1 ppm is sharper than the one at 13 ppm. An exact assignment of C-4 and C-5 has not been made because the observed line broadening may depend on other factors.[17,18]

The ^{13}C CP-MAS spectra of pyrimidine bases, viz., cytosine, uracil, and thymine, taken with Cu^{2+} doped samples, are presented in Figure 3, while those taken in the solution state are shown in Figure 4. The measured ^{13}C chemical shifts are given in Table II. The solution spectra of the nucleic acid bases have apparently not been reported, although purine and pyrimidine nucleosides have been studied in detail before.[20] We have included the solution spectra because of the fundamental importance of these compounds. The assignment of the ^{13}C resonances in the three bases proceeds directly following those in pyrimidine nucleosides.[20] The assignments from low to high field are: cytosine·H_2O C-4, C-2, C-6, and C-5; uracil C-4, C-2, C-6, and C-5; and thymine C-4, C-2, C-6, C-5, and CH_3.

Cytosine in fact exists as a monohydrate, as it does in the present study, when recrystallized from water.[21] Thus a shorter T_1^H, compared with a $T_1^H > 100$ s expected for a nonhydrate form,[8] is expected and is observed in our study (Table I). This is consistent with an efficient proton relaxation mechanism eminating from a twofold flipping of water molecules.[22] Cu^{2+} doping is seen to have a great effect as T_1^H is reduced to millisecond order and a CP-MAS spectrum easily obtained. In cytosine·H_2O the resonances of carbon bonded to nitrogen are either split (C-4) or broadened (C-2 and C-6), while the nonbonded one (C-5) remains sharp (Figure 3a). The CP-MAS spectrum of uracil (Figure 3b) also exhibits similar features in the resonances, C-4, C-2, and C-6 being broadened and C-5 being sharp as in cytosine·H_2O. Thymine, an important constituent of DNA, differs from uracil only in the presence of a methyl group in the 5-position of the ring and its CP-MAS spectrum is essentially the same as that of uracil except for the appearance of methyl resonance at 115 ppm. As seen, the resonances of C-5 and CH_3 are quite sharp as compared to the others. The broadening of the resonances of carbons bonded to nitrogen is therefore a common feature of the pyrimidine bases as well, and an explanation to this is to be sought in the ^{14}N quadrupole effects[16-18] discussed earlier.

It is also seen that though the assignment of the ^{13}C resonances is the same as in solution, the chemical shifts are not the same (Table II). Such differences are thought to be due to conformational variations in the solid state.

Conclusions

Paramagnetic doping can be used effectively to reduce T_1^H in the solid state and so one can obtain CP-MAS spectra routinely. At moderately low Cu^{2+} content (Cu^{2+}/host molecule molar ratio $\lesssim$0.4%) the carbon resonances do not shift nor are they broadened to any significant amount. The spectra so obtained exhibit features characteristic of the solid state, such as the absence of exchange processes, the broadening of ^{13}C resonances of the carbons bonded to nitrogen etc. It is suggested that T_1^H need not be reduced very much (millisecond order) as one has to use a recycle delay of about 5-10 s in most CP-MAS experiments. A reduction in T_1^H to about 2 s should be convenient for obtaining CP-MAS spectra. For most practical cases, it is good enough to dope with only 0.04% of paramagnetic impurity, and this amount of paramagnetic impurity definitely will not cause any shift or broadening in the ^{13}C NMR spectra. Also, the dissolved oxygen in the sample (at such low concentration) will not cause any confusion in the ^{13}C spectra as the effect will be negligibly small, obviating the need to use sealed samples.

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S-Adenosyl-L-methionine and *S*-Adenosyl-L-homocysteine, an NMR Study[1]

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Abstract: An analysis of the 360-MHz ^1H NMR spectra of the title compounds in ^2H_2O is presented. The ^3J values for the ribose vicinyl protons of (*S*)-adenosyl-L-methionine are consistent with a predominantly C_3-exo conformation and with one highly favored gauche-anti conformation about the C_4-$C_{5'}$ bond. The corresponding ^3J values for *S*-adenosyl-L-homocysteine imply a similar C_3-exo ribose ring conformation, but the orientation about the C_4-$C_{5'}$ bond is distributed between two gauche-anti rotamers. The methionine side chain of *S*-adenosyl-L-methionine has approximately equal populations of rotational isomers about the C_α-C_β and C_β-C_γ bonds, whereas the side chain of *S*-adenosyl-L-homocysteine exhibits a conformational preference for the gauche-anti conformations about the C_α-C_β bond. ^1H and ^{13}C NMR spectra of commercially available samples of (-)-*S*-adenosyl-L-methionine consistently reveal the presence of a small amount of the (+)-sulfonium diastereomer. This assignment was confirmed by synthesis of both the ^1H and ^{13}C methyl derivatives of *S*-adenosyl-L-homocysteine. Arguments are presented to explain the failure of previous workers to detect (+)-*S*-adenosyl-L-methionine in biological preparations.

S-Adenosyl-L-methionine (SAM) is ubiquitous and uniquely versatile. The extensive repertoire of this important cofactor includes methyl group transfer to oxygen, nitrogen, sulfur, or carbon in the chemical modification of members of every major class of biomolecule, including nucleic acids, proteins, lipids, and carbohydrates.[2] The coproduct of the methyl-transfer reaction is usually *S*-adenosyl-L-homocysteine (SAH).

Few extensive studies of the conformations of SAM and SAH have been reported. Klee and Mudd[3] examined the ORD curves

(1) Preliminary results presented at the Pacific Conference on Chemistry and Spectroscopy, San Francisco, Sept 28, 1978.

(2) For reviews on SAM see: (a) Salvatore, F. et al. "Biochemistry of Adenosylmethionine"; Columbia University Press: New York, 1977. (b) Cantoni, G. L. *Ann. Rev. Biochem.* **1975**, *44*, 435-452

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