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Solid-state ^{17}O NMR of thymine: a potential new probe to nucleic acid base pairing

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We report the first experimental solid-state ^{17}O NMR and theoretical (B3LYP/6-311++G) study of the ^{17}O electric-field-gradient and chemical shielding tensors in a free nucleic acid base, thymine.**

NMR spectroscopy is an important technique for studying structures of biological macromolecules. Most successful NMR applications have been based primarily on observation of spin-1/2 nuclei such as ^{1}H, ^{13}C and ^{15}N. Although oxygen is also an important element in biological molecules, ^{17}O ($S = 5/2$ and natural abundance = 0.037%) NMR studies are far less common.[1] To explore the potential of solid-state ^{17}O NMR spectroscopy in studying organic and biological compounds, we recently investigated a number of important oxygen-containing functional groups such as amides,[2] urea,[3] carboxylic acids,[4] phenols[4] and the oxonium ion.[5] Oxygen is also common in nucleic acids. Base pairing between nucleic acid molecules often directly involves oxygen atoms, *e.g.* G:C and A:U base pairing. To our knowledge, solid-state ^{17}O NMR has not been applied to compounds related to nucleic acids.

As a first step, we report solid-state ^{17}O NMR results for a free nucleic acid base, thymine; see Fig. 1. We synthesized [2-^{17}O]thymine and [4-^{17}O]thymine by acid-catalyzed exchange with $H_2{}^{17}O$ (40.9 atom% ^{17}O, ISOTEC, Miamisburg, Ohio) from 5-methyl-2-thiouracil and thymine, respectively. Solid-state ^{17}O NMR spectra were recorded on a Bruker Avance-500 spectrometer operating at 67.78 MHz for ^{17}O nuclei. Fig. 2 shows the experimental and simulated ^{17}O magic-angle spinning (MAS) NMR spectra of [2-^{17}O]thymine and [4-^{17}O]thymine. Analysis of these spectra yielded the following ^{17}O NMR parameters: O2, $\delta_{iso} = 200 \pm 5$ ppm, $C_Q = 6.65 \pm 0.02$ MHz, $\eta_Q = 1.00 \pm 0.02$; O4, $\delta_{iso} = 325 \pm 5$ ppm, $C_Q = 8.40 \pm 0.02$ MHz, $\eta_Q = 0.10 \pm 0.02$. Following the standard procedure,[2,6] we were also able to analyze the stationary ^{17}O NMR spectra shown in Fig. 2 and obtain the magnitude and relative orientation of the ^{17}O chemical shift (CS) tensors. The results are summarized in Table 1.

It can be seen from Table 1 that O2 and O4 exhibit drastically different ^{17}O NMR tensors. In particular, the amide-type oxygen, O4, shows a much larger C_Q, 8.40 MHz, than the urea-type oxygen, O2, 6.65 MHz. The difference between the ^{17}O CS tensors for O2 and O4 is also striking. The isotropic ^{17}O chemical shifts for O2 and O4 differ by 125 ppm. In addition, the span ($\Omega = \delta_{11} - \delta_{33}$) of the ^{17}O CS tensor for O4 is more than twice of that for O2. It is also apparent from Table 1 that, whereas the isotropic ^{17}O chemical shifts measured for O4 in the solid and solution states are essentially identical, the corresponding values for O2 differ by approximately 50 ppm! This is clearly a consequence of the strong intermolecular hydrogen-bonding interaction at O2 in crystalline thymine; see Fig. 1.

In order to evaluate quantitatively the influence of intermolecular hydrogen bonding interactions on the ^{17}O EFG and CS tensors in crystalline thymine, we chose to perform quantum chemical calculations using four different models. Model-I is simply an isolated thymine molecule. Model-II consists of two hydrogen-bonded thymine molecules, **1** and **2** as defined in Fig. 1. Model-III also consists of two thymine molecules, **1** and **3**. Model-IV is a trimeric cluster containing **1**, **2** and **3**. The experimental X-ray diffraction structure of thymine[7] was used in all the calculations. The positions of the hydrogen atoms were computed using the standard bond lengths and angles. The density functional theory (DFT) calculations were performed on a PC (400 MHz Pentium II processor, 128 MB RAM, 12 GB of

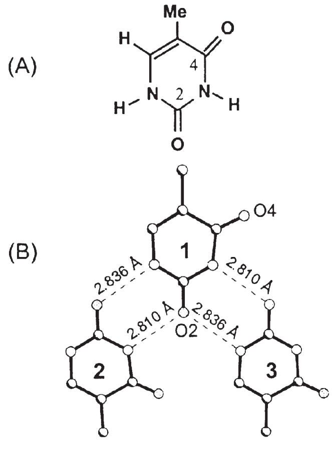

Fig. 1 (A) Chemical structure of thymine. (B) H-bond environment in crystalline thymine.[7] Thymine molecules are related by twofold screw axes. Hydrogen atoms are not shown for clarity.

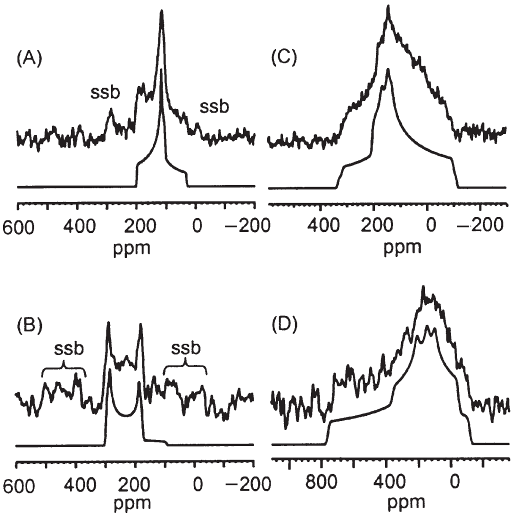

Fig. 2 Experimental (upper) and simulated (lower) ^{17}O MAS NMR spectra of (A) [2-^{17}O]thymine (3753 scans) and (B) [4-^{17}O]thymine (5236 scans). Experimental (upper) and simulated (lower) ^{17}O stationary NMR spectra of (C) [2-^{17}O]thymine (7400 scans) and (D) [4-^{17}O]thymine (5376 scans). The sample spinning frequency was 14.5 kHz. The B_1 field strength at the ^{17}O frequency was about 70 kHz. Spinning sidebands are marked as 'ssb'. A Hahn-echo pulse sequence was used in acquiring the stationary spectra. The recycle time was 10 s in all experiments.

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Table 1 Summary of experimental and theoretical (B3LYP/6-311++G**) ^{17}O NMR tensors in crystalline thymine

Compound	Model	δ_{iso}/ppm	δ_{11}/ppm	δ_{22}/ppm	δ_{33}/ppm	C_Q/MHz[a]	η_Q
[2-^{17}O]Thymine	I	298	441	406	48	8.42	0.55
	II	264	385	359	46	7.82	0.73
	III	250	365	344	40	7.72	0.79
	IV	226	327	306	45	7.12	0.99
	Exptl.	200 ± 2 (247.8)[b]	290 ± 5	270 ± 5	20 ± 5	6.65 ± 0.02	1.00 ± 0.02
[4-^{17}O]Thymine	I	387	698	487	−25	8.91	0.14
	II	399	685	480	−25	8.83	0.17
	III	380	734	496	−33	9.14	0.12
	IV	392	720	491	−34	9.08	0.15
	Exptl.	325 ± 2 (321.0)[b]	570 ± 5	360 ± 5	20 ± 5	8.40 ± 0.02	0.10 ± 0.02

[a] $C_Q = eQV_{zz}/h$ and $\eta_Q = (V_{xx} - V_{yy})/V_{zz}$. [b] Numbers in parentheses are the corresponding ^{17}O chemical shift values measured in DMSO solutions.

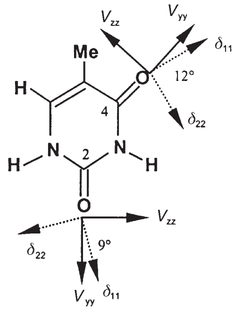

Fig. 3 Illustration of the orientations of the ^{17}O NMR tensors in thymine.

disk space) using Gaussian 98 program[8] with the standard 6-311++G** basis set and the B3LYP exchange functional.[9] The theoretical results are also presented in Table 1.

Close examination of the theoretical results reveals a remarkable difference between the ^{17}O NMR tensors for O2 and O4. In particular, both the ^{17}O quadrupole coupling tensor and the CS tensor at O2 exhibit a strong dependence on the cluster model used in the calculation, whereas the ^{17}O NMR tensors at O4 are essentially independent of the model. This clearly reflects the difference in the H-bond environment between O2 and O4. In the discussion that follows, we focus only on the ^{17}O NMR tensors for O2. As seen from Table 1, Model-I predicted $\Omega = 393$ ppm and $C_Q = 8.42$ MHz for O2, which are considerably larger than the observed values, $\Omega = 270$ ppm and $C_Q = 6.65$ MHz. When two H-bonds were considered in either Model-II or Model-III, smaller values were obtained for Ω and C_Q. When a complete H-bond network is included in the calculation (Model-IV), the theoretical results become much closer to the experimental values, $\Omega = 282$ ppm and $C_Q = 7.12$ MHz. The observed decrease in the isotropic ^{17}O chemical shift (increase in *shielding*) from Models I to IV results mainly from the changes in δ_{11} and δ_{22}. The large difference between the isotropic ^{17}O chemical shifts measured in the solid state and in solution, 200 *vs.* 247.8 ppm, was well reproduced by the calculations of Model-I and Model-IV. The quadrupole coupling constant exhibits a reduction of approximately 1.3 MHz upon hydrogen bonding, the ^{17}O EFG tensor increases monotonically from Models I, II, III to IV. Finally, the agreement between the calculated results from Model-IV and the experimental data is reasonable; but it is also clear that all calculated ^{17}O NMR parameters are larger than the observed values by approximately 10%. These discrepancies are likely due to the limitation of the current theory.

Another piece of useful information from the quantum chemical calculations is the absolute orientations of ^{17}O NMR tensors in the molecular frame. As seen from Fig. 3, the *orientations* of the ^{17}O NMR tensors for O2 and O4 are similar, despite the large difference in the magnitude of the individual tensor components. It should be noted that the relative orientation between the ^{17}O EFG and CS tensors shown in Fig. 3 is in agreement with the experimental determination from the analysis of static ^{17}O NMR spectra.

In summary, we have presented the first solid-state ^{17}O NMR study of a free nucleic acid base. The present study demonstrates that it is feasible to obtain solid-state ^{17}O NMR spectra for ^{17}O-labeled nucleobases and that ^{17}O NMR *tensors* are excellent indicators of H-bond formation. These features are potentially useful for probing base pairing in nucleic acids. With the availability of very high magnetic fields (18.8 T or higher) and the advances in solid-state NMR methodology, it is anticipated that solid-state ^{17}O NMR will become a new addition to the arsenal for studying biological macromolecules.

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