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 = 0.0375) NMR studies are far less common. 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, urea, carboxylic acids and the oxonium ion. 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 $^{17}$O-labelled $2$-[17]O-thymine and $4$-[17]O-thymine by acid-catalyzed exchange with $^{17}$O-enriched water ($90$ at% $^{17}$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: $O_2$, $\delta_o$ = 200 ± 5 ppm, $C_O$ = 6.65 ± 0.02 MHz, $\eta_o$ = 1.00 ± 0.02; $O_4$, $\delta_{oo}$ = 325 ± 5 ppm, $C_O$ = 8.40 ± 0.02 MHz, $\eta_o$ = 0.10 ± 0.02. Following the standard procedure, 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 $O_2$ and $O_4$ exhibit drastically different $^{17}$O NMR tensors. In particular, the amide-type oxygen, $O_4$, shows a much larger $C_O$, 8.40 MHz, than the urea-type oxygen, $O_2$, 6.65 MHz. The difference between the $^{17}$O CS tensors for $O_2$ and $O_4$ is also striking. The isotropic $^{17}$O chemical shifts for $O_2$ and $O_4$ differ by 125 ppm. In addition, the span ($\Omega = \delta_{oo} - \delta_{oo}$) of the $^{17}$O CS tensor for $O_4$ is more than twice that for $O_2$. It is also apparent from Table 1 that, whereas the isotropic $^{17}$O chemical shifts measured for $O_4$ in the solid and solution states are essentially identical, the corresponding values for $O_2$ differ by approximately 50 ppm! This is clearly a consequence of the strong intermolecular hydrogen-bonding interaction at $O_2$ 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, and 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 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. 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 $B_r$ 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 | $\delta_{xx}$/ppm | $\delta_{yy}$/ppm | $\delta_{zz}$/ppm | $\delta_{xy}$/ppm | $\delta_{xz}$/ppm | $\delta_{yz}$/ppm | $\Omega$/MHz | $\eta$/
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<tr>
<td>[2-$^{17}$O]Thymine</td>
<td>I</td>
<td>298</td>
<td>441</td>
<td>406</td>
<td>48</td>
<td>8.42</td>
<td>0.55</td>
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<td>II</td>
<td>264</td>
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<td>359</td>
<td>46</td>
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<td>III</td>
<td>250</td>
<td>365</td>
<td>344</td>
<td>40</td>
<td>7.72</td>
<td>0.79</td>
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<td></td>
<td>IV</td>
<td>226</td>
<td>327</td>
<td>306</td>
<td>45</td>
<td>7.12</td>
<td>0.99</td>
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<td></td>
<td>Expt.</td>
<td>200 ± 2</td>
<td>290 ± 5</td>
<td>270 ± 5</td>
<td>20 ± 5</td>
<td>6.65 ± 0.02</td>
<td>1.00 ± 0.02</td>
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<tr>
<td>[4-$^{17}$O]Thymine</td>
<td>I</td>
<td>387</td>
<td>698</td>
<td>487</td>
<td>−25</td>
<td>8.91</td>
<td>0.14</td>
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<td>II</td>
<td>399</td>
<td>685</td>
<td>480</td>
<td>−25</td>
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<td></td>
<td>III</td>
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<td>734</td>
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<td>−33</td>
<td>9.14</td>
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<td></td>
<td>IV</td>
<td>392</td>
<td>720</td>
<td>491</td>
<td>−34</td>
<td>9.08</td>
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<td>Expt.</td>
<td>325 ± 2</td>
<td>570 ± 5</td>
<td>360 ± 5</td>
<td>20 ± 5</td>
<td>8.40 ± 0.02</td>
<td>0.10 ± 0.02</td>
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</table>

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

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

disk space) using Gaussian 98 program\(^9\) 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 $\Omega$ 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 $\delta_{xx}$ and $\delta_{yy}$. 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 tensors increase 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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Notes and references