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Solid-State ^{17}O NMR as a Probe for Structural Studies of Proteins in Biomembranes

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A key experimental challenge to understand conformational details of membrane proteins is to provide unambiguous atomic-scale information about the molecular bonding arrangement and any changes that occur upon receptor activation. This demands the development of experimental probe techniques to deliver this information of biological and pharmaceutical importance. Solid-state NMR is a nonperturbing approach which can be used to study ligand−protein interactions where molecular size is not limiting and crystallinity is not a requirement.[1] As a first step in addressing this challenge by exploiting ^{17}O NMR in biomembrane applications, we report here the ^{17}O solid-state NMR spectra at high field of an ^{17}O selectively labeled transmembrane peptide in a biomimetic environment.

Oxygen plays a key role in the molecular conformation of biopolymers. Among the important information that can be obtained from ^{17}O, the only NMR-active oxygen isotope, is the isotropic chemical shift (δ_{iso}), the quadrupolar coupling constant (C_Q), and the asymmetry parameter (η). These parameters are extremely sensitive to the electronic distribution around the nucleus; more specifically, they are sensitive to its protonation state[2] and its involvement in hydrogen bonds. Furthermore, they contain structural information,[2−5] and several methods for determining internuclear distances between oxygen and other nuclei have been developed.[2,6,7] This suggests that oxygen could play a central role in biological NMR studies. However, ^{17}O has a low natural abundance (0.037%) and a spin ($I = 5/2$) with a corresponding quadrupole interaction that is manifested as significantly broadened signals in NMR spectra. Consequently, ^{17}O solid-state NMR studies are still relatively uncommon, and selective labeling is essential. Despite these difficulties, in recent years, with the advent of higher magnetic fields and techniques for improving resolution beyond magic angle spinning experiments (MAS), there has been a significant increase in ^{17}O NMR reports from inorganic materials, such as glasses and zeolites.[8,9] There has been much less ^{17}O NMR reported from organic materials since ^{17}O presents even more of a challenge due to the larger quadrupole interaction and, hence, larger line widths.[19] Recent reports of ^{17}O NMR from biologically relevant materials have included inorganic molecules interacting with hemeproteins,[10] polypeptides,[11,12] amino acids,[2] and nucleic acid bases.[13]

Here, we report the first example of ^{17}O NMR spectra from a selectively labeled transmembrane peptide, ^{17}O-[Ala12]-WALP23, as a lyophilized powder and incorporated in hydrated vesicles, opening up new possibilities for applications of ^{17}O solid-state NMR on real biological systems. WALP23 is a synthetic peptide which represents a consensus sequence for transmembrane protein segments.[14] This hydrophobic peptide forms well-defined and well-characterized transmembrane α-helices[14] and has special relevance for the packing of α-helices in polytopic proteins, such as G-protein-coupled receptors.

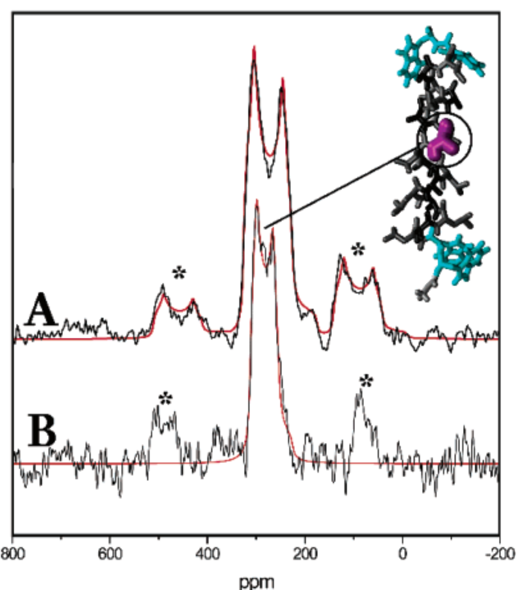

Figure 1. ^{17}O NMR spectra of lyophilized ^{17}O-[Ala12]-WALP23 at room temperature: (A) at 14.1 T, 4 mm rotor spinning at ~15 kHz, with simulation; (B) at 18.8 T, 2.5 mm rotor spinning at ~22 kHz, with simulation. Spinning sidebands are marked with an asterisk (∗).

Fmoc-L-alanine was prepared starting from ^{17}O uniformly enriched L-alanine following a previously described procedure[15] using $H_2{}^{17}O$ (70% enriched at ^{17}O). WALP23 was synthesized and transmembranously incorporated into multilamellar vesicles (MLVs) of 1,2-distearoyl-*sn*-glycerol-3-phosphatidylcholine (DSPC) lipids.[14] The ^{17}O NMR experiments were carried out on Chemagnetics Infinity 600 and Varian/Magnex 800 wide-bore 89 mm spectrometers at frequencies of 81.345 and 108.419 MHz for ^{17}O, respectively. MAS was performed at a spin rate of 11−22 kHz. Spectral simulations were carried out using DMFit software.[16]

The ^{17}O MAS NMR spectra of lyophilized ^{17}O-[Ala12]-WALP23, acquired at magnetic field strengths of 14.1 and 18.8 T, show a single resonance centered[17] at ~280−300 ppm from the single label at alanine-12 (Figure 1). Spinning sidebands of the central transition are also visible. The decrease of the second-order quadrupole broadening and the associated increase in spectral resolution with increasing applied magnetic field is clearly evident. The simulation parameters for ^{17}O in this alanine environment are summarized in Table 1.

Since WALP23 is lyophilized from a solution of 2,2,2-trifluoroethanol, the α-helical conformation should be retained. The isotropic chemical shift for the label (317.5 ppm) is very close to that of another polymer, poly-L-alanine, which also forms ideal α-helices (319 ppm[11]). The difference between these two samples

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Table 1. ^{17}O NMR Interaction Parameters for L-Alanine in Two Protonation States, [Ala12]-WALP23 Lyophilized and in Dry and Hydrated MLVs (1:10 peptide/lipid molar ratio), and Poly-L-alanine

site	C_Q (MHz)	η	δ_{iso} (ppm)	assignment
		L-Alanine[a]		
1	7.86 ± 0.05	0.28 ± 0.03	284.0 ± 0.5	O1
2	6.53 ± 0.05	0.70 ± 0.03	260.5 ± 0.5	O2
		L-Alanine·HCl[a]		
1	8.31 ± 0.02	0.00 ± 0.02	327.8 ± 0.5	C=O
2	7.29 ± 0.02	0.25 ± 0.02	176.7 ± 0.5	C−OH
		Fmoc Alanine[a]		
1	7.89 ± 0.05	0.16 ± 0.02	303.3 ± 0.5	C=O
2	6.95 ± 0.05	0.12 ± 0.02	175.7 ± 0.5	C−OH
		Lyophilized [Ala12]-WALP23		
1	8.45 ± 0.10	0.21 ± 0.03	317.5 ± 0.5	C=O
		[Ala12]-WALP23 in DSPC Vesicles		
1	8.42 ± 0.10	0.21 ± 0.03	311 ± 1	C=O
		[Ala12]-WALP23 in Hydrated DSPC Vesicles		
1	8.55 ± 0.15	0.24 ± 0.03	315 ± 1	C=O
		Poly-L-alanine[b]		
1	8.59	0.28	319	C=O

[a] From ref 2. [b] From ref 11.

is only ~1.5 ppm. This suggests only small differences in local environment for the two peptides, although poly-L-alanine is crystalline and ^{17}O-[Ala12]-WALP23 is not.

The ^{17}O MAS NMR spectrum of ^{17}O-[Ala12]-WALP23 in hydrated DSPC vesicles (Figure 2) also shows a single resonance from the label inserted in alanine-12 centered at ~280 ppm. There is a partial overlap between the spinning sidebands and the central transition because of the lower spinning speed, which was necessary for this sample to maintain vesicle integrity. The hydration water is also visible (0 ppm) but does not interfere with the main signal from the ^{17}O label. The lower spinning speed does not prevent determination of the ^{17}O NMR parameters, which are given in Table 1. This experiment, which was designed as a test to evaluate the sensitivity of the method, shows that it is possible to study ^{17}O selectively labeled peptides in hydrated biomembranes.

We recently obtained a correlation between the isotropic chemical shift and the C=O bond lengths determined by diffraction.[2] In addition, *ab initio* calculations on glutamic acid polymorphs also showed a correlation between these parameters.[18] These two correlations were very similar. On the basis of this data, the C=O bond length at the labeled site for lyophilized WALP is 1.217 Å, which is very close to the 1.220 Å bond length for an ideal α-helix. For the WALP peptide incorporated in hydrated vesicles, the corresponding value is 1.223 Å, an increase in C=O bond length of ~0.006 Å. To further test this correlation, it was applied on other polymers studied previously by ^{17}O solid-state NMR (polyglycine II and poly-L-alanine I and II; data not shown) and was shown to give bond lengths consistent with those of the known structures.

The data presented here are the first applications of ^{17}O solid-state NMR to characterize and estimate bond distances within a selectively labeled peptide of biological interest. The availability of high-field NMR spectrometers (here, up to 18.8 T, with 89 mm bore magnet) allows useable S/N ratios to be readily achieved. It should be emphasized that this included a sample with <5% of the amino acid residues labeled and which was further diluted by a factor of 10−20 because of the presence of lipids and water. The ability to detect and characterize a signal from a selectively labeled peptide suggests that ^{17}O NMR will be a fruitful experimental approach for studying hydrogen bonding in macromolecules. It could find widespread application in the study of selectively labeled peptides reconstituted in biological membranes, or selectively labeled organic molecules interacting with crystallized proteins or with membrane proteins in biological membranes. The high sensitivity of solid-state ^{17}O NMR to the local bonding environment also allows very accurate determination of small changes in C=O bond lengths.

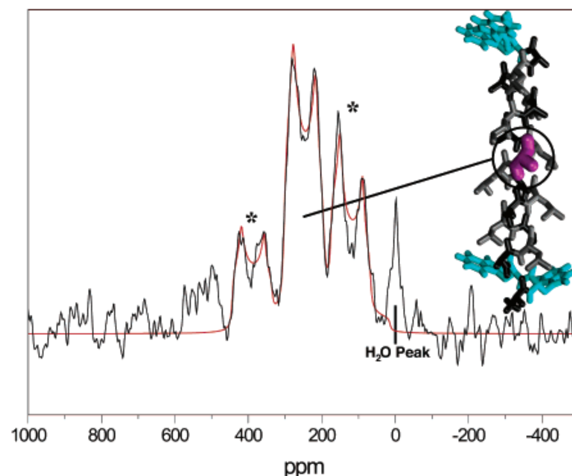

Figure 2. ^{17}O NMR spectra of ^{17}O-[Ala12]-WALP23 in hydrated DSPC vesicles (1:10 peptide/lipid molar ratio) at 14.1 T, 4 mm rotor spinning at ~11 kHz, with simulation. The lyophilized MLV sample was hydrated with one weight equivalent of water, and the spectrum was acquired at room temperature, with the lipids in the liquid−crystalline phase.

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Supporting Information Available: Details for the sample preparation and the NMR experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Watts, A. *Curr. Opin. Biotechnol.* **1999**, *10*, 48−53.
(2) Pike, K. J.; Lemaître, V.; Kukol, A.; Anupõld, T.; Samoson, A.; Howes, A. P.; Watts, A.; Smith, M. E.; Dupree, R. *J. Phys. Chem. B* **2004**, *108*, 9256−9263.
(3) Seliger, J. *Chem. Phys*. **1998**, *231*, 81−86.
(4) Asakawa, N.; Kameda, T.; Kuroki, S.; Kurosu, H.; Ando, S.; Ando, I.; Shoji, A. *Annu. Rep. NMR Spectrosc.* **1998**, *35*, 55−137.
(5) Kuroki, S.; Ando, S.; Ando, I. *Chem. Phys*. **1995**, *195*, 107−116.
(6) Chopin, L.; Vega, S.; Gullion, T. *J. Am. Chem. Soc.* **1998**, *120*, 4406−4409.
(7) Hu, K. N.; Iuga, D.; Griffin, R. G. Dynamic Nuclear Polarization Enhanced ^{17}O Solid State NMR Spectroscopy on Biological Solids. Presented at the 44th Experimental NMR Conference, Savannah, Georgia, Mar 30−Apr 4, 2003; p 198.
(8) MacKenzie, K. J. D.; Smith, M. E. *Multinuclear Solid State NMR of Inorganic Materials*; Pergamon Press: Oxford, 2002.
(9) Bull, L. M.; Bussemer, B.; Anupõld, T.; Reinhold, A.; Samoson, A.; Sauer, J.; Cheetham, A. K.; Dupree, R. *J. Am. Chem. Soc*. **2000**, *122*, 4948−4958.
(10) McMahon, M. T.; deBios, A. C.; Godbout, N.; Salzmann, R.; Laws, D. D.; Le, H.; Havlin, R. H.; Oldfield, E. *J. Am. Chem. Soc.* **1998**, *120*, 4784−4797.
(11) Yamauchi, K.; Kuroki, S.; Ando, I.; Ozaki, T.; Shoji, A. *Chem. Phys. Lett.* **1999**, *302*, 331−336.
(12) Yamauchi, K.; Kuroki, S.; Ando, I. *J. Mol. Struct*. **2002**, *602*−*603*, 171−175.
(13) Wu, G.; Dong, S.; Ida, R.; Reen, N. *J. Am. Chem. Soc.* **2002**, *124*, 1768−1777.
(14) de Planque, M. R.; Kruijtzer, J. A.; Liskamp, R. M.; Marsh, D.; Greathouse, D. V.; Koeppe, R. E., II.; de Kruijff, B.; Killian, J. A. *J. Biol. Chem.* **1999**, *274*, 20839−20846.
(15) Steinschneider, A.; Burgar, M. I.; Buku, A.; Fiat, D. *Int. J. Pept. Protein Res.* **1981**, *18*, 324−333.
(16) Massiot, D.; Fayon, F.; Capron, M.; King, I.; Le Calvi, S.; Alonso, B.; Durand, J.-O.; Bujoli, B.; Gan, Z.; Hoatson, G. *Magn. Reson. Chem.* **2002**, *40*, 70.
(17) For a quadrupolar nucleus, the second-order quadrupolar broadening means that the isotropic shift is near the left-hand edge of the line (see ref 8 above).
(18) Yates, J. R.; Pickard, C. J.; Paynes, M. C.; Dupree, R.; Profeta, M.; Mauri, M. *J. Phys. Chem. A* **2004**, *108*, 6032−6037.
(19) Lemaître, V.; Smith, M. E.; Watts, A. *Solid State Nucl. Magn. Reson.* **2004**, *26*, 215−235.

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