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Detection of Individual Carbon Resonances in Solid Proteins

Sir:

Natural abundance ^{13}C nuclear magnetic resonance spectroscopy is one of the most informative NMR approaches for the study of proteins.[1,2] A significant number of new ideas on the chemistry, structure, and dynamics of individual residues and the peptide backbone have resulted from ^{13}C NMR of proteins in solution. There are a number of reasons to expect NMR spectroscopy of proteins in the solid state will be of even greater value than the solution studies. The anisotropic character of nuclear spin interactions is retained in the absence of molecular motion;[3] therefore angular and distance parameters can be extracted with a variety of experiments. The ultimate ^{13}C resolution in solids may be significantly better than in solution because the isotropic chemical-shift dispersion is the same and experimental procedures can remove static line-broadening mechanisms but not relaxation-induced widths.[4] The relatively small globular proteins that are most amenable to high resolution solution NMR are exactly the same ones that crystallize most conveniently for X-ray diffraction analysis; so NMR of polycrystalline proteins will be complementary to diffraction studies. More importantly those proteins not readily crystallized and not water soluble can be studied as amorphous materials. A variety of structural, mechanical, and intrinsic membrane proteins fall in this category; thus solid-state NMR of proteins offers the promise of extending the range of the method.

There are formidable problems of sensitivity and resolution associated with natural abundance ^{13}C NMR of molecules as large and complex as proteins. The procedures that deal with the fundamental static nuclear spin interactions are now established experimental methods. These include the cross-polarization of carbon magnetization from protons and the decoupling of ^{1}H–^{13}C dipolar interactions during data acquisition.[5] Mechanical sample rotation at the magic angle removes the ^{13}C chemical-shift anisotropy.[6] A spectrum results with single resonance lines of approximately equal area, height, and width for each carbon.

Because of the large number of overlapping protein resonances, resolution enhancement is needed for both solution and solid-state studies to work with individual carbon signals. The aromatic amino acids are the focus of attention because they are relatively few in number with high functionality both chemically and biologically with resonances that appear in an uncluttered spectral region. In solution the nonprotonated aromatic carbons are narrower than the others, although this advantage is lost for solids; however, in both cases this restricted class of resonances can be selected experimentally. The solution studies of proteins rely on modulated off-resonance proton decoupling to broaden the protonated carbons.[7] A selection procedure for nonprotonated carbons of solids is described in the preceding paper that uses the stronger dipolar coupling of bonded protons to carbon to remove those resonances.[8] Not only do these selection procedures increase resolution by having 7 instead of 23 lines in the region 110–160 ppm from Me_4Si, they also provide partial assignments of resonances.

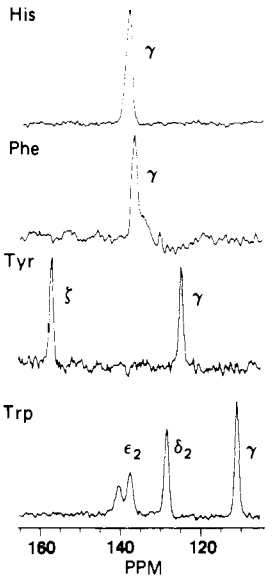

Figure 1. ^{13}C spectra of the nonprotonated ring carbons of the polycrystalline aromatic amino acids. All were obtained using the procedure of ref 8 with 1-ms mix time, 3-s recycle delay, 0.1-s acquisition time, and 300 acquisitions. The delay prior to data acquisition without decoupling to remove the protonated carbon resonances was 40 μs for His, Tyr, and Trp and 90 μs for Phe. All chemical shifts are relative to external Me_4Si.

Figure 1 contains the nonprotonated carbon spectra of the four polycrystalline aromatic amino acids. The assignments are readily made by comparison with the solution spectra. The line width of the aromatic resonances of amino acids is ~0.5 ppm (20 Hz) compared with 0.01 ppm for the same molecules in solution. This substantial line width remains in the presence of 3.0-mT ^{1}H decoupling and 4.0 kHz magic angle sample spinning and is a major limitation for ^{13}C NMR of solid proteins. Resonances from some carbons of amino acids have narrower lines (5–10 Hz), especially methyls of aliphatic side chains and carbonyl carbons. In general those carbons bonded to one nitrogen are split into an asymmetric doublet (e.g., Trp C_{ϵ_2} in Figure 1) in a 3.5-T field because the magic angle spinning does not completely average the dipolar coupling between the spin $^1/_2$ ^{13}C nucleus and the ^{14}N nucleus with its substantial quadrupole moment.[9] An exception is His C_γ (Figure 1) which is apparently unperturbed.

The solid-state spectra were obtained on a home-built double-resonance spectrometer with a 3.5-T magnet with ^{13}C resonance frequency of 37.83 MHz. The ^{1}H decoupling field used for the spectra shown was 1.5 mT. Magic angle spinning at 3.2 kHz was performed in 10-mm sample chamber Andrew rotors holding ~300 mg of material.[10] The solution spectrum was taken on a Nicolet NT-150 spectrometer with ^{13}C resonance frequency of 37.74 MHz.

We are investigating the bacteriophage fd by NMR, particularly the major coat protein.[11] This structural protein resides within the cell membrane during part of the virus life cycle and is completely intractable and insoluble when isolated. Without detergents or lipids the coat protein can only be studied in the solid state. The protein has 50 amino acids with

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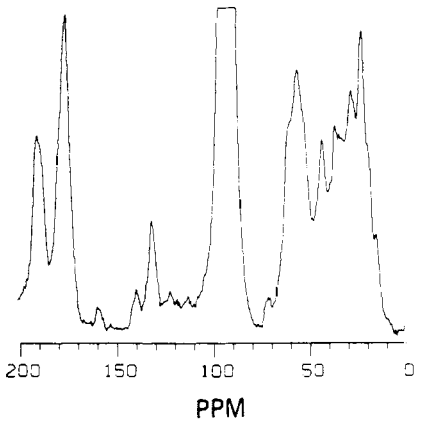

Figure 2. Natural abundance ^{13}C spectrum of isolated solid fd coat protein. Obtained from single-contact cross-polarization experiment with 1-ms mix time, 3-s recycle delay, 0.2-s acquisition time, and 12 000 transients. Signals from all carbons are present including the Delrin rotor at 90 ppm and its spinning side band at 195 ppm.

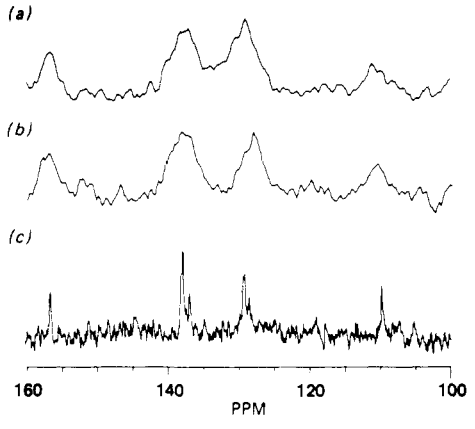

Figure 3. Nonprotonated aromatic carbon spectra of fd coat protein. (a) Isolated solid fd coat protein; cross polarized with 1-ms mix time, 3-s recycle delay, 0.1-s acquisition time, and a 90-μs delay without decoupling prior to data acquisition for 20 000 transients. (b) Solid fd virus: Same conditions as (a). (c) Detergent solubilized fd coat protein in water: sample is 6 mM in protein (10 mL) in 20-mm tube; weak noise-modulated proton decoupling is applied off-resonance and ^{13}C half echoes with 8-ms delay between pulses, 2-s recycle delay, and 0.4-s acquisition time for 64 000 transients were acquired.

a total of 10 nonprotonated aromatic resonances from 3 Phe, 2 Tyr, and 1 Trp.[12,13]

The spectrum in Figure 2 consists of signals from all the carbons of the isolated coat protein. The aliphatic carbons are in the region 10–70 ppm. All aromatic carbons are in the region 110–165 ppm with some separation of peaks apparent. The carbonyl carbons make up the band centered at 175 ppm. In this spectrum the only identifiable resonance is that of the two Tyr C_ζ at 160 ppm.

The nonprotonated aromatic carbon resonances of fd coat protein are shown in Figure 3. Spectrum a is from the same powder sample used in Figure 2, but was obtained with a 90-μs delay without proton decoupling prior to data acquisition to suppress the signals from carbons with bonded protons.[8] Spectrum b is of the intact virus which is 90% by weight coat protein; so the signals of the protein dominate. The protein solubilized in sodium dodecylsulfate detergent gives the ^{13}C spectrum c under conditions where only nonprotonated aromatic carbons appear.[14]

These three spectra can be compared directly. The most obvious difference is the narrower line widths of the detergent solubilized protein. The resonance at 160 ppm is from the two Tyr ζ carbons and in the best solution spectra two lines are completely resolved. The resonances at ~138 ppm are from the 3 Phe C_γ and the Trp ϵ_2 carbon; the contribution from the Trp carbon is probably reduced in the solid spectra owing to ^{14}N-induced splitting. Intensity at 130 ppm is due to the 2 Tyr C_γ and the Trp δ_2 carbon; these signals are completely overwhelmed by other ring carbons in the conventional proton-enhanced spectrum of Figure 2. Near 110 ppm in all three spectra is the resonance from the single γ carbon of Trp 26. Its assignment is unambiguous because there is only one Trp in the protein.[12,13] This is the only resonance that appears shifted in the solid compared with solution.

It is less time consuming to obtain a single carbon signal from a protein in the solid state than in solution. This is because of the greater ^{13}C magnetization resulting from cross-polarization than ^{13}C pulses and the recycling of data acquisition according to proton not carbon T_1. ^{13}C line widths are narrow enough in solid proteins to distinguish types of aromatic carbons. Resolution is sufficient for some experiments; for example, Tyr C_ζ pH titration over 12 ppm can be followed.

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Bond Switch with Participation of π-Bonded S^{IV} in a Thiadiazole Ring System[1]

Sir:

It has been reported that π-hypervalent sulfur plays an important role in effecting cycloaddition of iminothiazolines to activated acetylenes,[2] nitriles,[3] isothiocyanates,[4] and also in similar reactions involving 1,2-dithiole-3-thiones.[5] We recently reported a unique example of rapid equilibrium due to acid-catalyzed bond switch at π-hypervalent sulfur.[6] We now describe a ring-transformation equilibrium of the 1,2,4-thiadizole ring in neutral solution which can be understood as due to bond switch at π-hypervalent sulfur.

When 5-amino-3-methyl-1,2,4-thiadiazole (**1**) is heated with a nitrile in the presence of aluminum trichloride (molar ratio 1.0:1.0–2.0:1.0–1.5) at 80–110 °C for 0.5–1.5 h, the 1:1 adduct (**2**) was obtained in a moderate yield after usual workup[7] fol-

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