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Slow and Very Fast MAS Solid State NMR Study of Biopolymers

Tomasz Pawlak,[1] *Piotr Paluch*,[1] *Agata Jeziorna*,[1] *Grzegorz D. Bujacz*,[2] *Marek J. Potrzebowski**[1]

Summary: In the first part of article, the "NMR Crystallography" approach as tool to fine refinement of solid state structure of biopolymers is presented, employing the α polymorph of L-polylactide (PLLA) as model. Slow Magic Angle Spinning (MAS) technique (with spinning rate of sample in range from 1.2 kHz to 8.0 kHz) was used to assign ^{13}C isotropic chemical shifts and values of ^{13}C δ_{ii} principal elements of chemical shifts tensors (CST). Theoretical ^{13}C shielding parameters σ_{ii} were obtained employing GIPAW (Gauge Invariant Projector Augmented Wave) method and compared with experimental ^{13}C δ_{ii} elements. The computed and experimental ^{13}C CP/MAS spectra for WAND (Wide Angle Neutron Diffraction) geometry of powdered α PLLA were evaluated. It was revealed that the computed model of α PLLA model better fit to experimental NMR spectra.In the second part of article the applications of the new NMR methodology, so called very fast MAS (VF MAS) with sample spinning over 60 kHz are presented. The power of this approach is shown employing the ^{13}C and ^{15}N labeled protein, ubiquitin. We revealed that Cross-Polarization with Variable Contact (CPVC) time sequence under very-fast MAS condition performed in two-dimensional (2D) mode is very efficient method to measure accurately the C—H and N—H distances, and to analyze the dynamics of proteins with overlapped resonances in aliphatic and aromatic regions.

Keywords: NMR; polylactide; polymorphism; ubiquitin; very-fast MAS

Introduction

NMR spectroscopy is one of the most powerful techniques commonly used in the structural studies of different states of matter including gas phases, liquids and solids. The number of papers showing applications of NMR gradually increases each year. For instance, in 2012 ca 19 000 NMR papers were published, what means that each day over 50 articles report studies where the NMR experiments were performed.[1] For the apparent reasons the key position among different NMR techniques keeps the NMR spectroscopy in the liquid phase. However, in recent two decades the role of the solid-state NMR (SS-NMR) spectroscopy has been rapidly growing.[2]

The answer to the questions why solid state NMR has been so long in shadow of liquid NMR measurements is relatively easy. At the beginning SS NMR was treated as a rather "exotic" technique, mostly used by researchers with a "physics slant". Early NMR spectra recorded on solid-phase samples were very broad and of quality far from what chemists would normally expect. The big achievement in the field of SS NMR was introducing of Magic Angle Spinning (MAS) and heteronuclear decoupling techniques for obtaining narrow spectral lines.[3]

At the early stage of the development MAS NMR technique, powdered samples

[1] Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112 90-363, Lodz, Poland
E-mail: marekpot@cbmm.lodz.pl
[2] Institute of Technical Biochemistry, Technical University of Lodz, Stefanowskiego 4/10 90-924, Lodz, Poland

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placed in rotors with diameter 7 mm were routinely spun at the rate of few kilohertz. With the progress in the technique, 7-mm rotors, allowing sample rotation with frequency up to 6 kHz. The development of technique cause appearance of rotors with smaller diameters such as 4.0, 3.2 and 2.5 mm and increasing of rotational frequencies from the slow (6 kHz), to the medium (15–25 kHz) and fast (35 kHz) regimes. Actually, the "very-fast" regime of more than 60 kHz has been reached using commercially available 1.3-mm rotors. (Figure 1). In the very recent time has been presented rotors with 0.7 mm o.d. able to spin up to 110 kHz.[4] This frequency exceeds the strength of homonuclear proton dipolar coupling and is therefore expected to enter a new regime for spin dynamics.[5]

In this article we present some new ideas of solid state NMR spectroscopy and application of technique to study of synthetic biopolymers using α polymorph of L-Polylactide as an example and large-molecular weight natural polymers employing proteins as models. Paper is divided into two sections. The first one presents relatively new concept called as "NMR Crystallography" and first application of this strategy to analysis of polymers. The applicability of slow MAS NMR techniques in structural analysis of PLLA is clearly shown.

In the second section we present the power of very fast MAS NMR (VF MAS). It has to be stressed that very-fast MAS opens new perspectives for Cross Polarization (CP) techniques, which make use of low-power irradiation. Under VF MAS two coherent transfer pathways are accessible, depending on the experimental settings. This problem and applicability of Variable Contact Cross Polarization (CPVC) sequence to study of local molecular dynamics of ubiquitin will be discussed.

NMR Crystallography

NMR Crystallography is an idea which combines analysis of X-ray powder diffraction data, quantum mechanical calculations and NMR measurements.[6] This approach in pictorial form is shown in Scheme 1. Such a strategy, is usually used for the fine

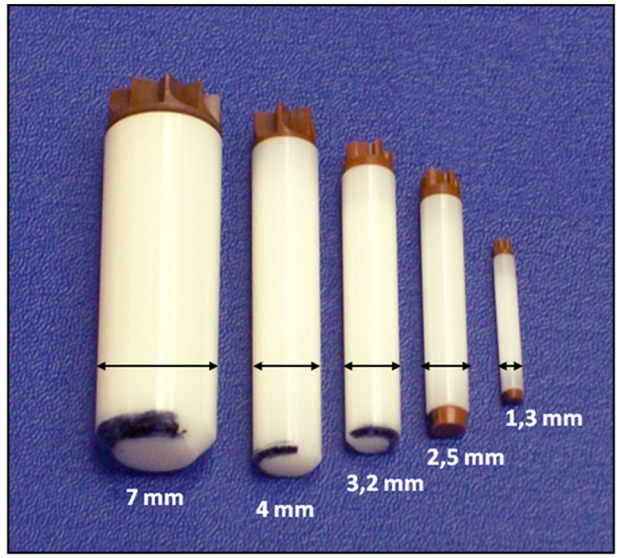

Figure 1.
Comparison of MAS rotors. Rotors of 1.3 mm o.d. have an active volume of 1.7 μL and can spin up to 67 kHz.[22]

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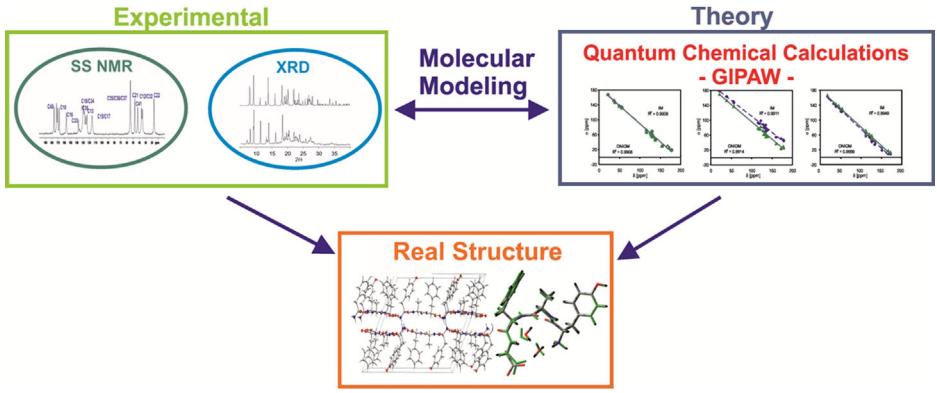

Scheme 1.

refinement of the structure of sample when the single crystals for X-ray or neutron diffraction studies are not available.

Solid-state NMR provides rich set of constraints, which are extremely useful in structural analysis of condensed matter. First, spectra provide a "fingerprint" of the local structure and represent the local electronic environment for each nucleus under investigation. NMR responds to the short-range environment of relevant atoms and is not directly influenced by long-range order. Most important NMR parameter, chemical shift gives information about intermolecular interactions. Analysis of principal elements of chemical shift tensor δ_{ii} provides detailed information about electronic distribution around each individual nucleus. Inter- and intra-molecular hydrogen-bond linkages can be identified. Information on crystallographic asymmetric units is especially readily available, usually merely by counting lines. Polymorphs are usually easily distinguished. Phase transitions can be monitored. Crystallographic disorder is detectable, and distinctions between spatial and temporal disorder can be made. Measurement of dipolar coupling constants yields through-space inter-atomic (i.e. internuclear) distances, though these will be modulated by local mobility.

A number of spectacular applications of NMR crystallography in structural studies of small molecules, mostly pharmaceuticals, have been published thus far by the groups of Emsley, Brown, Harris and others.[7] In our project, for the first time NMR Crystallography approach was employed for refinement of biopolymer, α polymorph of L-Polylactide.[8]

Shown in Figure 2 is structure of helix of α-PLLA chain obtained by means of WAXD (Wide-Angle X-ray Diffraction) and WAND techniques.[9]

Such sample was used as a starting material for NMR measurements. Figure 3 shows ^{13}C CP/MAS spectra of α-PLLA

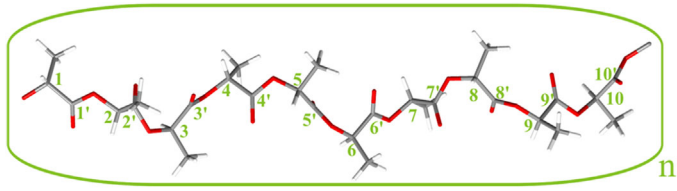

Figure 2.
10^3-Helix α-PLLA and its numbering system. Carbonyl groups are labeled with primes (n′).[9]

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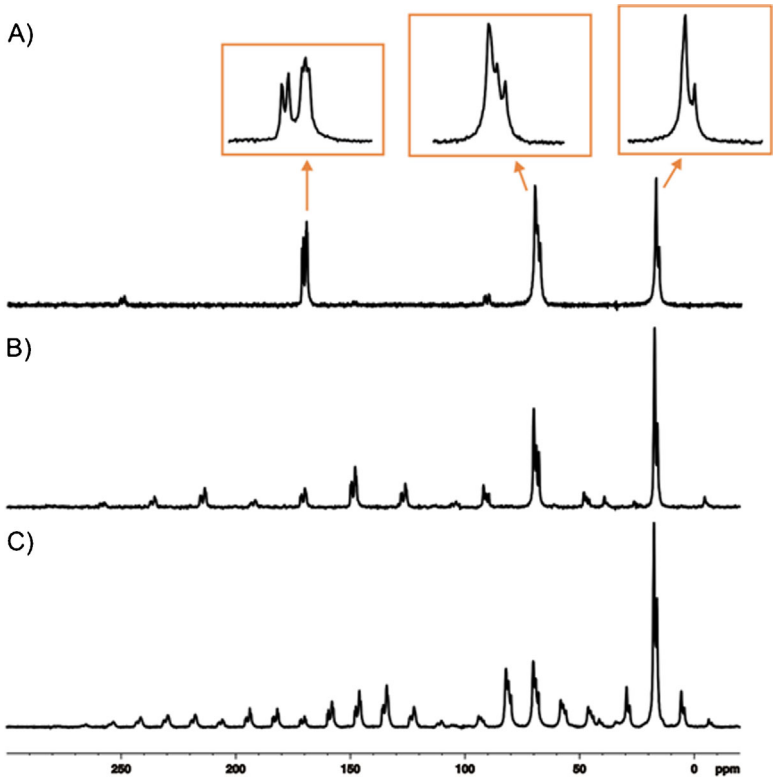

Figure 3.
^{13}C CP MAS NMR spectra of α-PLLA recorded with a spinning rate of A) 8 kHz, B) 2.2 kHz and C) 1.2 kHz. Insets in Figure A show expanded signals with splitting typical of a highly crystalline sample.

recorded with spinning rate 8 kHz (Figure 3A), with spinning rate 2.2 kHz (Figure 3B) and 1.2 kHz (Figure 3C). The difference between spectra is apparent. The upper spectrum shows only isotropic resonances while for middle and the bottom spinning sidebands which reflect chemical shift anisotropy for each resolved position are clearly seen. The spinning sidebands were further employed for assignment of values of principal elements of Chemical Shift Tensors (CST) ^{13}C δ_{ii} employing 2D PASS experiment.[10]

In the next step of our project we have computed ^{13}C NMR parameters using as an input file coordinates taken from paper of Wasanuk et. al.[8] The second set contained coordinates after full optimization of position of heavy atoms and hydrogens. The size of the unit cell was preserved during computing. Theoretical calculations were carried out employing CASTEP program[11,12] which incorporate the spatial repetition of the unit cell inherent in crystals[13] and implement the Gauge Invariant Projector Augmented Wave (GIPAW) method.[14]

Further, for both models obtained ^{13}C shielding parameters σ_{ii} we have converted to ^{13}C chemical shift δ_{ii} parameters in order to generate theoretical spectra. Figure 4A and Figure 4B show the isotropic part of theoretical spectra simulated with WAND coordinates and with fully optimized coordinates. Experimental spectrum is shown in Figure 4C. As one can see there is significant inconsistency between spectra 4A and 4C. It means that WAND coordinates for α-PLLA do not represent true structure with sufficient accuracy. Much

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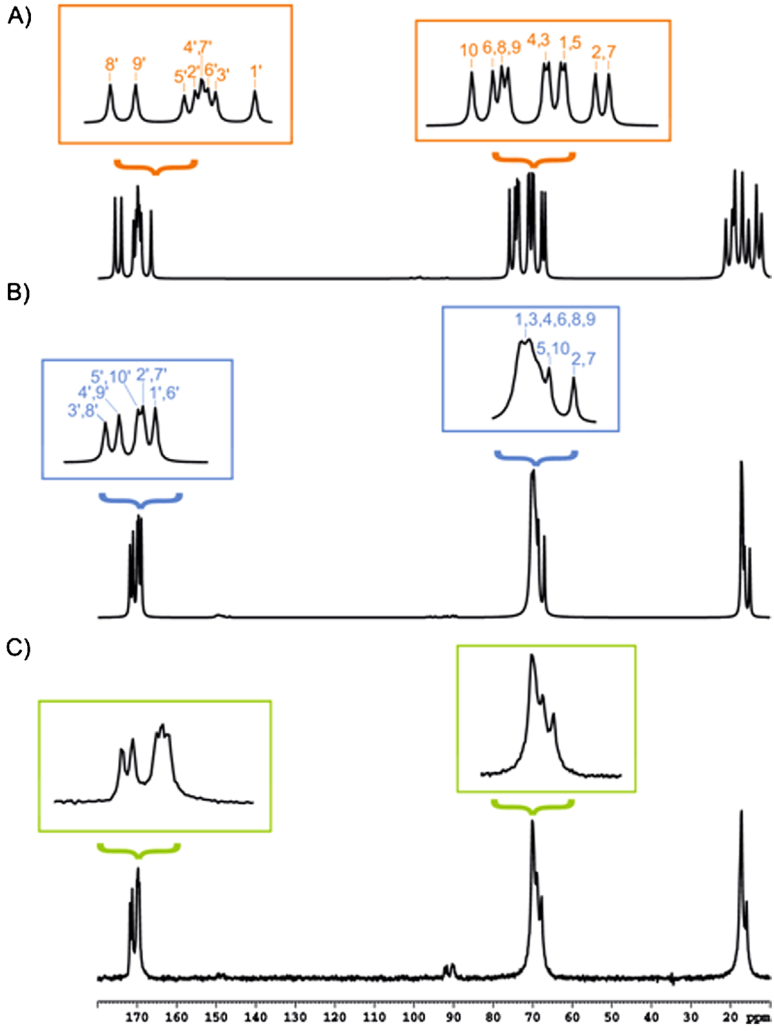

Figure 4.
Calculated and experimental 1D ^{13}C CP/MAS NMR spectra of α-PLLA measured/computed with a spinning rate of 8 kHz. Figure A shows the theoretical spectrum computed for structure with coordinates obtained by Wasanuk et al. (ref. [8]) the assigned signals correspond to the notation system given in Figure 3. Figure B shows the calculated spectrum obtained with fully optimized structure. Figure C displays the experimental ^{13}C CP/MAS spectrum.[9]

better correlation we obtained for sample with fully optimized geometry of PLLA helix.

Similar conclusion we have drawn testing the anisotropic pattern for spectra simulated with spinning rate 2.2 kHz (Figure 5). As in previous case much better correlation was obtained for sample with fully optimized geometry of PLLA helix. Figure 6 shows the differences between the literature WAND crystal structure and the α-PLLA structure optimized using the GIPAW method. At the first glance the differences seem to be very small but in fact the distinction in position of atoms can generate significant changes in the NMR spectral pattern. It means that NMR spectroscopy is very sensitive to local

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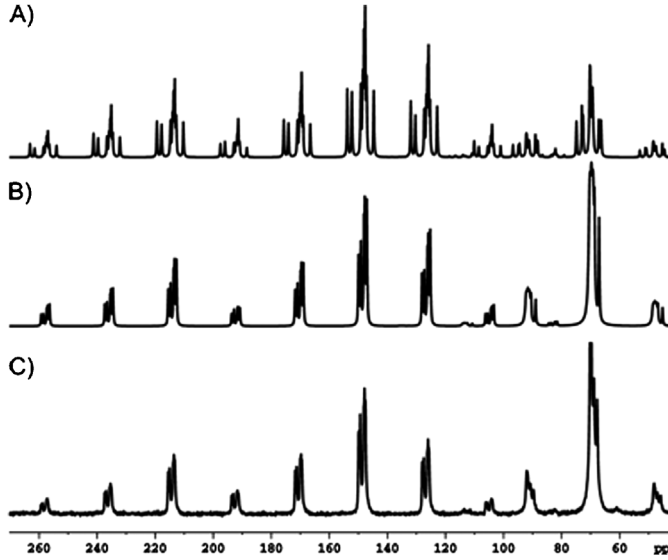

Figure 5.
Calculated and experimental and 1D ^{13}C CP MAS NMR spectra of α-PLLA collected at a spinning rate of 2.2 kHz. Figure A shows the theoretical spectrum computed for structure with coordinates obtained by Wasanuk et al. (ref. [8]), and Figure B shows the calculated spectrum obtained with fully optimized structure. Figure C displays the experimental ^{13}C CP/MAS spectrum.[9]

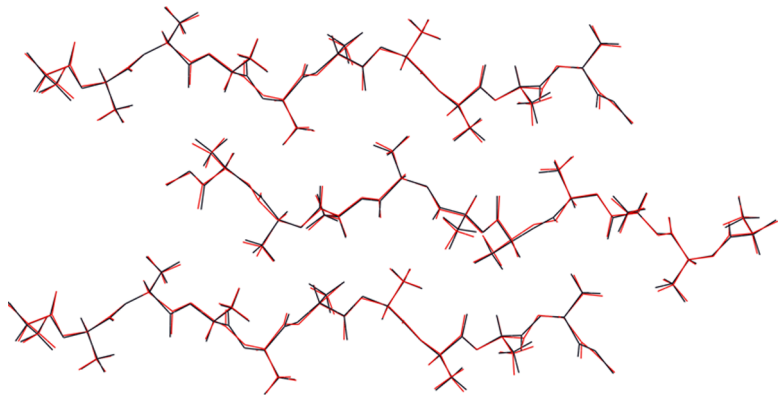

Figure 6.
The comparison between the literature WAND crystal structure (black, ref. [9]) and the α-PLLA structure optimized using the GIPAW method (red)).[9]

environment and can be used to fine refinement of polymer structure for which coarse geometry is known.

Very Fast MAS SS NMR

The last few years have witnessed an incredible hardware improvement in the design of solid state NMR probe-heads. Today it is possible to spin samples up to $\nu_R = 67$ or even 110 kHz with 1.3 or 0.75 mm diameter rotors.[15] This so-called very-fast (VF: $\nu_R > 60$ kHz) MAS regime opens new solid-state NMR possibilities for analyses of the condensed matter. The size of the 1.3 mm rotor is significantly smaller compared to others. Thus usually few

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milligrams of sample fills the rotor space (see Figure 1). Hence this technique is perfectly suited to measurement of samples which are difficult to obtain or very expensive or both. The natural products, e. g. selectively or uniformly ^{13}C, ^{15}N enriched proteins belong to this group.

VF-MAS has introduced new challenges because some effects which are not important under moderate sample spinning then become crucial under this new regime, or reciprocally, desired effects are reduced or eliminated. For instance, under VF-MAS, all hetero-nuclear dipolar couplings are completely averaged out,[16] and even the strong multi-spin dipole–dipole couplings between protons in organic solids are reduced to two-spin correlations.

Under VF-MAS condition two coherent transfer pathways (Hartman-Hahn match) for X–^{1}H spin pair are accessible, depending on the experimental settings.[17] At low MAS frequencies ($\nu_R = 10\,kHz$) and large RF-fields, the most efficient coherent transfers are dominated by the zero-quantum (ZQ) coherences. In contrast, at high MAS frequencies ($\nu_R = 20\,kHz$) and/or small RF-fields, the transfer could be carried out by double-quantum (DQ) coherences. In case of on-resonance irradiations, ZQ-CP occurs when $\nu_{1H} - \nu_{1X} = n\nu_R$ and gives rise to peaks with positive intensity, whereas DQ-CP occurs when $\nu_{1H} + \nu_{1X} = n\nu_R$ and gives rise to peaks with negative intensity. However, to achieve an effective coherence transfer for all resonances, the experimental parameters have also to take into account the resonance offsets (Ω):

$$(\Omega_H^2 + \nu_{1H}^2)^{1/2} + \varepsilon(\Omega_X^2 + \nu_X^2)^{1/2} = n\nu_R \quad \text{with: } n = 0,\ \pm 1,\ \pm 2,\ \pm 3,$$

where $\varepsilon = +1$ or -1 for DQ and ZQ transfers, respectively, and ΩH and ΩX are chemical shift offsets. The effects of off-resonance irradiation decrease with increasing RF-fields. Playing with different setup of Hartman-Hahn condition we can extent the palette of experimental possibilities. The unique feature of ultra-fast MAS NMR spectroscopy is the possibility of using the band-selective CP experiment. This approach was recently reported by several groups for sequential assignments of proteins.[18] As revealed, by employing spectrally induced filtering in combination with CP (SPECIFIC–CP) (see Figure 7), it

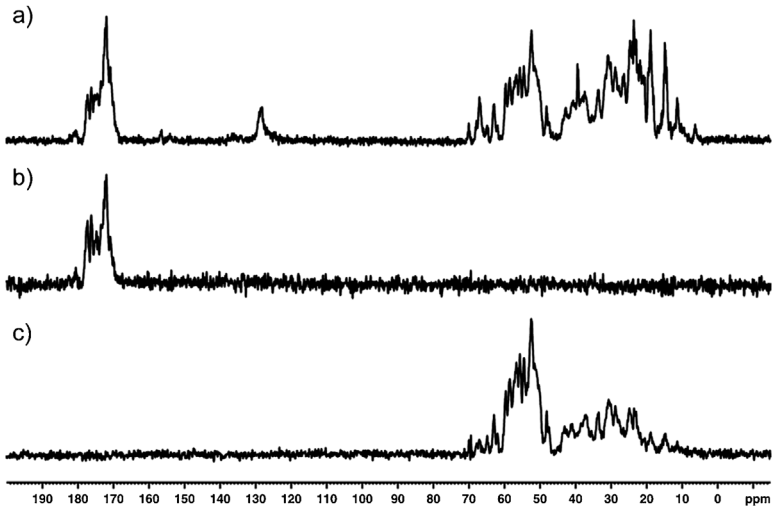

Figure 7.
Spectra of ^{13}C, ^{15}N labeled ubiquitin at 60 kHz MAS. a) standard (hard) ^{13}C CP; b) Specific-CP optimized for C=O region; c) Specific-CP optimized for Cα region.

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is possible to direct dipolar coherence transfer between N and C spins, based on the difference in the chemical shifts of the carbon resonances.

In our recent paper we have demonstrated that a simple experiment, Cross-Polarization with Variable Contact-time (CPVC), is very efficient at very-fast MAS (ν_R >60 kHz) to measure accurately the C-H and N-H distances, and to analyze the dynamics of bio-molecules.[19] This experiment can be performed with samples that are either ^{13}C or ^{15}N labeled or without any labeling. The power of the methodology we tested employing labeled protein, ubiquitin. Ubiquitin is one of the small regulatory proteins and consists of 76 amino acids (molecular weight 8.5 kDa). The NMR studies used to characterization of ubiquitin in liquid state were reported in number of papers.[20] Due to good stability, spectral dispersion and relaxation parameters, ubiquitin is currently widely used as standard for routine setup of sequences for NMR experiments in solution. The NMR analysis of protein in solid state is more challenging problem. According to our knowledge only few papers concern structural characterization of ubiquitin by solid state NMR.[21]

Figure 8 shows CPVC spectrum recorded in 2D mode with sample spinning equal to 60 kHz. The full spectrum is shown on the left while expanded aliphatic region on the right. The F2 projection represents the distribution of the ^{13}C chemical shifts. The F1 reflect the values of C-H dipolar couplings and/or dynamic processes. The selected cross sections and corresponding the 1D slices with line-shapes of Pake doublets are shown in Figure 9 as a bottom traces. The values of splitting are labeled.

As we proved in cited work,[18] in CPVC experiment for static sample after Fourier transformation we obtained a splitting of 16.2 kHz for CH spin pair, which corresponds to the theoretical scaling factor of $\sqrt{2}$ assuming a CH distance of 1.09 Å, and hence a dipolar coupling of 22.8 kHz. As one can see the values of splitting shown in Figure 9 are very different. In ubiquitin sequence there are only four aromatic residues, Phe4, Phe45, Tyr 59 and His68. Analysis of splitting suggests the static aromatic groups for Tyr and His and very mobile of Phe residues. Similar conclusion can be drawn for residues found in aliphatic region. The overlapped mobile resonances detected at $\delta = 39.1$ ppm and $\delta = 26.3$ ppm can be analyzed only employing 2D approach.

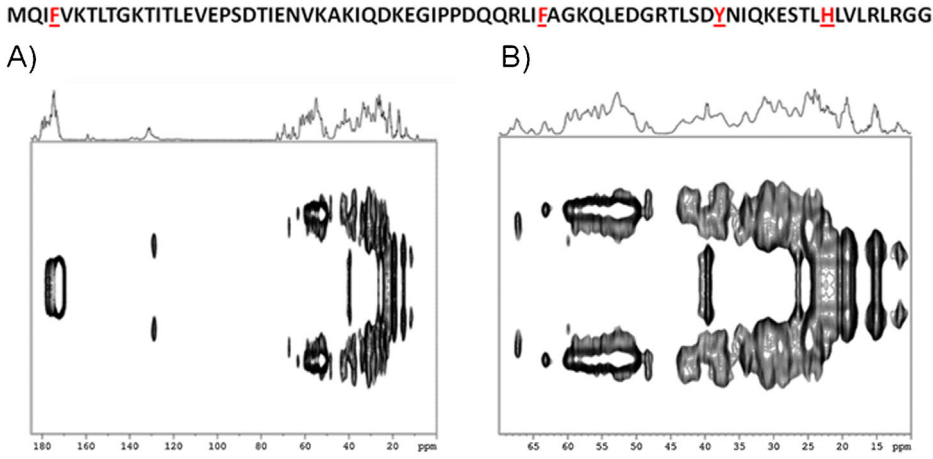

Figure 8.
^{13}C CP-VC 2D spectrum for 98% ^{13}C, 98% ^{15}N enriched Ubiquitin sample, recorded with $\nu_R = 60$ kHz, $\nu_{1H} = 50$ kHz, and $\nu_{15N} = 110$ kHz (n = −1), a) full spectrum, b) aliphatic region.

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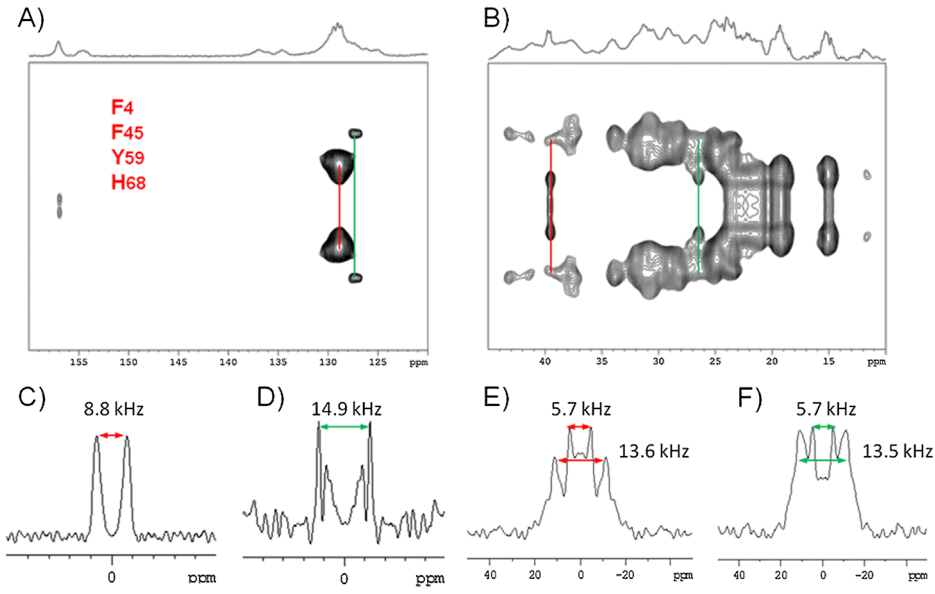

Figure 9.
^{13}C CP-VC 2D spectrum for 98% ^{13}C, 98% ^{15}N enriched Ubiquitin sample, recorded with $\nu_R = 60$ kHz, $\nu_{1H} = 50$ kHz, and $\nu_{15N} = 110$ kHz ($n = -1$) selected regions (a, b). Slices along F1 are shown for amino acid signals: F4 (c), F45 (d), Y59 (e), H68 (f).

Conclusion

In this article we have presented small piece of recent applications of high-resolution SS NMR in structural studies of biopolymers. With rapid, recent progress in software and hardware technologies, the possibilities of NMR spectroscopy in structural studies of polymers have been greatly extended. Today, N-Dimensional SS NMR spectroscopy, Fast MAS and Very Fast MAS experiments have become the routine approach to investigation of condensed matter.

In many cases, solid-state spectra are similar to those recorded in the liquid phase, but usually contain a wider range of information than is available in liquid NMR spectroscopy. Analysis of the tensorial nature of the chemical shifts of the active bonding centers provides subtle structural information. Strategies based on dipolar recoupling indicate a number of ways in which dipolar coupling constants can be measured, to yield direct data on internuclear distances and/or local molecular dynamics. This approach, combined with advanced theoretical calculations, traces new trends in structural studies of polymers in the solid state.

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