Solid State Nuclear Magnetic Resonance 72 (2015) 9–16

Contents lists available at ScienceDirect

Solid State Nuclear Magnetic Resonance

journal homepage: www.elsevier.com/locate/ssnmr

Evolution of CPMAS under fast magic-angle-spinning at 100 kHz and beyond

Ayesha Wickramasinghe [a], Songlin Wang [a], Isamu Matsuda [a], Yusuke Nishiyama [c,d], Takahiro Nemoto [c], Yuki Endo [c], Yoshitaka Ishii [a,b,*]

[a] Department of Chemistry, University of Illinois at Chicago, Chicago, IL 60607, United States
[b] Center for Structural Biology, University of Illinois at Chicago, Chicago, IL 60607, United States
[c] JEOL RESONANCE Inc., 3-1-2 Musashino, Akishima, Tokyo 196-8558, Japan
[d] RIKEN CLST-JEOL Collaboration Center, Yokohama, Kanagawa 230-0045, Japan

ARTICLE INFO

Article history:
Received 12 September 2015
Received in revised form
1 October 2015
Accepted 3 October 2015
Available online 8 October 2015

Keywords:
^{1}H decoupling
Solid-state NMR
Ultra-fast MAS
WALTZ
Composite-pulse decoupling
Micro-analysis
^{1}H detection

ABSTRACT

This article describes recent trends of high-field solid-state NMR (SSNMR) experiments for small organic molecules and biomolecules using ^{13}C and ^{15}N CPMAS under ultra-fast MAS at a spinning speed (ν_R) of 80–100 kHz. First, we illustrate major differences between a modern low-power RF scheme using UFMAS in an ultra-high field and a traditional CPMAS scheme using a moderate sample spinning in a lower field. Features and sensitivity advantage of a low-power RF scheme using UFMAS and a small sample coil are summarized for CPMAS-based experiments. Our 1D ^{13}C CPMAS experiments for uniformly ^{13}C- and ^{15}N-labeled alanine demonstrated that the sensitivity per given sample amount obtained at ν_R of 100 kHz and a ^{1}H NMR frequency (ν_H) of 750.1 MHz is ~10 fold higher than that of a traditional CPMAS experiment obtained at ν_R of 20 kHz and ν_H of 400.2 MHz. A comparison of different ^{1}H-decoupling schemes in CPMAS at ν_R of 100 kHz for the same sample demonstrated that low-power WALTZ-16 decoupling unexpectedly displayed superior performance over traditional low-power schemes designed for SSNMR such as TPPM and XiX in a range of decoupling field strengths of 5–20 kHz. Excellent ^{1}H decoupling performance of WALTZ-16 was confirmed on a protein microcrystal sample of GB1 at ν_R of 80 kHz. We also discuss the feasibility of a SSNMR microanalysis of a GB1 protein sample in a scale of 1 nmol to 80 nmol by ^{1}H-detected 2D ^{15}N/^{1}H SSNMR by a synergetic use of a high field, a low-power RF scheme, a paramagnetic-assisted condensed data collection (PACC), and UFMAS.

1. Introduction

High-resolution ^{13}C solid-state NMR (SSNMR) using a CPMAS scheme has marked a milestone of 40 years since the scheme was originally introduced by Schaefer and Stejskal for characterization of polymers and other organic systems at a spinning speed of 3 kHz [1]. The work not only demonstrated a creative integration of earlier inventions of cross polarization (CP) [2] and magic-angle-spinning (MAS) [3], but also involved development of hardware such as a spinning system (see Fig. 1; a photograph courtesy of Prof. Schaefer). With enormous progress after this discovery, high-resolution SSNMR spectroscopy is generally considered as a matured field. Nevertheless, recent advances in SSNMR have markedly improved its capabilities. In particular, novel developments for fast MAS systems have increased the available spinning speed from 30 kHz to 100 kHz or higher over the past decade [4–11]. Engineering needs for ultra-fast MAS (UFMAS) at 80 kHz or higher demand a small MAS rotor having a diameter of 1 mm or less (see Fig. 2). Because of its very restricted sample volume ($\leq$1 μL), there was a natural skepticism about the sensitivity and feasibility of biomolecular SSNMR using such a UFMAS system in the NMR community. Nevertheless, recent studies have demonstrated that SSNMR using UFMAS at 80–100 kHz in a high field offers a practical tool for structural biology [11–13]. These findings signify a crucial development in biological SSNMR as a production of a protein and other biological sample in a large quantity is often prohibitive for systems of biological interest. The new development was prompted by a series of sensitivity enhancement approaches that are compatible with UFMAS. These approaches include ^{1}H indirect detection [5,14–18], paramagnetic assisted condensed data collection (PACC) [6,19–22], usage of a high field [12,13,23–25], and deuteration and other unique isotope

* Corresponding author at: Department of Chemistry, University of Illinois at Chicago, Chicago, IL 60607, United States.
E-mail address: yishii@uic.edu (Y. Ishii).

http://dx.doi.org/10.1016/j.ssnmr.2015.10.002
0926-2040/

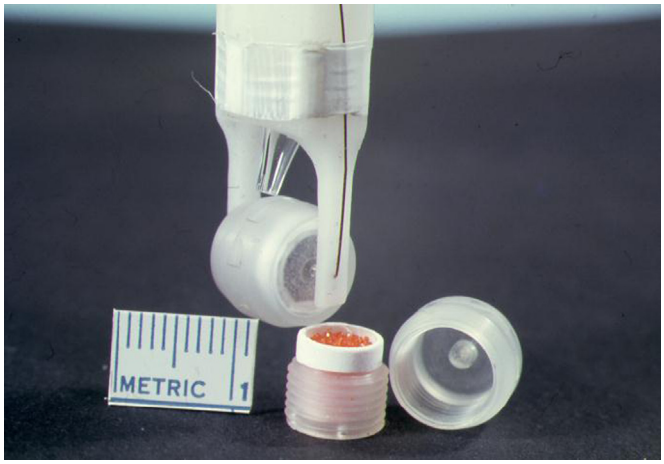

Fig. 1. Spinning system for the original CPMAS experiment [1]. The photograph provided courtesy of Prof. Jacob Schaefer at the Washington University in St. Louis. "The rotor was suspended from two phosphor–bronze wires held to the "stator" by teflon tape. A pipette brought the drive air in. A rotor would only last through 2 or 3 stop–start cycles. It took Ed (Stejskal) a day to make a rotor," according to a note from Prof. Schaefer.

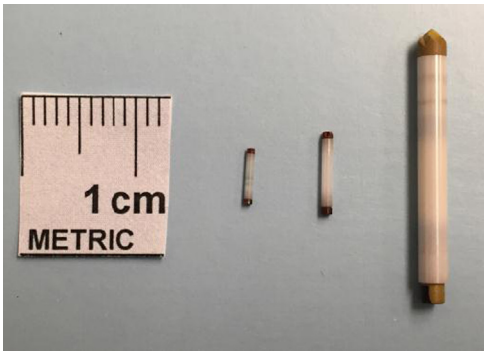

Fig. 2. A picture of three MAS rotors used for this study in a comparison with a metric ruler. (Left) JEOL 0.75-mm rotor, (middle) JEOL 1-mm rotor, and (right) Varian 2.5-mm rotor.

labeling schemes [12,18,26,27]. Importantly, sensitivity enhancement factors by these approaches can be compounded since many of these methods can be integrated together without any major interferences. Thus, despite a limited sample volume, recent studies using UFMAS indicate unparalleled mass-sensitivity (i.e. sensitivity per sample amount) for a 3D SSNMR analysis of a mass-limited protein sample [12,13]. Moreover, unlike dynamic nuclear polarization (DNP) approach [28]. UFMAS-based methods offer an analysis of a biological sample in a hydrated state. Another important aspect in SSNMR spectroscopy using UFMAS is that a drastic change in the spin physics allows one to explore new types of pulse sequences such as low-power ^{1}H decoupling [14,15,29,30] and low-power cross-polarization sequences [6,8,31].

In this article, we discuss the present status and a prospect of biomolecular SSNMR using CPMAS under UFMAS. We review how basic building blocks of a CPMAS scheme such as CP and ^{1}H decoupling have been evolved under UFMAS. Then, we discuss an alternative ^{1}H low-power decoupling scheme using composite-pulse decoupling schemes such as WALTZ-16, which were previously ineffective for ^{1}H decoupling in SSNMR. We also demonstrate that a SSNMR microanalysis of proteins in a 1–100 nmol scale is feasible through examining the sensitivity and resolution of ^{1}H-detected 2D ^{1}H/^{15}N SSNMR experiment for a GB1 microcrystalline sample under UFMAS.

2. Material and methods

Unless otherwise mentioned, all the SSNMR experiments were performed on a Bruker Avance III 750 MHz spectrometer at the UIC Center for Structural Biology using a JEOL 0.75-mm or 1-mm ^{1}H/^{13}C/^{15}N/^{2}H quad-resonance MAS probe. The experiment in Fig. 4b was performed on a Bruker Avance III 400 MHz spectrometer using a homebuilt 2.5-mm ^{1}H/^{13}C/^{15}N CPMAS probe. The sample temperatures were maintained at $\sim$15 °C by applying a cooled VT air at $-$15°C in order to compensate for the temperature increase by sample spinning at 100 kHz. Fig. 3 shows (a) a CPMAS pulse sequence for the UFMAS experiment and (b) the sequence for the conventional CPMAS experiment in Fig. 4b. In the 1D ^{13}C CPMAS experiments using UFMAS, ^{13}C spin polarization was prepared with double-quantum adiabatic cross polarization (DQ-CP) using an amplitude-modulated shaped pulse with an upward tangential ramp for the ^{13}C channel and a rectangular pulse for the ^{1}H channel [8]. The ^{13}C RF field strength was swept from 55 kHz to 95 kHz with the average rf field at $\sim 3\nu_R/4$ while the ^{1}H RF field amplitude was set kept constant at 25 kHz ($\sim\nu_R/4$), where the sum of the average RF fields was matched to ν_R for DQ-CP, where ν_R denotes a spinning speed. The ^{13}C signals were acquired under low-power WALTZ-16 ^{1}H decoupling [32] at 5 kHz in Fig. 4a. The same CP sequence was used for Figs. 4–6 with

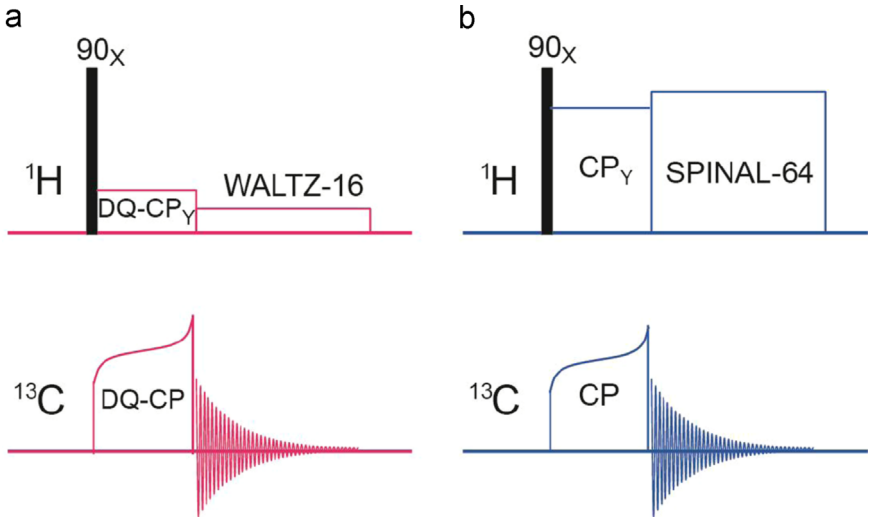

Fig. 3. Pulse sequences of (a) a low-power CP scheme suited for a UFMAS condition and (b) a high-power CP scheme used for a conventional CPMAS.