Available online at www.sciencedirect.com
SCIENCE DIRECT®
Solid State Nuclear Magnetic Resonance 29 (2006) 119–124
Solid State Nuclear Magnetic Resonance
www.elsevier.com/locate/ssnmr

A combined ^{17}O RAPT and MQ-MAS NMR study of L-leucine

Subramanian Prasad[a], Ted M. Clark[a], Ramesh Sharma[a], Hyung-Tae Kwak[a], Philip J. Grandinetti[a,*], Herbert Zimmermann[b]

[a]*Department of Chemistry, The Ohio State University, 120 W. 18th Avenue, Columbus, OH 43210-1173, USA*
[b]*Max-Planck Institut for Medical Research, Jahnstrasse 29, 69120 Heidelberg, Germany*

Received 28 June 2005; received in revised form 4 October 2005
Available online 15 November 2005

Abstract

We report the application of rotor-assisted population transfer (RAPT) to measure the quadrupolar coupling constant (C_q) for spin $\frac{5}{2}$ nuclei. Results from numerical simulations are presented on the magnitude of enhancement factor as a function of frequency offsets, i.e. the RAPT profile. Experimental ^{17}O RAPT profile is traced for the amino acid L-leucine. In addition, results from MQ-MAS experiments are incorporated to determine the quadrupolar asymmetry parameter (η_q). Unlike previous reports, the ^{17}O NMR parameters for an amino acid, L-leucine, is reported at a relatively low field of 9.4 T.

Keywords: Amino acids; ^{17}O; RAPT; MQ-MAS

1. Introduction

Oxygen-17, which is a spin $\frac{5}{2}$ nucleus, has never been as successful as a probe of structure in organic molecules as spin $\frac{1}{2}$ nuclei such as ^{13}C NMR. The combination of a low natural abundance (0.037%), and the large quadrupolar couplings of oxygen in organic molecules have made the ^{17}O NMR measurement a daunting task. There have been a number of renewed efforts in the study ^{17}O NMR of small organic molecules of biological interest in the solid state. This stems from the advent of high-magnetic field strengths ($\geqslant$ 500 MHz ^{1}H frequency), fast spinning rotors and resolution enhancement techniques such as multiple-quantum magic-angle spinning (MQ-MAS) [1]. These recent reports vary from amino acids (alanine, L-glutamic acid), nucleosides (thymine and uracil), monosodium glutamate, polyglycines and polyalanines [2–5]. The magnitude of nuclear quadrupolar coupling constants are typically large, on order of 7–8 MHz. Carbonyl groups located in the α-helix and β-sheet have been distinguished in polypeptides [2,3], and five distinct oxygen environments have been identified in monosodium glutamate [5]. Small differences in the quadrupolar coupling constants have been attributed to the differences in the H-bonding strengths in alanine and in polypeptides. Recently, Dupree and co-workers have compiled ^{17}O NMR parameters for a series of amino acid salts [6] and a transmembrane peptide [7]. Also, a theoretical study has been made to correlate the ^{17}O shielding parameters to hydrogen bonding distances in glutamic acid polymorphs [8].

Here we report NMR data on the amino acid L-leucine. By combining the increased sensitivity of rotor-assisted population transfer (RAPT) [9–11] with high-speed MAS (20 kHz) we can obtain high-quality spectra and extract the NMR parameters at a relatively lower magnetic field (400 MHz ^{1}H frequency) than used in the previous studies.

2. Materials and methods

L-leucine was synthesized by acid catalyzed exchange of oxygen in ^{17}O labelled water at 80 °C. The resulting solution was neutralized with aniline to precipitate the free amino acid [12]. The purity of the sample was determined by powder X-ray diffraction and ^{13}C MAS NMR measurements. All NMR measurements were made on a Bruker Avance 400 MHz spectrometer using 2.5 mm MAS probehead and spinning rates of 20 kHz. One-dimensional

*Corresponding author. Fax: +1 614 292 1685.
E-mail address: grandinetti.1@osu.edu (P.J. Grandinetti).

0926-2040/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ssnmr.2005.10.007

^{17}O MAS spectra were measured using the sensitivity enhancement scheme RAPT; to obtain the RAPT sensitivity enhancement, frequency switched Gaussian pulses ($\sigma = 2.855\,\mu s$, $\tau_p = 16\,\mu s$; 20 pulse pairs) were applied at pre-determined frequency offsets [9–11]. A 2 μs delay between each pulse in the RAPT pulse train was used to allow time for the transmitter phase to stabilize. A radiofrequency field strength of 35 kHz was used for the RAPT pulse train and a recycle delay of 500 ms. The RAPT-enhanced MAS spectra were acquired with 8192 scans using a RAPT frequency offset of ±500 kHz, 256 scans were acquired to trace the RAPT profile with varying frequency offsets. Shifted-echo method [13,14] was used for acquiring and processing triple quantum MQ-MAS data [15]. A radiofrequency field strength of 72 kHz was used for the excitation and the FAM conversion pulses [16], and 22 kHz for the π pulse. $64t_1$ points (9600 scans) were acquired with an increment of 24 μs at a recycle delay of 100 ms. T_1 measured using saturation recovery method was 25 ms. Tap water (0.0 ppm) was used to calibrate the radiofrequency field and as chemical shift reference.

2.1. Simulations

For simulating the RAPT enhancement profiles we have employed numerical density matrix simulations of a polycrystalline sample containing spin I nuclei to calculate the evolution of an initial density operator $\boldsymbol{\rho}_0$ in the rotating frame according to

$$\boldsymbol{\rho}(\Omega, t) = \mathbf{U}(\Omega, t, 0\boldsymbol{\rho}_0\mathbf{U}^{\dagger}(\Omega, t, 0), \tag{1}$$

where

$$\mathbf{U}(\Omega, t, 0) = \mathbf{T}\,\exp\left\{-i\hbar\int_0^t \mathbf{H}(\Omega, s)\,ds\right\} \tag{2}$$

and $\mathbf{T}$ is the time ordering operator. The time-dependent Hamiltonian in a frame rotating at the transmitter frequency is

$$\mathbf{H}(\Omega, t) = \mathbf{H}_q(\Omega, t) + \hbar\omega_1(t)[\mathbf{I}_x\cos\phi(t) + \mathbf{I}_y\sin\phi(t)] + \hbar\Delta\omega\mathbf{I}_z, \tag{3}$$

where $\mathbf{H}_q(\Omega, t)$ contains the first- and second-order quadrupolar Hamiltonians given by [17]

$$\mathbf{H}_q^{(1)}(\Omega, t)/\hbar = \omega_q A_{2,0}(\Omega, t)\mathbf{T}_{2,0} \tag{4}$$

and

$$\mathbf{H}_q^{(2)}(\Omega, t)/\hbar = -\frac{\omega_q^2}{\omega_0}\sum_{k=1,2}\frac{A_{2,k}(\Omega, t)A_{2,-k}(\Omega, t)[\mathbf{T}_{2,k}, \mathbf{T}_{2,-k}]}{k}. \tag{5}$$

Here $\Omega = (\alpha, \beta, \gamma)$ is a set of Euler angles describing the orientation of the principal axis system of the electric field gradient with respect to the rotor axis system. In all simulations, we will follow the evolution of the powder average of the fictitious spin half [18,19] observables according to

$$\langle\mathbf{I}_\alpha^{r-s}(t)\rangle = \int \mathrm{Tr}\,\boldsymbol{\rho}(\Omega, t)\mathbf{I}_\alpha^{r-s}\}\,d\alpha\,\sin\beta\,d\beta\,d\gamma. \tag{6}$$

For the simulations in this investigation, we typically start with $\boldsymbol{\rho}_0 = \mathbf{I}_z$ and observe $\mathbf{I}_z^{r-s}$.

When dealing with a time-dependent Hamiltonian we adopt the conventional numerical approximation of discretizing the time-dependence into small time-independent periods, and write the propagator matrix in Eq. (2) as

$$\mathbf{U}(\Omega, t, 0) = \prod_n \mathbf{U}(\Omega, n\Delta t, n\Delta t - \Delta t),$$

where

$$\mathbf{U}(\Omega, n\Delta t, n\Delta t - \Delta t) = \exp\{-i\hbar\mathbf{H}(\Omega, n\Delta t)\Delta t\}$$

and the Hamiltonian is assumed to be time independent during the time interval Δt. In this approximation $1/\Delta t$ needs to much larger than the highest frequency in a Fourier expansion of the time-dependent rotating frame Hamiltonian.

3. Results and discussion

For odd-half-integer quadrupolar nuclei such as ^{17}O, the observed total frequency shifts in the MAS spectra (δ_{iso}^{Total}) are directly related to the isotropic chemical shifts (δ_{iso}^{CS}) and isotropic second-order quadrupolar shifts (δ_{iso}^{2Q}) by

$$\delta_{iso}^{Total} = \delta_{iso}^{CS} + \delta_{iso}^{2Q} \tag{7}$$

The second-order isotropic shift is a function of the quadrupolar product (P_q), the Larmor frequency (ν_L) and the spin quantum number (I).

$$\delta_{iso}^{2Q} = \frac{-3[I(I+1) - 3/4]}{40\nu_L^2 I^2(2I-1)^2}P_q^2 \times 10^6, \tag{8}$$

where the quadrupolar product (P_q) is related to the quadrupolar coupling constant (C_q), which in turn is related to the quadrupolar frequency (ν_q) as

$$P_q = C_q\sqrt{1 + \frac{\eta_q^2}{3}}, \tag{9}$$

$$C_q = \frac{e^2qQ}{h}, \tag{10}$$

$$\nu_q = \frac{3C_q}{2I(2I-1)}. \tag{11}$$

Sensitivity enhancement schemes are particularly useful in the study of low natural abundant ^{17}O isotope. The method of RAPT enhances the central transition signal intensity by the saturation of the satellite transitions [20]. The method has been applied to higher spin systems [21] and exploited to enhance the signal intensity in the RIACT-MQ-MAS experiment [22–24]. The enhanced RAPT sequence consists of a train of Gaussian pulses with alternating off-resonant frequencies of $\pm\nu_{off}$ [9–11].