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Heteronuclear proton double quantum-carbon single quantum scalar correlation in solids

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ABSTRACT

A new NMR experiment that exploits the advantages of proton double quantum (DQ) NMR through a proton DQ-carbon single quantum (SQ) correlation experiment in the solid state is proposed. Analogous to the previously proposed 2D ^{1}H (DQ)–^{13}C refocused INEPT experiment (Webber et al., 2010), the correlation between ^{1}H and ^{13}C is achieved through scalar coupling evolution, while the double quantum coherence among protons is generated through dipolar couplings. However, the new experiment relies on ^{13}C transverse coherence for scalar transfer. The new experiment dubbed MAS-J-^{1}H (DQ)–^{13}C-HMQC, is particularly suited for unlabeled molecules and can provide higher sensitivity than its INEPT counterpart. The experiment is applied to four different samples.

1. Introduction

Proton NMR spectroscopy is routinely used in solution state for the characterization of structure and dynamics of a variety of molecules [1] due to its inherent high sensitivity, high natural isotopic abundance and the ubiquitous nature of hydrogen atoms. In solids, the study of proton chemical shifts becomes more interesting because they are very sensitive to inter-molecular packing and are involved in interactions such as hydrogen bonding and aromatic π–π interactions. However, the dense network of strong homonuclear dipolar couplings among protons leads to broad and featureless resonances in solids due to which ^{1}H chemical shifts are not easily accessible. In recent years, three different approaches have been employed to obtain high-resolution ^{1}H spectra namely (i) fast-magic angle spinning [2–5], (ii) combined rotation and multiple pulse spectroscopy (CRAMPS) [6–8] and (iii) isotopic dilution with deuterium [9–14]. In the last year MAS frequencies on the order of 100–120 kHz have become available. However, even with such high spinning speeds, the ^{1}H resonances of highly mobile molecules like adamantane remain relatively broad compared to the spectra in solution due to residual homonuclear ^{1}H–^{1}H dipolar coupling [15]. The CRAMPS approach at high MAS frequency provides a marginal improvement in ^{1}H line widths compared to only MAS spectra at 80 kHz [16,17]. The third strategy for obtaining narrow ^{1}H resonances relies on diluting the protons by replacing protons with deuterons and leaving only a small fraction of protons at predesigned sites [11,18,19]. This strategy applied to proteins in the solid state has yielded resolution similar to that observed in solution.

For the study of systems at natural abundance such as polymers [20,21], pharmaceutical molecules [22] and peptides [23], fast MAS and CRAMPS are the only practical ways to obtain proton chemical shifts. These methods are often further supplemented by two-dimensional proton–proton SQ–SQ or DQ–SQ experiments [6,23,24]. However, due to limitations in proton resolution, this approach is not optimal when there are many protons or they have a narrow distribution of chemical shifts. To boost resolution, the ^{1}H SQ/DQ dimension can be concatenated with a ^{13}C dimension while the transfer between ^{1}H and ^{13}C can be mediated through scalar [25–29] or dipolar couplings [30–34]. One of the main problems with the dipolar coupling mediated experiment is the lack of sufficient selectivity in the magnetization transfer i.e., only to directly bonded carbon nuclei and not to carbon nuclei that are further away. These correlation peaks between non-bonded pairs can significantly complicate the initial analysis of the 2D spectrum and hence are not desirable [25]. So far, the scalar coupling mediated 2D ^{1}H (DQ)–^{13}C correlation experiments have been done with a refocused INEPT element for heteronuclear polarization transfer [26]. In this approach, the proton T_2' plays an important role in

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determining the efficiency of the heteronuclear INEPT transfer step and can be a limiting factor under conditions of short proton T_2's [26,28].

Herein, we propose a new experiment that is based on the design of the MAS-J-HMQC experiment and relies on ^{13}C T_2' to achieve a 2D ^1H (DQ)–^{13}C correlation spectrum. The ^{13}C transverse coherences are typically longer lived than proton coherences and therefore in naturally abundant samples (where ^{13}C–^{13}C scalar couplings play no role) the proposed experiment has a higher sensitivity than the refocused INEPT based experiment [35]. The general advantages of a 2D ^1H (DQ)–^{13}C correlation experiment are the following: (a) The use of the DQ coherence can give rise to multiple correlation peaks and thus increase the precision of proton chemical shift measurement, since in this scheme, even protons bonded to ^{12}C carbons can contribute to signal through a DQ coherence generated with another proton bonded to a ^{13}C carbon. (b) In favorable cases, it is possible to obtain chemical shifts of protons that are not attached to a carbon, such as NH or OH protons. This obviates the need for performing a separate nitrogen–proton heteronuclear correlation experiment. (c) The presence of the double-quantum peaks could be used to infer proximity between two protons. (d) Since protons in most cases are located on next neighbor carbons, the ^1H–^{13}C SQ correlation spectrum can be used to trace the carbon connectivity in naturally abundant samples. The MAS-J-^1H (DQ)–^{13}C-HMQC experiment is applied here to four different molecules, namely alanine, histidine, glutathione and 4-hexyloxybenzoic acid to demonstrate its general applicability and utility.

2. Pulse sequence

In the MAS-J-^1H (DQ)–^{13}C-HMQC pulse sequence (Fig. 1), after ^1H–^{13}C CP, the carbon magnetization evolves under the influence of scaled heteronuclear scalar couplings during the delay τ. During this period proton–proton dipolar couplings are removed by homonuclear decoupling (here DUMBO-1) [36], whereas the chemical shift anisotropy and heteronuclear dipolar couplings are averaged out by magic angle spinning (MAS). For a pair of covalently bonded ^1H–^{13}C spins, the carbon magnetization evolves during τ from in-phase S_x coherence into anti-phase $(-2I_zS_y)$ coherence. At this point the homonuclear recoupling sequence POST-C7 [37] is applied for duration τ_{exc} to excite ^1H–^1H DQ coherences. During t_1, proton double quantum coherences evolve only under the effect of the proton chemical shifts, as proton–proton dipolar couplings are removed by the homonuclear decoupling. The 180° pulse refocuses the ^1H–^{13}C J couplings during t_1 and the ^{13}C chemical shifts

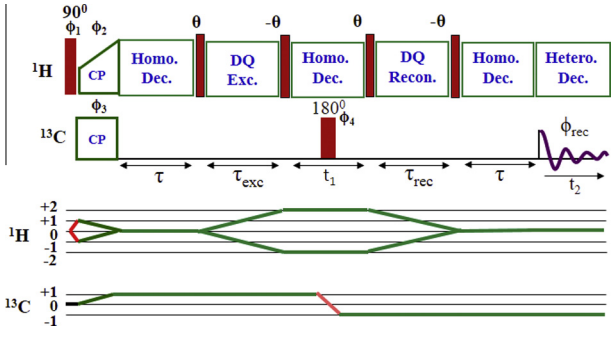

Fig. 1. Pulse scheme for the two-dimensional MAS-J-^1H (DQ)–^{13}C HMQC correlation experiment. The coherence transfer path way diagram is given below. The following 8-step phase cycle was used: ϕ_1 = +*x*, +*x*, +*x*, +*x*, −*x*, −*x*, −*x*, −*x*; ϕ_2 = +*y*; ϕ_3 = +*x*; ϕ_4 = +*y*, +*y*, +*y*, +*y*, +*y*, +*y*, +*y*, +*y*, −*y*, −*y*, −*y*, −*y*, −*y*, −*y*, −*y*, −*y*, receiver = +*x*, −*x*, +*x*, −*x*, −*x*, +*x*, −*x*, +*x*. The reconversion block has been phase cycled in 4-steps to select DQ coherence.

over the 2τ period. After the t_1 period, the double quantum (DQ) proton coherences are converted back into single quantum (SQ) coherences during the period τ_{rec}. The second τ period refocuses the anti-phase coherence back to in-phase observable carbon coherence, which is detected in the t_2 period under heteronuclear decoupling.

3. Experimental

Unlabeled samples of the amino acids alanine, histidine and glutathione reduced tripeptide were purchased from Sigma and used without further purification. 4-hexyloxybenzoic acid (HBA) was purchased from Aldrich and was further purified by recrystallization from heptane. Spectra were recorded on a Bruker Avance-III NMR spectrometer operating at ^1H Larmor frequency of 500.18 MHz. All the experiments were performed using a Bruker 4 mm triple-resonance MAS probe, operating in double-resonance mode with samples spinning at 11 kHz. A proton r.f field amplitude of 62.5 kHz was used for the ^1H flip pulses and heteronuclear decoupling (SPINAL-64) [38]. The ^1H–^{13}C CP was 2 ms long, employed a ramp on proton and had an r.f amplitude of 62.5 kHz on ^{13}C. Using an acquisition time of 35 ms, 16 transients for alanine and 32 transients for histidine, glutathione reduced tripeptide and HBA were coadded. A recycle delay of 4 s was employed between transients. For obtaining individual proton chemical shifts, single quantum ^1H–^{13}C correlation experiments were carried out using the MAS-J-HMQC pulse sequence reported earlier [25]. For the carbon dimension, the adamantane methylene peak at 29.5 ppm was used as the chemical shift reference while for the proton dimension the methyl proton peak of L-alanine at 1.4 ppm was used as the chemical shift reference.

Two-dimensional MAS-J-^1H (DQ)–^{13}C-HMQC spectra of all the compounds were recorded using the pulse sequence of Fig. 1. The phase modulated homonuclear decoupling scheme DUMBO-1 was applied in the τ and t_1 periods respectively. A single cycle of DUMBO-1 was constructed with an r.f field amplitude of 100 kHz from 64 steps of 500 ns each, yielding a total duration of 32 μs. Under DUMBO-1 decoupling, the effective field lies along an axis inclined at an angle θ with respect to the B_0 field. The flip-angle pulses associated with the τ periods serve to increase the sensitivity of the experiment, while those associated with t_1 serve to minimize axial peaks and quadrature images. The phase ϕ and orientation θ of the effective field were calibrated as 90° and 36° respectively, from one-dimensional experiments described earlier [36]. The DUMBO decoupling sequence was optimized by observing the scalar multiplet structure on the carbons for samples such as camphor and adamantane. The t_1 increment was set to 32 μs corresponding to one basic DUMBO-1 cycle. For the POST-C7 pulse sequence, the duration of both the excitation (τ_{exc}) and the reconversion (τ_{rec}) period was 52 μs, corresponding to two basic POST-C7 elements. The corresponding proton r.f field amplitude was set to equal to seven times the spinning frequency of 11 kHz at 77 kHz. A τ evolution period of 2.0 ms for all the samples provided maximum ^1H–^{13}C through bond polarization transfer. Spectra were acquired in the States-TPPI method [39]. All other experimental details are given in the respective figure captions.

4. Results and discussion

The relative advantage of the MAS-J-^1H (DQ)–^{13}C-HMQC approach vis-à-vis the 2D-DQ–SQ INEPT [26] method can be inferred from a comparison of the corresponding SQ correlation experiments namely, MAS-J-HMQC and 2D-INEPT–HSQC. A previous comparison of signal to noise obtained for ^{13}C labeled samples showed that the INEPT–HSQC polarization transfer was more